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Immunohistochemical study of the pineal glial cells in the postnatal development of the rat pineal gland

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Abstract: The developmental expression of the glial antigens, vimentin (VIM), glial fibrillary acidic protein (GFAP), and S-100 protein is described in the rat pineal gland from the first postnatal day to adulthood. Thick VIM immunopositive cell cords forming a network throughout the pineal gland were observed from the first postnatal days. These cords progressively disappeared during the first postnatal month as their cells dispersed into the pineal parenchyma. From 20 to 25 postnatal days, pineal glial cells appeared as isolated star-shaped VIM immunopositive cells. Immunostaining for GFAP and S-100 protein showed a similar developmental expression pattern. Both antigens appeared later than VIM (15–20 postnatal days) and were restricted to the pineal glial cells located in the proximal third of the gland, close to the pineal stalk.

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Introduction

The pineal gland in mammals contains two types of parenchymal cells: the main cell type or pinealocyte, and a second cell type variously named as interstitial cell, type II pinealocyte, etc. [Wolfe, 1965; Arstila, 1967; Pevet, 1977; for review see Vollrath, 1981].

In 1978, Møller et al. showed the presence of glial fibrillary acidic protein (GFAP) immunopositive cells within the parenchyma of the rat pineal gland. These cells were identified as interstitial cells and a glial nature for them was proposed in rodents. Further studies have confirmed the presence of GFAP in the second pineal cell type of different mammals [Huang et al., 1984; Schachner et al., 1984; Calvo et al., 1988; Li and Welsh 1991]. Moreover, the expression of other antigens considered as glial cell markers has been widely documented in the second pineal cell type in rodents [Schachner et al., 1984; Calvo et al., 1988; López-Muñoz et al., 1992]. All the immunohistochemical studies so far reported on this pineal cell type were performed mainly in adult animals. Information on the developing immunohistochemical characteristics of this cell type is scarce. To our knowledge, there is only one report describing the postnatal development of GFAP immunoreactivity in the pineal gland of the hamster and gerbil [Li and Welsh, 1991].

In previous investigations, it has been demonstrated that the second pineal cell type expresses three antigens present in glial cells, i.e., the intermediate filament proteins Vimentin (VIM), GFAP, and the cytoplasmic calcium-binding protein S-100 [Møller et al., 1978; Schachner et al., 1984; Calvo et al., 1988; López-Muñoz et al., 1992]. In the present investigation, we studied the developmental expression of these three antigens in the rat pineal gland from birth to adulthood.

Materials and methods

Sixty Wistar albino rats of both sexes between 1 day and 6 months old were used in this study. Animals were kept under routine laboratory conditions, with food and water ad libitum and in a natural light environment. Four rats at each age stage from 1, 2, 3, 5, 7, 10, 15, 20, 25, 30, 45 days, 2, 3, 4, and 6 months were killed by decapitation under ether anesthesia. Pineal glands were quickly removed and fixed by immersion in Bouin's fixative or Metha-

carn (60% methanol, 30% chloroform, 10% acetic acid) for 12–16 hr at 4°C and embedded in paraffin. Tissue blocks were oriented to obtain sagittal sections of the pineal gland. Serial tissue sections 5 μm thick were mounted on chromegel-coated slides for immunolabelling. The peroxidase anti-peroxidase (PAP) method was carried out for the demonstration of GFAP and S-100 protein. VIM was demonstrated with an indirect immunoperoxidase method according to Taylor [1986]. The following primary antisera (Dakopatts, Denmark) were used: 1) 1:300 polyclonal rabbit anti-bovine GFAP, 2) 1:320 polyclonal rabbit anti-bovine S-100 protein, and 3) 1:50 monoclonal mouse anti-human vimentin. Negative controls consisted in the substitution of the primary antiserum for non-immune serum of the same species or an irrelevant antibody. Positive controls were brain and cerebellum sections; furthermore, the immunostaining of astrocytes, ependyma, and subcommissural organ of the nervous tissue adjacent to the pineal gland served as an intrinsic positive control. After incubating in 3-3' diaminobenzidine, sections were briefly counterstained with haematoxylin for better demonstration of the pinealocyte nuclei.

Results

Vimentin

VIM immunoreactivity is present from the first postnatal days in cells of the rat pineal gland arranged in thick cords distributed throughout the gland (Fig. 1). At higher magnification, these cords are made up by numerous VIM immunopositive (VIM+) cells located close to the pineal connective tissue spaces (Fig. 2). This cord-like pattern is very striking during the first 5 postnatal days, but progressively disappears up to the age of 20–25 days (Figs. 3, 5, 7). During this period, VIM+ cell cords seem to break up into small cell clusters located near the connective tissue spaces (Figs. 3, 5, 7). The disappearance of the cord-like pattern of VIM immunoreactivity is concomitant with a progressive increase in the population of isolated VIM immunopositive cells within the pineal parenchyma. At earlier stages (5–10 days), these cells only exhibit one or two cell processes (Fig. 4). In the ongoing evolution, both an increased number of single cells and a progressive development of their cell processes are observed (Figs. 5–8).

At the age of 30 days, the rat pineal gland shows the characteristic immunocytochemical pattern that will be maintained during the adult period (Figs. 7, 9). Cell cords are no longer observed, although some scattered small cell clusters of VIM+ cells can be seen occasionally (Figs. 7, 8). A notable population of star shaped VIM+ cells are evenly distributed throughout the pineal parenchyma (Fig. 9). Immunoreactive cell processes form a network among pinealocytes (Fig. 10) and reach the connective tissue spaces.

GFAP

GFAP immunoreactivity in the pineal gland is first observed in 20 day-old rats. GFAP+ cells displayed a typical shape and location that remain unchanged during the period studied. Thus, GFAP+ cells appear star shaped, with large somata and long processes being exclusively located in the proximal third of the gland, close to the pineal stalk (Figs. 11, 12). In older animals, a slight increase in these GFAP+ structures is found.

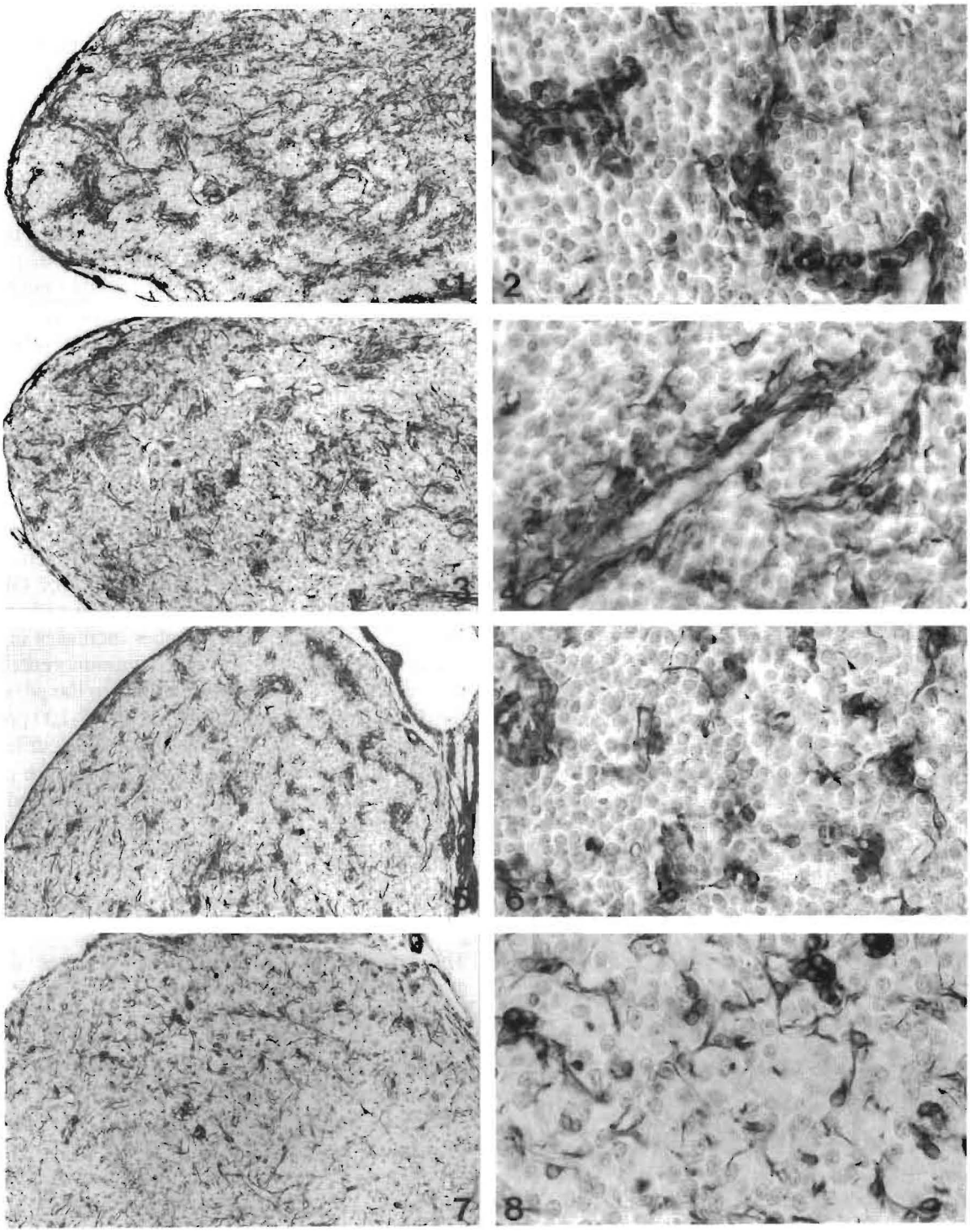
S-100 Protein

The evolution of S-100 protein immunoreactivity in the pineal gland is very similar to that of GFAP. S-100 protein is first detected in a few cells at the age of 15–20 days. Their number increases in later stages but, similarly to GFAP immunoreactivity, this cell population is also restricted to the proximal third of the gland (Fig. 13). However, S-100 protein immunopositive cells show a broader distribution than GFAP+ cells (Fig. 13). Moreover, the population of S-100 protein positive somata and cell processes were both clearly larger than GFAP+ elements (Fig. 14).

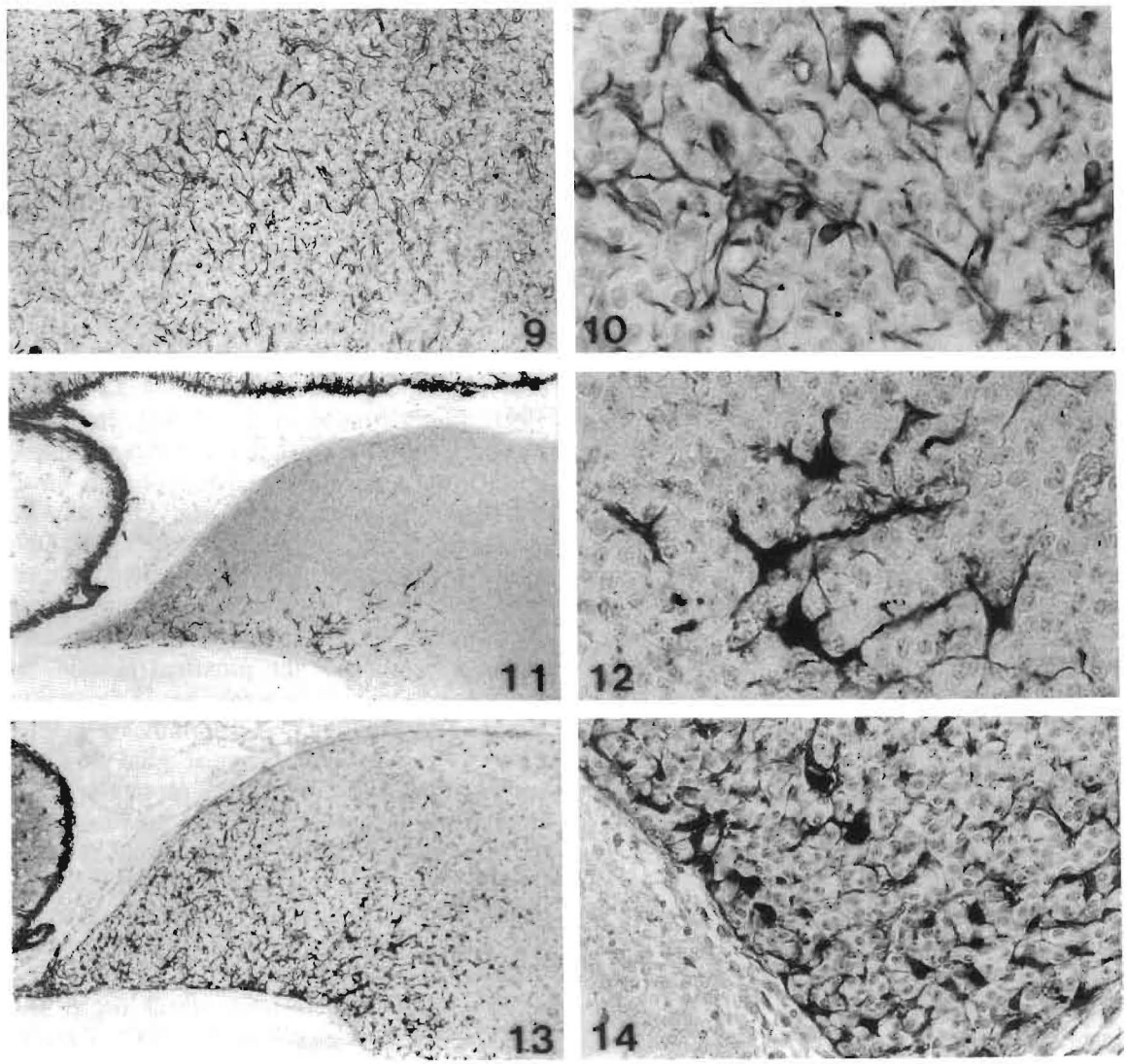
Discussion

The results reported here show changes in the expression of the glial antigens VIM, GFAP, and S-100 protein occurring during the postnatal development of the rat pineal gland. To date, the presence of these antigens had been reported only in pineal glial cells of adult rats [Møller et al., 1978; Schachner et al., 1984; Calvo et al., 1988; López-Muñoz et al., 1992].

The presence of abundant VIM+ elements is a consistent finding throughout the postnatal development. Nevertheless, large changes in the number, location, and arrangement of VIM+ cells are noted during pineal gland maturation. Thus, VIM immunoreactivity is present from the 1st day after birth, although maximal expression is reached during the first postnatal week (2–5 days). Throughout this week, VIM+ cells are arranged in thick cords, mainly located close to the pineal connective tissue spaces. While connective tissue elements also express VIM, previous morphological studies have demonstrated that pineal connective tissue stroma is



Figs. 1–8. Developmental expression of VIM in the rat pineal gland during the first postnatal month at low (×100, Figs. 1, 3, 5, 7) and high magnification (×450, Figs. 2, 4, 6, 8). The characteristic cord-like pattern of the first postnatal week (Figs. 1, 2) progressively disrupts (Fig. 1, 3, 5, 7). The thick cellular cords (Fig. 2) fragment in small cell clusters (Figs. 4, 6), that are very sparse at 30 days (Fig. 8). At the same time, VIM+ cells are detached from the cords, and appear like isolated cells within the pineal parenchyma. In early stages (Fig. 4), single cells are sparse and display few processes. Later on, an increase in both the number of isolated cells and their processes is observed (Figs. 6, 8). Figs. 1, 2: 5 days-old. Figs. 3, 4: 10-day-old. Figs. 5, 6: 15-day-old. Figs. 7, 8: 30-day-old.



Figs. 9–10. 60-day-old rat. VIM. VIM+ glial cells with numerous processes form a dense network throughout the pineal parenchyma. No cell clusters are observed. Fig. 9: ×100. Fig. 10: ×450.
Figs. 11–12. 60-day-old rat. GFAP. GFAP immunopositivity is restricted to the proximal third of the pineal gland. At higher magnification (Fig. 12), GFAP+ cells display thick processes resembling astrocytes. Fig. 11: ×75. Fig. 12: ×450.
Figs. 13–14. 60-day-old rat. S-100 protein. The pattern of immunoreactivity is similar to that of GFAP, although a broader area of S-100 protein positivity is noted. At higher magnification, S-100 protein positive cells form a more dense network than GFAP. Fig. 13: ×75. Fig. 12: ×280.

very poorly developed during the first postnatal weeks [Calvo and Boya, 1984] and thus, VIM+ cell cords should be mainly made up by parenchymal glandular cells. In fact, both the arrangement and distribution of these structures fully correlate with immature cells or pinealoblast cell cords previously described with light and electron microscopy [Calvo and Boya, 1983, 1984].

VIM expression in immature cell cords could lead to the consideration that these structures are exclusively made up by pineal glial cell precursors. However, the population of VIM+ cells during the first postnatal weeks clearly outnumbers that observed in the pineal parenchyma of the adult rat (10–15% of the parenchymal cells) [Calvo and Boya, 1984; López-Muñoz et al., 1991]. Former studies with light and electron microscopy have also documented the cytodifferentiation of immature cells to pinealocytes during early stages of postnatal life [Karasek, 1974; Steinberg et al., 1981; Calvo and Boya, 1983, 1984]. VIM expression in immature cells is a common finding during embryonic development [Van Muijen et al., 1987]. In neuroectodermal tissues, VIM expression has been observed in neuroepithelial cells with pluripotential ability to differentiate toward neurons and glial cells

[Schnitzer et al., 1981]. Therefore, VIM immunopositivity in the cells forming the pineal cords may be probably due to the immature nature of these cells rather than indicating that the cell cords are completely made up by glial cell precursors. In later stages, some of these cord-like cells would differentiate to pinealocytes losing VIM immunoreactivity, whereas the remainder will differentiate to pineal glial cells retaining VIM expression. Cell death could also explain the decrease in VIM+ cells of these cords; however, very few degenerating VIM+ cells were observed in our study.

VIM+ cell cords progressively disrupt during the first postnatal month. In the course of this process, immature cells are commonly arranged in small clusters, confirming previous light microscope studies [Calvo and Boya, 1984]. During the first postnatal week, pineal glial cells displaying short processes were observed. These cells were usually seen near the immature cell clusters. In later stages, there is an increase in this population of isolated cells. Their cell processes, widely distributed, form a dense network. As described before, the ultrastructural differentiation of the pineal glial cells in the rat also starts during the first postnatal week, and is fully developed in the first postnatal month [Calvo and Boya, 1983].

The development of GFAP and S-100 protein expression exhibits an analogous pattern during the postnatal maturation of the rat pineal gland. Both antigens appear later than VIM (15–20 days) and, from the very first stages, they are exclusively present in the proximal third of the gland, close to the pineal stalk. In the hamster and gerbil [Li and Welsh, 1991], GFAP immunoreactivity first appears on days 6 and 10 after birth, respectively, being more conspicuous in the proximal portion of the gland (deep pineal).

In the adult rat [Schachner et al., 1984; Calvo et al., 1988; López-Muñoz et al., 1992] and other rodents including hamster [Huang et al., 1984] and mouse [Schachner et al., 1984], VIM is a ubiquitous marker for pineal glial cells. The present results reinforce that VIM is a generic marker for pineal glial cells, since the expression of this antigen is present from early stages of their cytodifferentiation.

In the rat, glial pineal cells could constitute diverse cell subtypes according to their antigen expression patterns. Thus, VIM+ glial cells are evenly distributed throughout the gland, whereas GFAP and S-100 protein are also expressed in the glial cells located in the proximal region of the pineal [Calvo et al., 1988; López-Muñoz et al., 1992]. These three antigens could be present in the same cells; in fact, it has been reported that, at least, VIM and GFAP are coexpressed in pineal glial cells in the proximal portion of the rat pineal gland [López-Muñoz et al., 1992]. According to the present investigation, the heterogeneity of pineal glial cells in the rat is established during the postnatal development. Until 15 days after birth, only VIM is detected in developing pineal glial cells. From the third week, S-100 protein and GFAP+ cells first appear near the pineal stalk and later spread all over the proximal third of the gland.

In previous reports, pineal glial cells have been considered similar to immature astrocytes of the central nervous system [Møller et al., 1978; Huang et al., 1984; Schachner et al., 1984; Li and Welsh, 1991; López-Muñoz et al., 1992]. In the pineal parenchyma it seems that a maturation gradient of pineal glial cells occurs. Therefore, in the distal region of the gland, VIM+, GFAP− pineal glial cells, resembling astroglial precursors (radial glia) in the central nervous system [Schnitzer et al., 1981], are found. On the other hand, the expression of VIM, GFAP, and S-100 protein is observed in pineal glial cells of the proximal portion, as described in more mature astrocytes [Schnitzer et al., 1981]. The stimulus that determines a more complete differentiation of pineal glial cells in the proximal third of the gland is presently unknown. However, a relationship exists between the anatomic localization of the pineal gland, the distribution of pineal glial cells within the glandular parenchyma, and their level of immunocytochemical differentiation. Thus, GFAP+ glial cells have been observed distributed throughout the gland in some species with deep located pineal organ [Møller et al., 1978; Lowenthal et al., 1982; Zang et al., 1985]. In contrast, the superficial pineal gland of rats contains GFAP+ glial cells exclusively in the pineal stalk and proximal region of the gland. Commisural fibers could drive the hypothetical stimuli that induce the astroglial differentiation of pineal glial cells. However, the recent description of the development of GFAP+ pineal cells in pineal grafts from neonatal hamsters transplanted beneath the renal capsule by Li and Welsh (1991) suggests that other mechanisms are involved in the regulation of the cytodifferentiation of pineal glial cells.

The presence of glial cells in the pineal gland is an unusual characteristic in endocrine glands. In the present report, we have described the postnatal maturation of these glial cells in the rat pineal gland. Further investigations should clarify the mechanisms that regulate the differentiation of pineal glial cells.

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