THE ANATOMICAL RECORD 199:543-553 (1981)

Ultrastructural Study of the Embryonic Development in the Rat Pineal Gland

J. CALVO AND J. BOYA
Department of Histology and General Embryology, Faculty of Medicine
University Complutense, Madrid, Spain

ABSTRACT The ultrastructure of the albino rat embryo pineal gland was studied from day 13 of development through birth. In the first stages (13–16.5 days of development) the pineal evagination presents a barely differentiated epithelium. From 17 days onward the transformation of the pineal gland from a tubular evagination into a compact organ occurs. The obliteration of the recess takes place by means of two mechanisms: (a) multiple foldings of the epithelium which determine an approximation and fusion of the walls of the recess, and (b) occupation of the lumen by cells extruded from the pineal epithelium. Embryos of 18–21 days of gestation still show remains of the pineal recess.

From day 16.5 onward elements of the pineal parenchyma have been found outside the pineal epithelium contour. They contact with the mesenchymal cells without a basal lamina separating both elements.

Day 20 marks the beginning of recognizable differentiation of pineal cellular types. However, in the newborn rat these types are not yet clearly established.

Several authors have described the ultrastructure of the adult albino rat pineal gland (Wolfe, 1965; Arstila, 1967; Tapp and Blumfield, 1970; Matsushima and Reiter, 1975a). Nevertheless, the embryonic development of the pineal gland has been scarcely studied in this species. Only two studies have been published (Clabough, 1973; Boucher and Bourges, 1975) on the ultrastructure of the embryonic rat pineal gland, both of which describe some of the ultrastructural features of young rat pinealocytes.

In a previous study (Calvo and Boya, 1980), we described the embryonic development of the rat pineal gland using light microscopy. An ultrastructural study of rat pineal development, however, may provide a better understanding of adult pineal morphology.

MATERIALS AND METHODS

Albino rats (Wistar) have been used for our study. Female rats in estrous phase were mated with the males. Eight hours later the presence of sperm was demonstrated by vaginal smear. In this way, the fecundation time was calculated within an approximation of 4 hours.

The pregnant rats were sacrified at intervals of 12 hours from day 13 until day 21 postcoitum. The mother was anesthetized and the fetus removed from the uterine horns. Various fetuses from at least three litters were used to cover the developmental stages studied. Moreover, the pineal glands of six newborn rats from two different litters were included in the study.

The pineal glands were fixed by immersion in cold 0.1 phosphate-buffered 3% glutaraldehyde, pH 7.4. They were then rinsed in phosphate buffer, postfixed in phosphate-buffered 1% osmium tetroxide, and embedded in Epon (Luft, 1961). Semithin and ultrathin sections were obtained from an LKB ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined in a Philips 201 electron microscope.

RESULTS

Although the primordium of the pineal gland appears at 13.5 days of development (Calvo and Boya, 1979), its small size makes it difficult to locate with the electron microscope. At 15.5 days it may already be identified. It has a large lumen (recess) and its epithelium is composed of tall cells placed perpendicularly

Received January 10, 1980; accepted October 17, 1980.

0003-276X/81/1994-0543$03.50

to the basal lamina (Fig. 1). The pinealoblast nuclei tend to locate themselves in the central zone of the epithelium, leaving a basal and apical zone composed of cellular processes (Figs. 1, 2). These pinealoblasts appear undifferentiated and no cellular types may be identified. The pinealoblasts are very rich in free polyribosomes throughout their cytoplasm and their mitochondria are usually small. Occasionally, lipid droplets, small dense bodies, and coated vesicles have been found. In the vicinity of the pineal recess the pinealoblasts display junctional mechanisms, especially zonulae adhaerentes (Fig. 2). The apical cytoplasm located above the band of junctional complexes usually adopts a clublike form and occasional basal bodies and cilia may be found in it (Fig. 2).

At 16 – 16.5 days of development the pineal anlage has increased in size, still showing a morphology similar to the one previously described. The apical zone displays abundant mitotic figures. The nuclear zone shows an increase in the number and size of the nucleoli. Basal bodies and cilia are more frequent in the terminal clubs of the pinealoblasts although ciliary profiles are still rarely found in the lumen. The cilia lack the central pair of microtubules (Fig. 3a). Longitudinal sections of the cilia frequently show a sudden narrowing near the base (Fig. 3b,c). Also, certain images have been found that suggest the existence of widenings in some of the cilia.

At 16.5 days, groups of pinealoblast processes have been found outside the pineal epithelium contour (Fig. 4). The surface of these groups of processes, which faces the mesenchyma, contacts directly with mesenchymal cells without a basal lamina separating both elements. On the contrary, the surface of the group of processes facing the recess wall does display a basal lamina. In some cases a continuance may be seen between the groups of processes and the pineal epithelium (Figs. 4, 5). In these cases the basal lamina of the epithelium continues directly along the surface of the group of processes which face the epithelium (Fig. 5).

At 17 days the obliteration of the pineal recess takes place. Sections of the proximal portion of the gland still show a large recess similar to the one described in previous stages. In the distal zone, no large lumens may be found. In some cases, the lumen is reduced to a narrow cavity mostly occupied by pinealoblast terminal clubs (Fig. 3). In other cases, the decrease in lumenal size is due to the appearance of cells inside the lumen. These cells, forming groups of different sizes, are located above the apical pinealoblast projections (Figs. 6, 7). Although polyhedral and practically lacking processes, these cells have an ultrastructural appearance similar to the pinealoblasts of the recess wall. In some areas interruptions have been found in the band of junctional mechanisms, at which level there seems to be a continuity between the lumen cells and the rest of the pineal epithelium (Fig. 6).

Other distal sections of pineal glands of 17 days of development show total obliteration and fragmentation of the recess. They display localized groups of junctional complexes limiting a space totally occupied by pinealoblast apical processes. Some of the longer groups of junctional complexes show these complexes only in one of the two contact surfaces (Fig. 8). The cells around these complexes are placed radially, forming "rosettes." Mitotic figures are frequently associated to these formations (Fig. 8).

Capillaries of immature appearance begin to be found in pineal glands of 17 days of development (Fig. 9). A narrow space may be seen separating the endothelial cells from the pinealoblasts. The basal lamina of the parenchyma is incomplete in many cases.

Numerous interruptions of the basal lamina are found along the basal surface of the pineal epithelium. Now, groups of pinealoblasts as well as their processes may be found outside the basal lamina retaining a continuity with the pineal epithelium (Fig. 10). The external surface of these cell groups, in contact with mesenchymal cells, lacks a basal lamina (Fig. 10a).

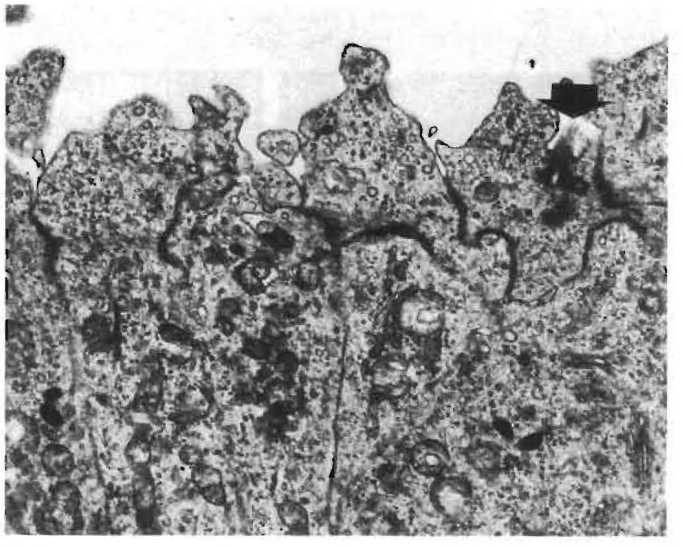
The obliteration of the pineal recess advances rapidly toward the proximal portion of the gland. From 18 days of development onward, throughout most of the gland the previous lumenal spaces have been obliterated. There are only pinealoblasts forming "rosettes" around groups of junctional complexes.

The number of intrapineal vessels increases progressively. Also, small connective tissue spaces lacking a vascular component may be observed (Fig. 9).

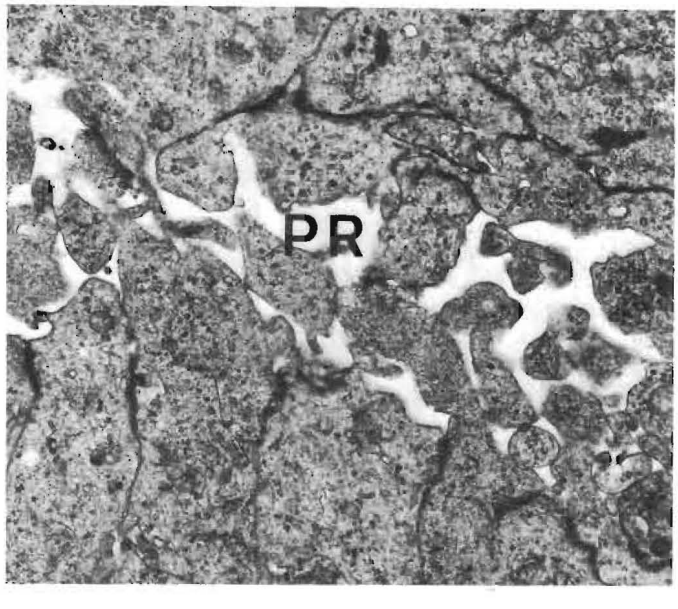
At 18 days there is a clear increase in pineal cellularity. Pinealoblasts become gradually smaller, having processes which are distributed throughout the parenchyma. After 19.5 – 20 days of development differences begin to be observed in pinealoblast cytoplasmic density (Fig. 11).

The pineal gland of the newborn rat appears solid. The lumenal remnants described during the embryonic period may no longer be found.

1



2



3

Fig. 1. embryo of 15 days. Pineal recess wall. The pineal epithelium presents an apical zone (AZ), a very large nuclear zone (NZ), and a basal zone (BS) near the mesenchyma (M). PR: Pineal recess; arrow: image of cellular degeneration. × 4,800.

Fig. 2. Embryo of 15 days. Apical zone of the pineal epithelium. The pinealoblast apical processes show junctional mechanisms. Above the band of complexes, terminal clubs may be observed, one of which presents a cilium (arrow). × 11,800.

Fig. 3. Embryo of 17 days. Obliteration process of the pineal recess (PR). The recess walls have approximated without contacting. The lumen is reduced to a narrow fissure. × 12,800. A. Transverse section of the pinealoblast cilium. × 26,300. B. Longitudinal section of a pinealoblast cilium. × 13,600. C. Longitudinal section of a pinealoblast cilium. Its diameter suddenly decreases near the origin. × 12,000.

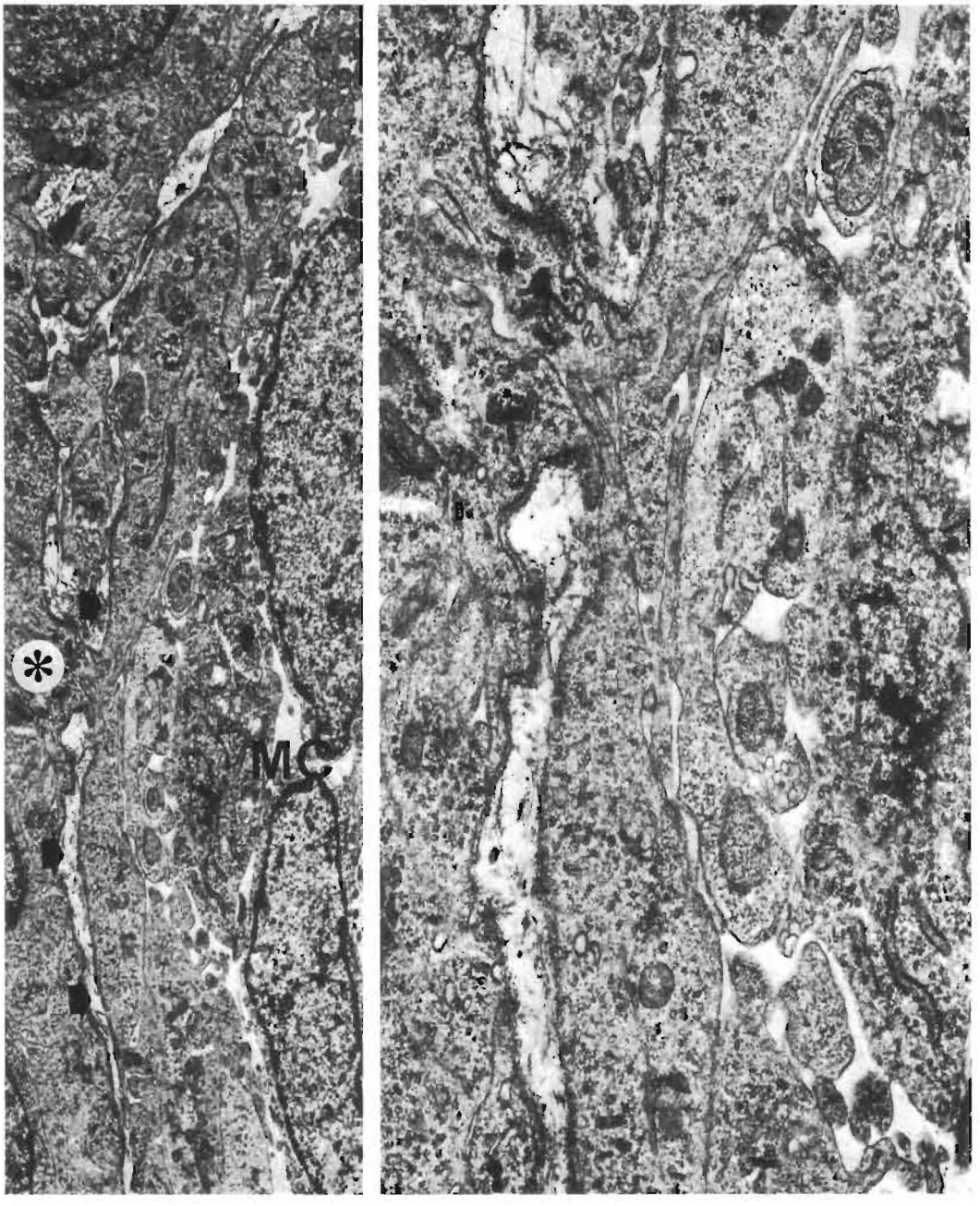
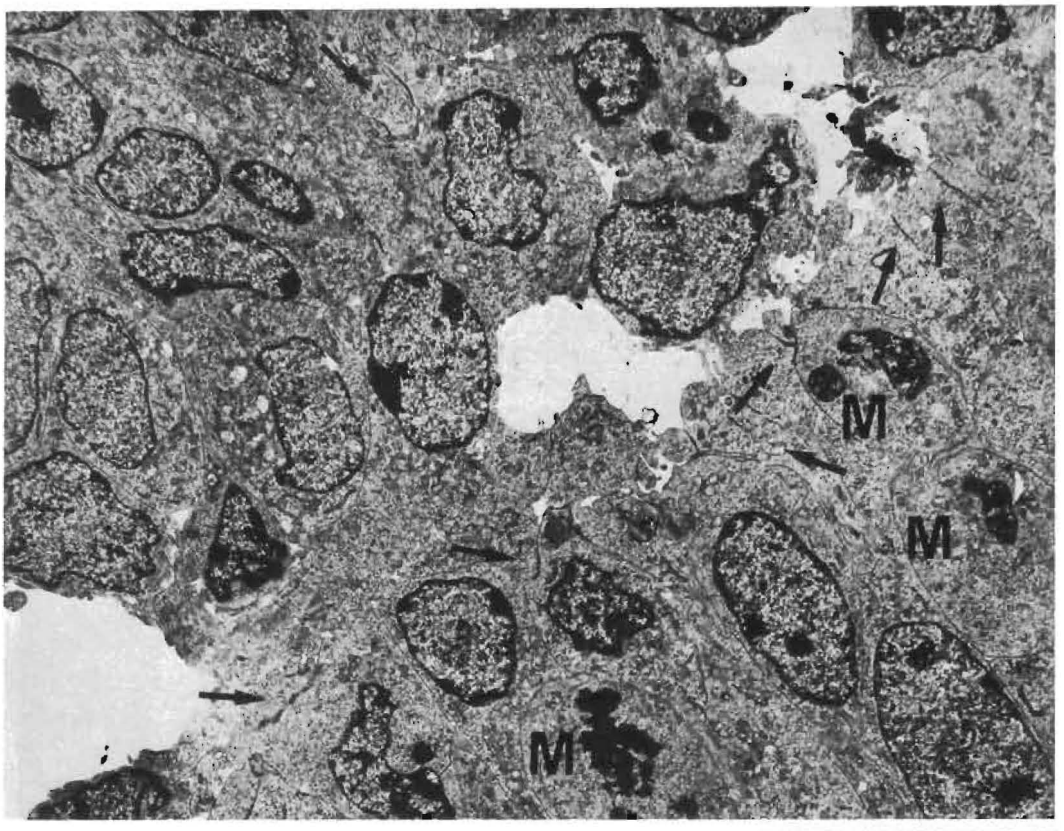
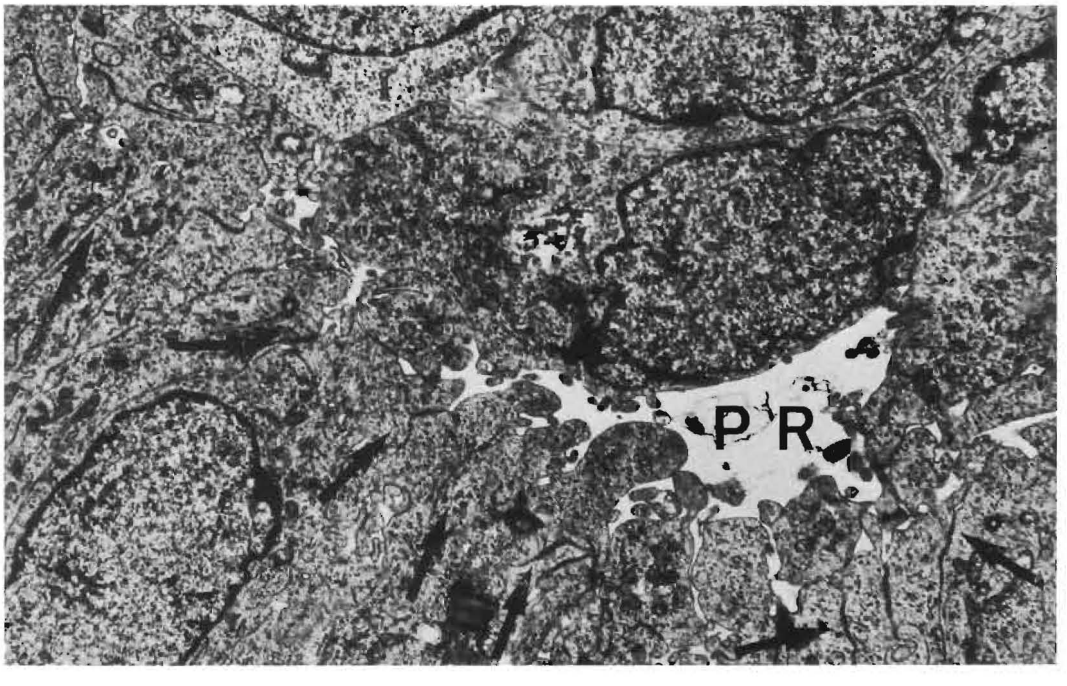


Fig. 4. Embryo of 16.5 days. Basal limit of the pineal epithelium having a basal lamina. At the point (*) pinealoblast processes penetrate into the neighboring mesenchyma. The basal lamina continues along the process surface which faces the epithelium (arrows). The outer surface, adjacent to mesenchymal cells (MG), has no basal lamina. × 8,900.

Fig. 5. Embryo of 16.5 days. Detail at higher magnification of Figure 4. × 22,900.



6

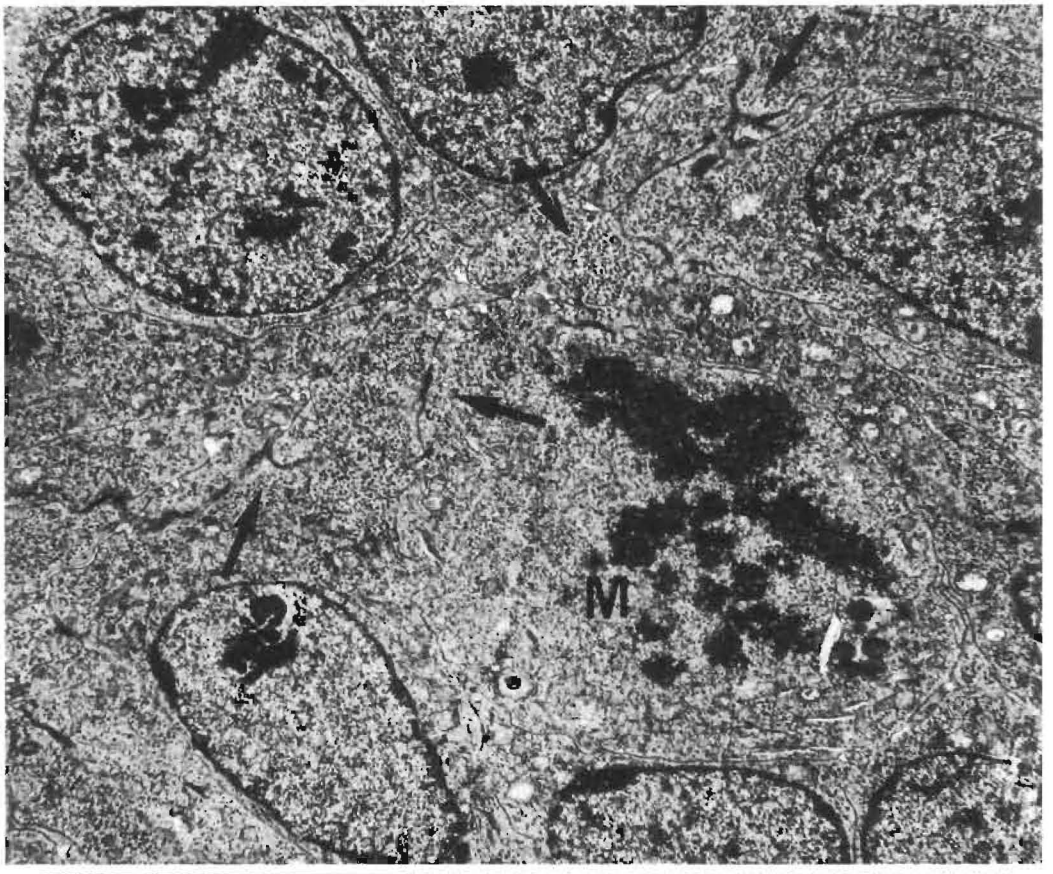


7

Fig. 6. Embryo of 17 days. Obliteration process of the pineal recess. Fragmentation of the pineal recess by a pinealoblast bridge. The junctional complexes (arrows) of the recess wall seem to be missing at the bridge level. The bridge pinealoblasts lack the typical polarity of the pineal epithelium cells. In the apical zone of the epithelium, various mitotic cells are observed (M). × 4,900.

Fig. 7. Embryo of 17 days. Obliteration process of the pineal recess. The pineal recess (PR) appears limited by polarized pinealoblast apical processes having junctional complexes (arrows) and terminal clubs. Most of the lumen is occupied by pinealoblasts without signs of polarization. × 7,800.

8



9

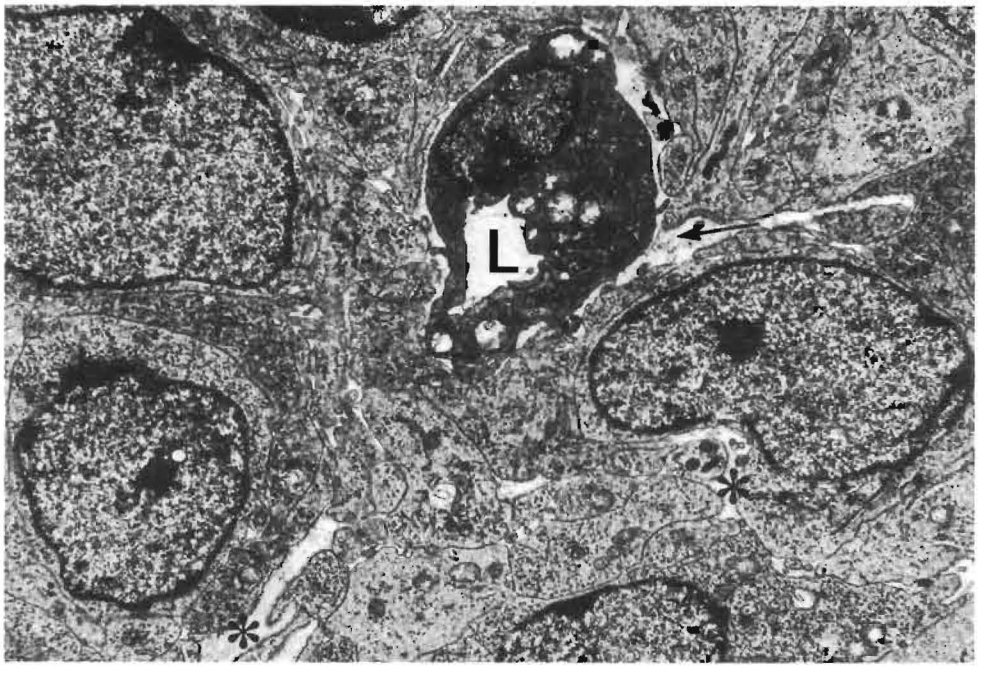


Fig. 8. Embryo of 17 days. Group of junctional complexes. The junctional complexes seem to locate themselves in only one of the two surfaces (arrows), which contains a mitotic figure (M). × 8,600.

Fig. 9. Embryo of 17 days. Intrapineal capillary of immature appearance separated from the pineal parenchyma by an incomplete basal lamina. Several small connective tissue spaces are observed (*), one of which communicates with the vascular-connective tissue space (arrow). L, Lumen. × 8,200.

Occasionally, a "rosette" may be seen. The structures most frequently observed are isolated junctional complexes without a tendency for pinealoblast polarization around them.

The pinealoblasts of the newborn rat are small cells from which abundant processes extend. Due to the small cellular size, there is a high cellular population at this stage. The processes are distributed apparently at random throughout the parenchyma, showing a tendency to end along connective tissue spaces. Numerous mitotic figures are still found.

At birth, two different pinealoblast types, clear and dark, may be differentiated (Fig. 11). There are, however, numerous cells of an intermediate appearance. The clear pinealoblast displays an ovoid nucleus of disperse chromatin and clear nuceloplasm (Fig. 11). The perinuclear cytoplasm is abundant and the cellular processes tend to be wide. In the cytoplasm, free polyribosomes and rough endoplasmic reticulum forming long cisterns are obvious (Fig. 12). Mitochondria are small, and the well-developed Golgi systems have numerous associated vesicles. Coated vesicles are present throughout the cytoplasm (Fig. 12). Small round or ovoid dense bodies are also found. Occasionally, lipid droplets may be observed (Fig. 12). This cell type also contains microfilaments, sometimes very abundant, and some microtubules.

The dense pinealoblast is more scarce than the clear one, and it may appear isolated or forming bands (Fig. 11). The nucleus is smaller than that of the clear cell and its chromatin is disposed in groups inside a dense nucleoplasm. The perinuclear cytoplasm is scarce and electron dense (Figs. 11–13). The processes tend to be thinner than those of the clear type. Some organelles seem less numerous, especially the endoplasmic reticulum and the free ribosomes. Lipid droplets and microfilaments may also be found, being less obvious due to the higher density of the cytosol (hyaloplasm). Characteristic features of the dense pinealoblasts are centrioles, diplosomes, and even cilia (Fig. 13). Also, small dense granules of different shapes may be observed (Fig. 14). The dense content of the granule is separated from its membrane by a thin clear halo. These dense granules are constantly found in this cellular type, both in its perinuclear cytoplasm and in its processes (Figs. 14, 15).

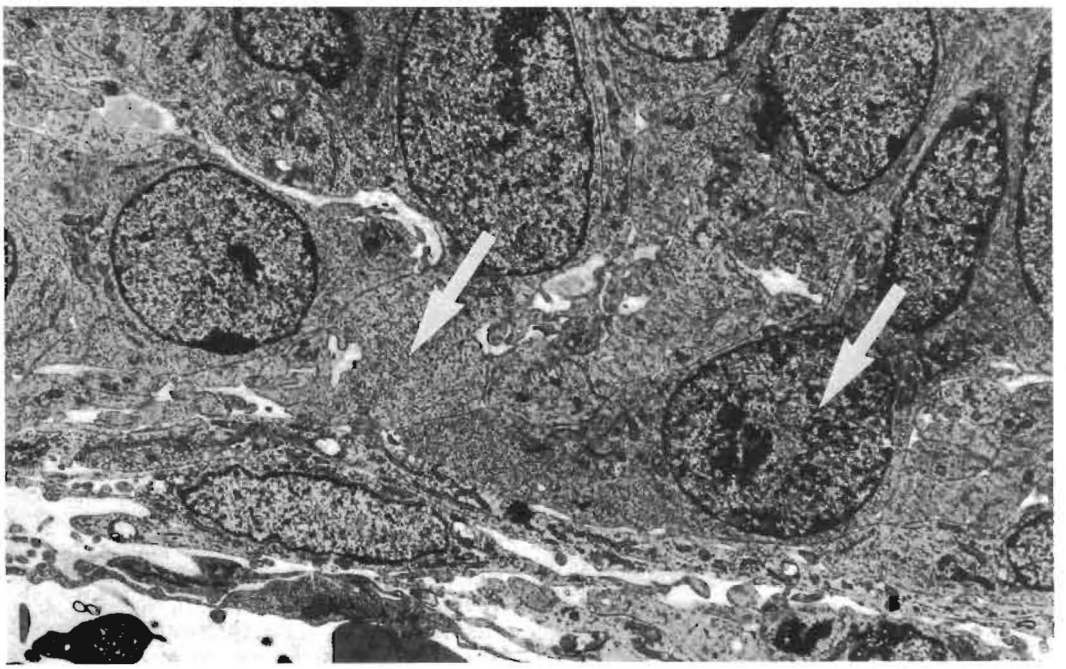
The pineal gland of the newborn rat shows a great development of its connective tissue spaces. Some of them display capillaries and even connective tissue cells. Numerous capillaries already show an ultrastructural appearance similar to that described for the adult pineal (Wolfe, 1965; Arstila, 1967; Matsushima and Reiter, 1975b), although capillaries of immature appearance may still be found. These vascular-connective tissue spaces usually display numerous small projections lacking vessels and cells. The basal lamina is incomplete in many cases (Fig. 14).

DISCUSSION

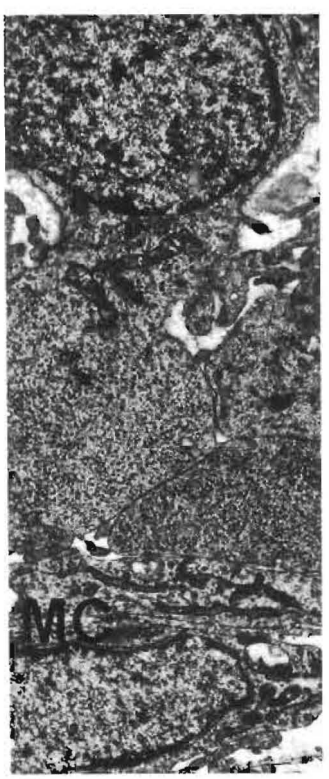
In embryos of 15 to 16.5 days of development the pineal epithelium presents an ultrastructural appearance comparable to that described with the light microscope (Kappers, 1960; Clabough, 1973; Calvo and Boya, 1980). The pinealoblasts appear as scarcely differentiated cells. This appearance agrees with that of previously published ultrastructural descriptions (Clabough, 1973; Boucher and Bourges, 1975).

From 15 days of development lipid droplets are observed in the rat pinealoblasts. Lipid droplets are a characteristic component of adult rat pinealocytes (Wolfe, 1965; Arstila, 1967; Tapp and Blumfield, 1970; Matsushima and Reiter, 1975a). The presence of cilia and junctional complexes in the apical zone of the pineal epithelium has been described by Clabough (1973) in rat embryos of 18 days. According to our results, these structures are present from the first stages of pineal development.

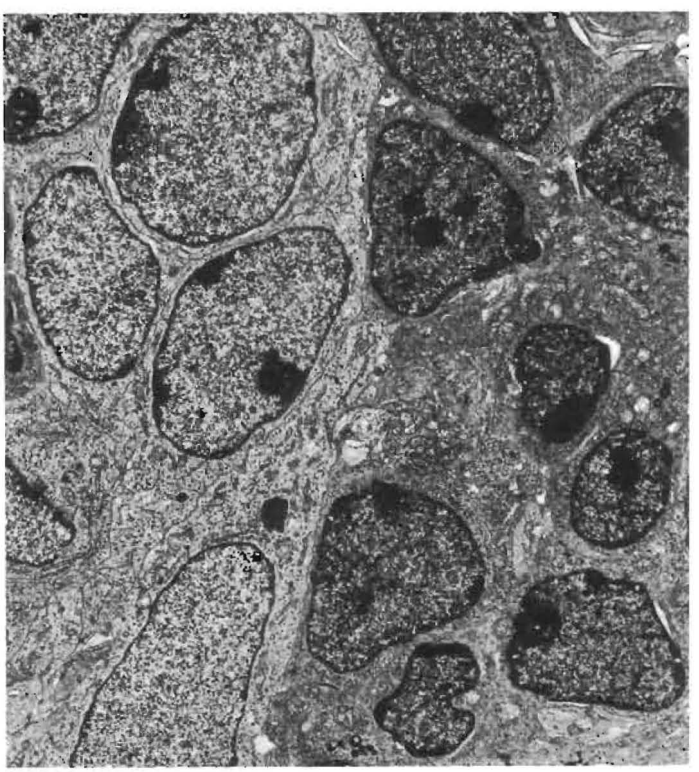
From 17 days of development onward, the obliteration of the pineal recess takes place. Clabough (1973) only cites the obliteration of the pineal recess in embryos of 18 days. The obliteration of the recess begins by an approximation and later a fusion of its walls. This process is partly due to the mechanism of infolding of the pineal epithelium previously described by us with light microscopy (Calvo and Boya, 1980) in rat embryos of this age. It is also due to the mechanism of occupation of the lumen by pinealoblasts. Although the location of these cells inside the lumen could be attributed to tangential sections of the recess, several findings argue against this possibility; (1) The cells are frequently located outside and in contact with an epithelium whose structural pattern, polarity toward the lumen, and appearance of junctional complexes and terminal clubs indicate that it is not a tangential section; (2) the pinealoblasts located inside the lumen show no signs of polarity toward it;



10



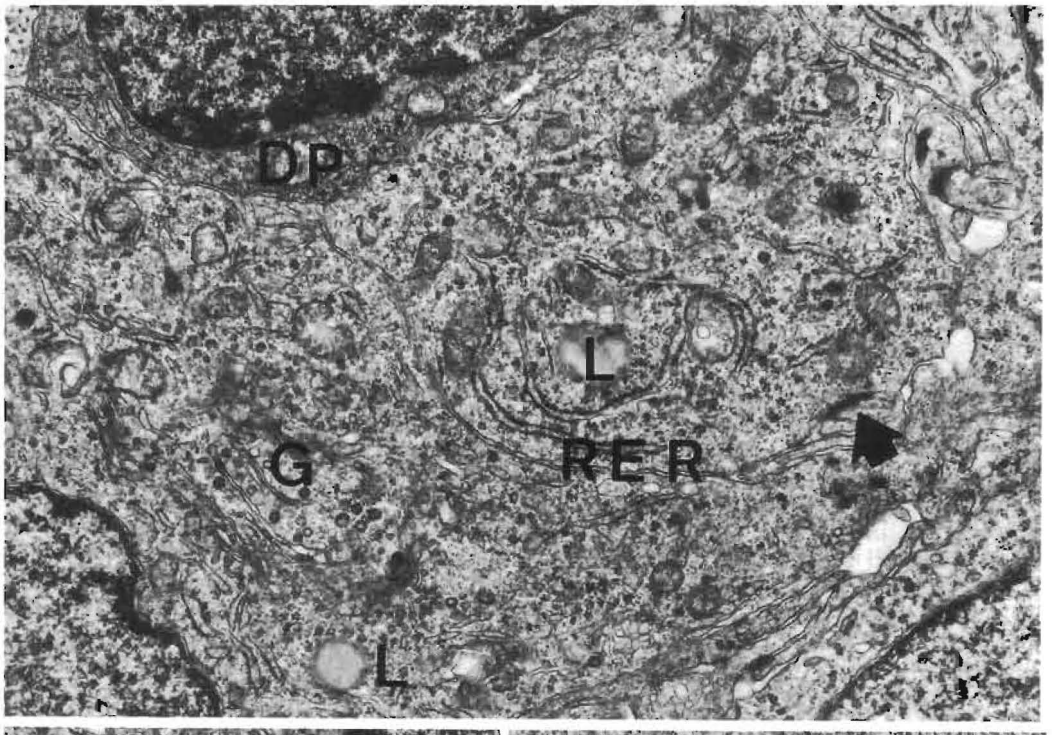
10A



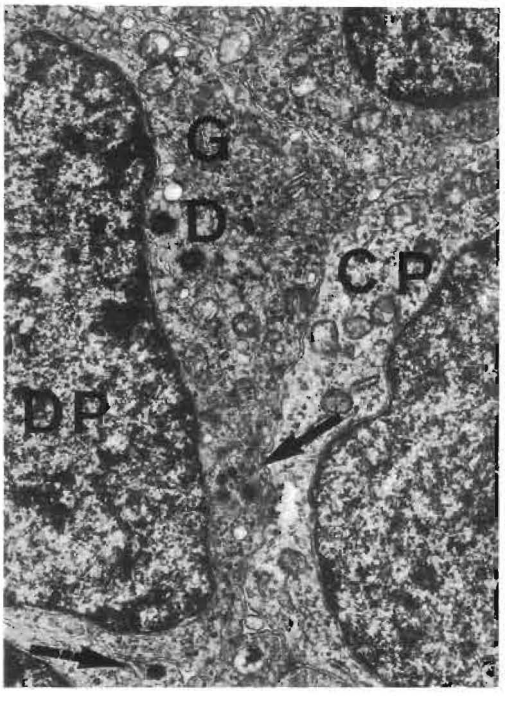
11

Fig. 10. Embryo of 17 days. Basal zone of the pineal epithelium. At several points (arrows) parenchymal element may be seen to penetrate the neighboring mesenchyma. A. Detail of the previous figure. The pinealoblast surface in contact with mesenchymal cells (MC) lacks a basal lamina. × 10,600.

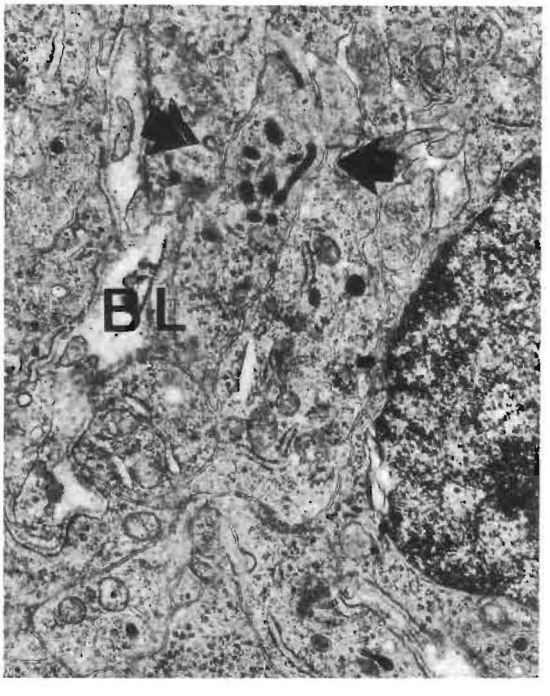
Fig. 11. Embryo of 21 days. Clear and dense pinealoblasts in groups. × 4,600.



12



13



14

Fig. 12. Newborn rat. Clear pinealoblasts having large cytoplasm rich in organelles, and part of a dense pinealoblast (DP). G: Golgi system; RER: rough endoplasmic reticulum; L: lipids; arrow: isolated junctional complex. × 17,000.

Fig. 13. Newborn rat. Dense pinealoblasts (DP) and clear pinealoblasts (CP). G: Golgi system; D: diplosome; arrows: dense granules. × 12,100.

Fig. 14. Newborn rat. Minimal connective tissue spaces having a basal lamina (BL). Pinealoblast processes, some of which present dense granules (arrows), in contact with the basal lamina. × 12,800.

neither do they display junctional complexes nor terminal clubs along their luminal surface. Any oblique or tangential section of the recess would show a lumen totally surrounded by junctional complexes but never an isolated nuclear zone of the epithelium inside the lumen not surrounded by junctional complexes; and (3) zones of discontinuity have been found in the band of junctional complexes which seem to be the points at which the pinealoblasts enter the lumen. All the zones of discontinuity found are associated to groups of pinealoblasts located inside the lumen.

As a consequence of these events, the recess is reduced to a series of elongated cavities limited by junctional complexes, whose lumens are totally occupied by pinealoblast apical processes. Frequently, only one of the two surfaces of contact presents junctional complexes. In these cases, the cavity was probably formed by the process of cellular occupation of the lumen. Thus, the surface lacking junctional complexes would correspond to the cells which previously invaded the lumen. Eventually, the elongated cavities undergo a process of fragmentation giving way to the formation of small aligned cavities.

The appearance of cavities in the rat pineal has been previously described with the light microscope by Kappers (1960) and Clabough (1973), being interpreted as a neoformation of follicles from the recess wall. According to our previous descriptions (Calvo and Boya, 1978; 1979), this mechanism does exist in the embryonic development of the chick pineal. However, our previous results with the light microscope (Calvo and Boya, 1980), and the present study, indicate that the cavities observed in the rat pineal are remnants of the pineal recess obliteration. This statement is based on the following findings: (1) The cavities are found where the recess was previously located; (2) the cavities present the features (junctional complexes, terminal clubs, cilia) characteristic of the apical zone of the epithelium limiting the recess; (3) none of these characteristics has been found in the depth of the epithelium; and (4) the first cavities observed are large, and they evolve toward a progressive fragmentation until they disappear. If neoformation of cavities existed, the inverse evolution would be observed.

In embryos of 16.5 and 17 days of development, images have been found which indicate that elements of the pineal parenchyma penetrate into the mesenchyma which surrounds the pineal anlage. Initially (16.5 days), only cellular processes are found, but in later stages complete cells may be observed. The absence of a basal lamina at this level suggests that this is not a simple irregularity of the pineal parenchyma basal contour. The discontinuity and even absence of a basal lamina separating the parenchyma from the stroma seems to be one of the characteristics of the adult rat pineal gland (Wolfe, 1965; Arstila, 1967; Wartenberg, 1968). The pinealocyte processes of the adult rat end in connective tissue spaces without a basal lamina separating both elements (Wolfe, 1965; Wartenberg, 1968). In other mammals, the parenchymal basal lamina is continuous (Anderson, 1965; Wartenberg, 1968) even during the embryonic period (Anderson, 1965). Thus, the images found in the basal surface of the rat embryo pineal anlage could indicate the early appearance of a special relation between the parenchyma and the stroma characteristic of the pineal gland in this species. The penetration of pineal parenchymal elements into the mesenchyma is closely related to the intense foldings of the pineal epithelium which accompany the obliteration of the pineal recess (Calvo and Boya, 1980). The mechanical tensions associated with the infoldings could possibly be related to the penetration of the basal lamina by epithelial elements.

The invasion of the pineal anlage by connective tissue begins in 17.5-day embryos but it mainly develops in later stages. Invaginations and infoldings are generally occupied by blood vessels which course from the periphery of the pineal. In the first stages, the basal lamina of the parenchyma is either incomplete or lacking in these invaginations. In the newborn rat some of the capillaries already display an ultrastructure similar to that described in adult animals (Wolfe,1965; Artstilla, 1967; Matsushima and Reiter, 1975b). Apart from the large connective tissues spaces containing blood vessels, numerous small spaces are found presenting collagen microfibrils and a basal lamina which is sometimes incomplete. Many of these are probably sections of fingerlike projections of a large vascular-connective tissue space.

The changes in pineal apithelial configuration and the invasion of the connective tissue stroma result in a loss of the regular structural pattern characteristic of young embryos. Thus, from day 18 onward rosettes near a connective tissue space have been found as well as mitotic figures in contact with a basal lamina, etc. These images could be interpreted as the

neoformation of cavities in the rat pineal. However, their tendency to decrease in size and disappear in later days suggests that they are related to the process of disappearance of the pineal recess. Thus, the location of "rosettes" near a basal lamina seems to be a consequence of pineal invasion by stroma.

From day 19.5 of development, differences begin to become visible in the ultrastructural appearance of the pinealoblasts. At birth, two contrasting types of pinealoblasts may be distinguished. The differences in cellular density in the last stages of development have been indicated by Clabough (1973) and Boucher and Bourges (1975). What we have described as clear and dense pinealoblasts only represent two extreme forms between which numerous pinealoblasts display intermediate morphologic characteristics. Thus, a dual classification of pineal cells may not be established at birth. These two pinealoblast types do not seem to show a clear relation with the two adult cellular types described by various authors (Wolfe, 1965; Arstila, 1967; Tapp and Blumfield, 1970; Matsushima and Reiter, 1975a). We thus consider that cytologic differentiation at birth is not sufficiently defined as to be able to classify cellular types in relation to those of the adult pineal.

LITERATURE CITED

Anderson, E. (1965) The anatomy of bovine and ovine pineals. Light and electron microscopic studies. J. Ultrastruct. Res. Suppl., *8*:1–180.

Arstila, A.U. (1967) Electron microscopic studies on the structure and histochemistry of the pineal gland of the rat. Neuroendocrinology, *2*:1–101.

Boucher, D., and M. Bourges (1975) Ultrastructure de la glande pinéale de rat au cours de son dévelopment embryonnaire. J. Microscopie, *23*:83–92.

Calvo, J., and J. Boya (1978) Embryonic development of the pineal gland of the chicken. Acta Anat. (Basel), *101*:289–303.

Calvo, J., and J. Boya (1973) Ultrastructural study of the embryonic development of the pineal gland of the chicken. Acta Anat. (Basel), *103*:39–74.

Calvo, J., and J. Boya (1980) Embryonic development of the rat pineal gland. Anat. Rec. (in press).

Clabough, J.W. (1973) Cytological aspects of pineal development in rats and hamsters. Am. J. Anat., *137*:215–230.

Kappers, J.A. (1960) The development, topographical relations and innervation of the epiphysis cerebri in albino rat. Z. Zellforsch., *52*:163–215.

Luft, J.H. (1961) Improvements in epoxi resin embedding methods. J. Biophys. Biochem. Cytol., *9*:409–414.

Matsushima, S., and R.J. Reiter (1975a) Comparative ultrastructural studies of the pineal gland of rodents. In: Electron Microscopic Concepts of Secretion: Ultrastructure of Endocrine and Reproductive Organs. M. Hess, ed., J. Wiley, New York, pp. 335–356.

Matsushima, S., and R.J. Reiter (1975b) Ultrastructural observations of pineal gland capillaries in four rodent species. Am. J. Anat., *143*:265–282.

Reynolds, E.S. (1963) The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J. Cell Biol., *17*:208–212.

Tapp, E., and M. Blumfield (1970) The parenchymal cells on the rat pineal gland. Acta Morphol. Neerl. Scand., *8*:119–131.

Wartenberg, H. (1968) The mammalian pineal organ: Electron microscopic studies on the fine structure of pinealocytes, glial cells and on the perivascular compartment. Z. Zellforsch., *86*:74–97.

Wolfe, D.E. (1965) The epiphyseal cell: An electron microscopic study of its intercellular relationships and intracellular morphology in the pineal body of the albino rat. Prog. Brain Res., *10*:332–376.