Calcium Binding Proteins Immunohistochemistry and Identification of Neurons in the Mammalian Pineal Gland of the African Giant Rat: *Cricetomys gambianus*

E. BASTIANELLI[1], K. MOUTAIROU[2], M. T. AKÉLÉ-AKPO[3], R. DARBOUX[3] AND R. POCHET[1]

1 *Laboratoire d'Histologie, Faculté de Médecine, Université Libre de Bruxelles, Bruxelles, Belgique*
2 *Département de Biologie cellulaire, Faculté des Sciences et Techniques, Université Nationale du Bénin, Cotonou*
3 *Unité d'Anatomie Pathologique, Faculté des Sciences de la Santé, Université Nationale du Bénin, Cotonou*

Abstract. The presence of true neurons in the rodent pineal gland is still a matter of controversy In this work, by using immunohistochemistry with five antibodies against calcium-binding proteins (calbindin-D28k, calretinin, calmodulin, neurocalcin and S-100β) and *Cricetomys gambianus*, a rodent belonging to Muridae family living in Africa, we were able to illustrate the presence of neurons in the pineal gland. Anti-calbindin-D28k and anti-calretinin labelled neurons belonging to two neural ganglia. One ganglion was localized in the anterior part of the gland near the pineal stalk and the other one in the posterior portion of the organ. Immunoreactive neurons are medium in size (15–20 μm) and have long thick processes running towards the stalk. Calretinin and calbindin-D28k positive neurons stained with different intensities. Thin processes were detected by anti-calretinin whereas thick processes were preferentially calbindin-D28k positive. Neurocalcin labelled a few smaller neurons and many thin processes within the ganglion. Calmodulin could not be detected immunochemically. Within the ganglia many astrocytic processes were S-100β positive The afferent and the efferent pathways of the pineal ganglia remain to be elucidated.

Key words: Calcium – Calbindin – Calmodulin – Calretinin – Pinealocytes – Neurons

Correspondence to Dr Roland Pochet, Laboratoire d'Histologie, Faculté de Médecine, Université Libre de Bruxelles, 808 route de Lennik, B–1070 Bruxelles, Belgique
E-mail rpochet@ulb.ac.be

Introduction

In rat, the most intensively studied rodent, the pineal gland is composed of two main cell subclasses the pinealocytes which account for 90% of the cell total and the interstitial cells which include mainly astrocytes, pericytes and mast cells (Arstila 1967) Immunohistochemical studies have discriminated among pinealocytes and categorized them in distinctive subsets Specific retinal antigen, S-antigen (Korf et al 1985), rhodopsin (Huang et al 1992) and recoverin (Korf et al 1992, Bastianelli and Pochet 1994) recognize different pinealocyte subpopulations Some pinealocytes express these proteins whereas others do not Synaptophysin (Redecker and Bargsten 1993) and neuron-specific enolase (Mockett and Lapwood 1994) also label different subsets of pinealocytes Recently, using calbindin-D28k, we were able to discriminate, in the rat, between type I and type II pinealocytes, or intraparenchymatous and perivascular pinealocytes (Bastianelli et al 1993, Pochet et al 1994) Interstitial cells in the pineal of rodents do not show such a diversity, but subsets have been described using GFAP (Borregón et al 1993), S-100 (Moller et al 1978), vimentin (Chakravarty 1992), calbindin-D28k (Bastianelli and Pochet 1995)or cal retinin (Redecker et al 1996) The presence of neurons, a third class of pineal cells, has not, until recently, been clearly shown and is not "generally accepted" ["The pineal of the rat does not contain any neurons" as stated in the recent textbook The Rat Nervous System by Paxinos (1995), p 399] However, already in 1973 David et al have described the presence in the ferret of an intrapineal ganglion and the presence of intrapineal neurons has been noted in the rabbit (Romijn 1975), the ground squirrel (Matsushima and Reiter 1978), bats (Bhattacharya et al 1990) and the chipmunk (Karasek et al 1982) The presence of true neurons in the pineal gland increases the polysynaptic pathway conveying photic information whose physiological importance could be trough a regulatory role on the rhythmic secretion of melatonin Recently, by using anti-calretinin immunohistochemistry, we and another group (Novier et al 1996) provided morphological description strongly suggesting that neurons were indeed present in rat pineal gland (Pochet et al 1994, Bastianelli et al 1995) In this work we present evidence for the presence of neurons clustered in two ganglia in a rodent pineal

Calretinin like parvalbumin and calbindin-D28k occurs in distinct subpopulations of neurons and both have been employed as neuroanatomical markers (Celio 1990, Jacobowitz and Winsky 1991, Résibois and Rogers 1992, Andressen et al 1993, Floris et al 1994, Seto-Ohshima 1994) These proteins have an advantage over other neuronal markers in that, by virtue of their high solubility, they are usually present throughout the cytosol, even in the thin processes of neurons, therefore facilitating studies of neuronal shape and connectivity The provide new and powerful tools to classify neurons, to reveal the mode of distribution of distinct neuronal populations, and to analyse cell class-specific morphological features at both the

light and electron microscopic level. This is best illustrated by the recent discovery and precise morphology, thanks to calretinin, of ultrastructural features of unipolar brush cells (UCB). The first precise description of this "novel" neuronal type was made after calretinin immunohistochemistry of the adult human cerebellar cortex (Braak and Braak 1993). Mugnaini and Floris (1994) by utilizing a variant of the classical Golgi/Del Rio-Hortega procedure which affords a much higher rate of impregnation have described the same new type of neuron they named unipolar brush cells and concommitantly used calretinin immunohistochemistry to analyse their distribution and ultrastructural features (Floris et al. 1994). Unipolar brush cells are the cortical neurons that are most densely immunostained with antiserum to calretinin. They have rounded or ovoidal cell bodies (9–12 μm in diameter) that are intermediate in size between granule and Golgi cells, send out a thin axon, 0.3–0.5 μm in diameter, and commonly give rise to a single, short stubby dendrite, 2–3 μm in diameter, which usually divides only at the tip, where it forms a tightly packed group of branchlets covered by thin, spine-like appendages, ressembling a paintbrush. The field occupied by the brush tip is rounded, ovoid, boxy or cap-shaped and its diameter is 10–30 μm in average, forming a neuropil island. The unipolar brush cells are highly concentrated in the flocculo-nodular lobe, the ventral ovula and the ventral paraflocculus. They are also in the cochlear nucleus and form a pathway between the flocullus and the cochlear nucleus. Electron microscopic analysis after calretinin immunostaining revealed that the soma, the dendritic stem and specially the dendrioles which terminate the brush-like tips send out short, non synaptic appendages. The dendrioles represent the main synaptic apparatus of the UCB and articulate tightly with a single mossy fiber rosette forming a glomerular array characterized by an extraordinarily extensive synaptic contact. Electrophysiological and electron microscopic observations (Mugnaini et al. 1997) indicate that the unusual synaptic ultrastructure may produce entrapment of neurotransmitter in the synaptic cleft. *Cricetomys gambianus* belongs to Muridae, is a wild animal, and is common throughout tropical Africa. These animals are omnivorous rodents whose physiological and morphological characteristics are poorly known.

Cricetomys gambianus lives in savannah and fallow areas. Adult animals weight between 1.2 and 2.0 kg. They generally do not drink water and eat fresh food (green fodder and palm nuts). In captivity, cannibalism and coprophagy are sometimes observed. Female cricetomes give birth four times a year and have two to four offsprings at each birth (Moutairou et al. 1996).

Materials and Methods

Tissues

Experiments were performed on 6 adult (aged 3 to 9 months) *Cricetomys gambianus*. The pineal gland was dissected and rapidly fixed in Helly's fluid (68 mmol/l

potassium dichromate, 70 mmol/l sodium sulphate, 180 mmol/l $HgCl_2$ and 4% formaldehyde) Fixed tissues were routinely dehydrated, embedded in paraffin, and sectiones at 5μm or 7μm thick were prepared

Antibodies

Rabbit antiserum raised against chick duodenal calbindin-D28k was prepared as described (Spencer et al 1976) and routinely used at 1/6000 dilution Its specificity was demonstrated previously (Pasteels et al 1990, Pochet et al 1991) Polyclonal antibody raised against S100β, was obtained from J Baudier (Grenoble, France) (Cocchia 1981, Baudier et al 1983) and used at 1/3000 dilution Polyclonal antibodies raised against calretinin (Schwaller et al 1993) were obtained from SWant (Belinzona, Switzerland) and used at 1/1000 dilution Three different rabbit polyclonal anti-calmodulin antibodies were used They were prepared as described previously (Van Eldik and Watterson 1981, Van Eldik et al 1983) and termed 449, 465 and 860 Antibodies 449 and 465 were produced against per formic acid-oxidized vertebrate calmodulin and 860 was made against a synthetic peptide of the COOH terminus (residues 134 148) of vertebrate calmodulin Purified IgG fractions were used at 1/2000 dilution Number 465 was routinely used as it gave higher signal versus background Rabbit anti-recoverin was prepared as described (Polans et al 1993) and used at 1/1000 dilution Polyclonal rabbit anti serum raised again rat brain neurocalcin was prepared by Nakano et al (1992) and used at 1/1000 dilution Anti-synaptophysin antibodies were obtained from Dako (Glostrup, Denmark) and used at 1/300 dilution Endogenous peroxydase staining was abolished by H_2O_2 (0,3%) pre-treatment (30 min at room temperature) Con trols for specificity were performed using preincubated antibodies with an excess (2μg/ml) of pure antigen No labelling occurred (not shown) S100β, calbindin and calretinin were purchased from SWant, calmodulin from Sigma (St Louis, USA), and neurocalcin was donated by Dr K Okasaki (Nagoya)

Immunohistochemical staining

Dewaxed and hydrated sections were processed for immunohistochemistry accord ing to a peroxidase-antiperoxydase (PAP) procedure as described previously (Pasteels et al 1990, Pochet et al 1991) Serum dilutions were made up in Coons Veronal Buffered Saline (CVBS 10 mmol/l sodium veronal, pH 7 2, NaCl 0 9%) supplemented with 5% (v/v) normal sheep serum The immunostaining sequence consisted of the following steps 1) rinse in CVBS, 2) preincubation in 5%(v/v) normal sheep serum, 3) incubation with primary antibodies for 48 h at 4°C in a moist chamber, 4) incubation with sheep anti-rabbit immunoglobulin G (IgG) serum 1 100 dilution (Laboratoire d'Hormonologie et Immunologie, Marloie, Belgium), and 5) incubation with soluble rabbit PAP complex 1 300 dilution (DAKO, Glostrup, Denmark) Between each step, sections were thoroughly rinsed for 10

min in CVBS. After the last rinse, staining was performed in a citrate phosphate buffer, pH 6.2, containing 0.5 mg/ml 3,3'-diaminobenzidine- HCl (DAB; Sigma, St. Louis, USA) and 0.01% H_2O_2. All steps, except step 3, were performed at room temperature.

Results

General Description

By macroscopic examination, the *Cricetomys gambianus* pineal gland can be subdivided, as for other rodents, into a superficial and a deep portion. Medium length, measured in six animals, was 4 mm with a diameter of 1 mm. As in albino rats, a substantial portion of the parenchyma of the gland extends from the diencephalon to the vicinity of the cerebellum. Meningeal attachments were observed in the supero-posterior parts of the superficial gland which were connected by a thin pineal stalk to the cerebellum. This *Cricetomys gambianus* pineal gland can be described as a proximo-intermediate-distal gland corresponding to the $\alpha\beta$C type (Reiter 1981; Vollrath 1981).

At the light microscope level, the pineal of adult animals was constituted to

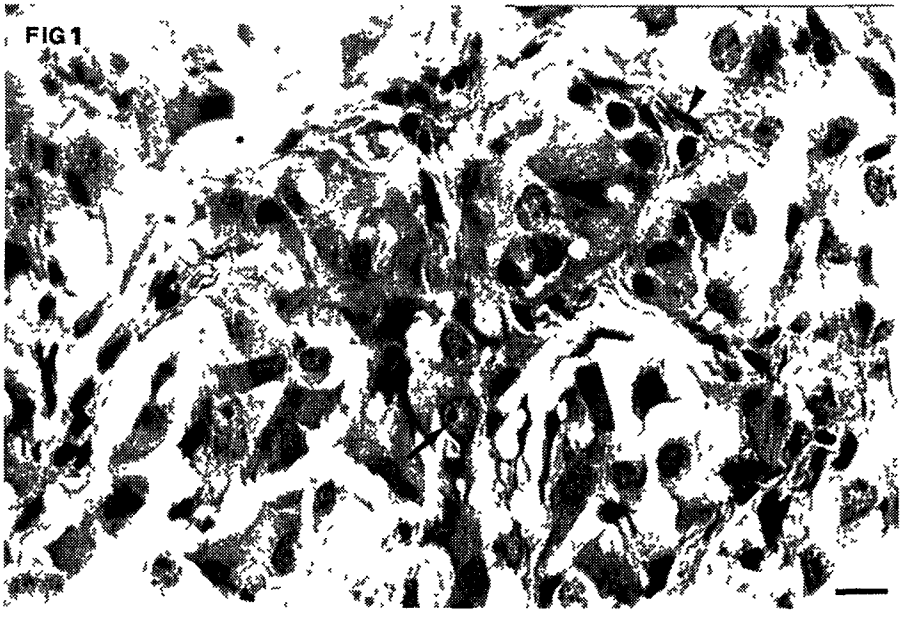

Figure 1. Hematoxylin-eosin staining illustrating the general architecture of the pineal gland The arrow points to a pinealocyte with its large nucleus and central prominent nucleolus The capillary lumen (asterisk) is surrounded by small spindle-shape interstitial cells (arrowhead) Scale bar = 12 μm

2/3 of an anterior cellularized portion and the remaining 1/3 of a posterior more fibrous portion. The posterior portion was composed of a fibrous stroma, sometimes hyalinized, containing rare fibrocytes. The anterior portion was characterized by small plain follicular structure surrounded by capillaries (Fig. 1).

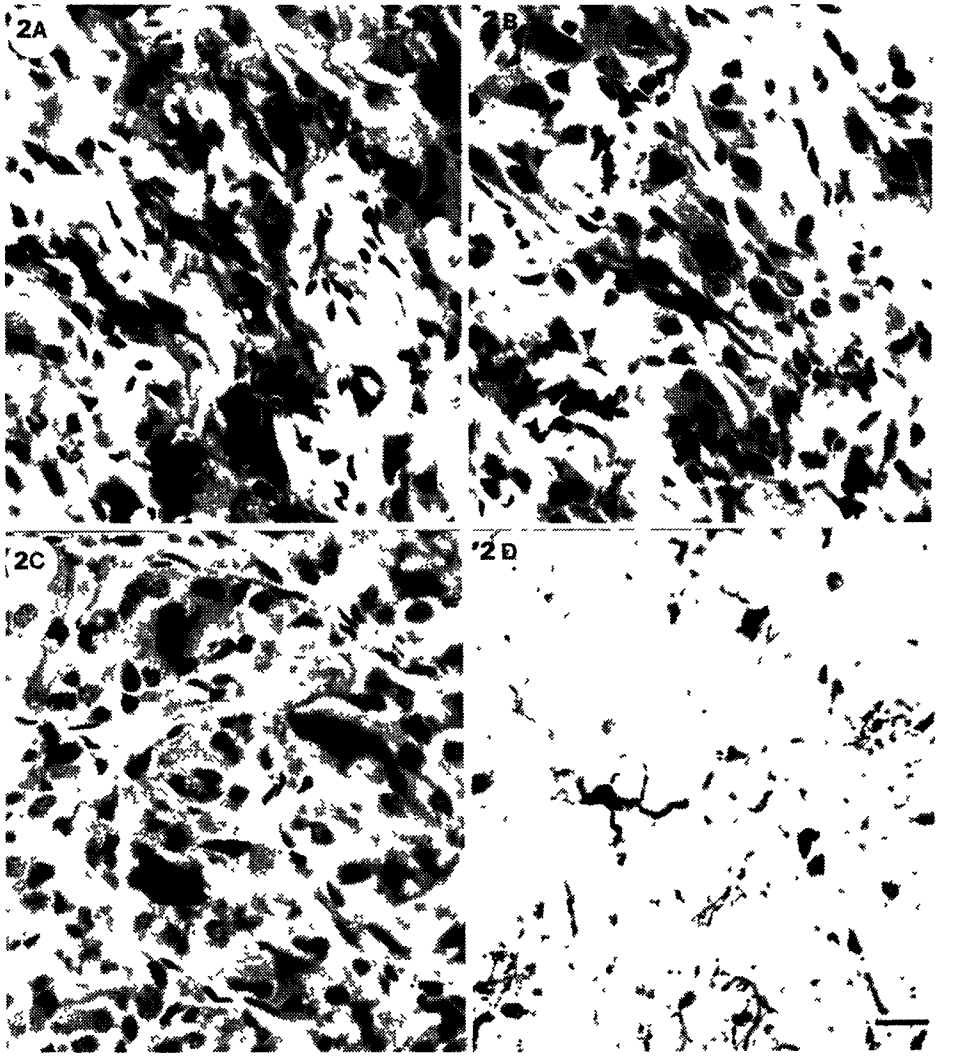

Figure 2. Immunohistochemistry of the anterior part of *Cricetomys gambianus* hematoxylin counterstained and using anti-calretinin (*A*), anti-calbindin (*B*), anti-synaptophysin (*C*) and anti-S-100β (without counterstaining) (*D*) Only pinealocytes are immunoreactive for calretinin, calbindin and synaptophysin Near the stalk some interstitial cells are S-100β-Ir Scale bar = 10 μm

Immunohistochemistry

Further characterization was achieved by using immunohistochemistry and antibodies against 7 proteins: calbindin-D28k, calmodulin, calretinin, neurocalcin, recoverin, S-100β and synaptophysin. Calmodulin and recoverin could not be detected in any cell type (not shown). Calbindin and calretinin-Ir were detected in many pinealocytes (Fig. 2*A* and *B*) but also in two neuronal ganglia (a superior and an inferior) in which both fibers and perikarya could be visualized (Fig. 3*A* and *B*) These two ganglia were located between the anterior and posterior portions of the superficial pineal body. Synaptophysin-Ir was also detected in pinealocytes (Fig 2*C*) whereas neurocalcin and S-100β labelled many processes within the ganglia (Fig. 4*A* and *B*). Immunohistochemical data allowed to distinguish three cell types: pinealocytes, interstitial cell and neurons.

Pinealocytes

Hematoxylin-eosin staining revealed that pinealocytes were distributed in small

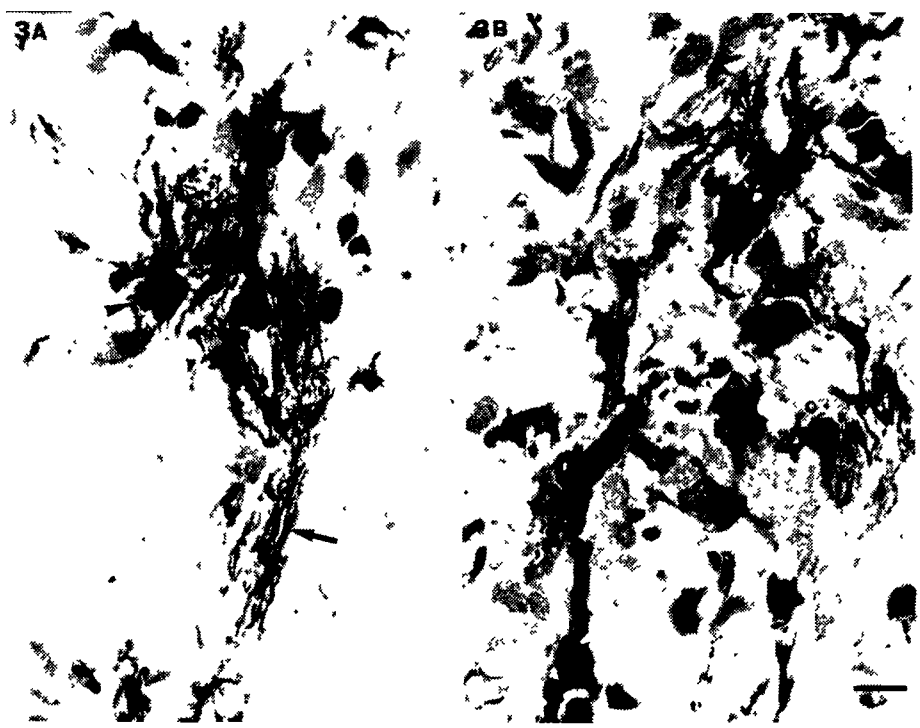

Figure 3. Immunohistochemistry showing calretinin (a) and calbindin (b) positive neurons clustered into the ganglia Neurons were stained with different intensity Fibre arrays were strongly positive Scale bar = 15 μm

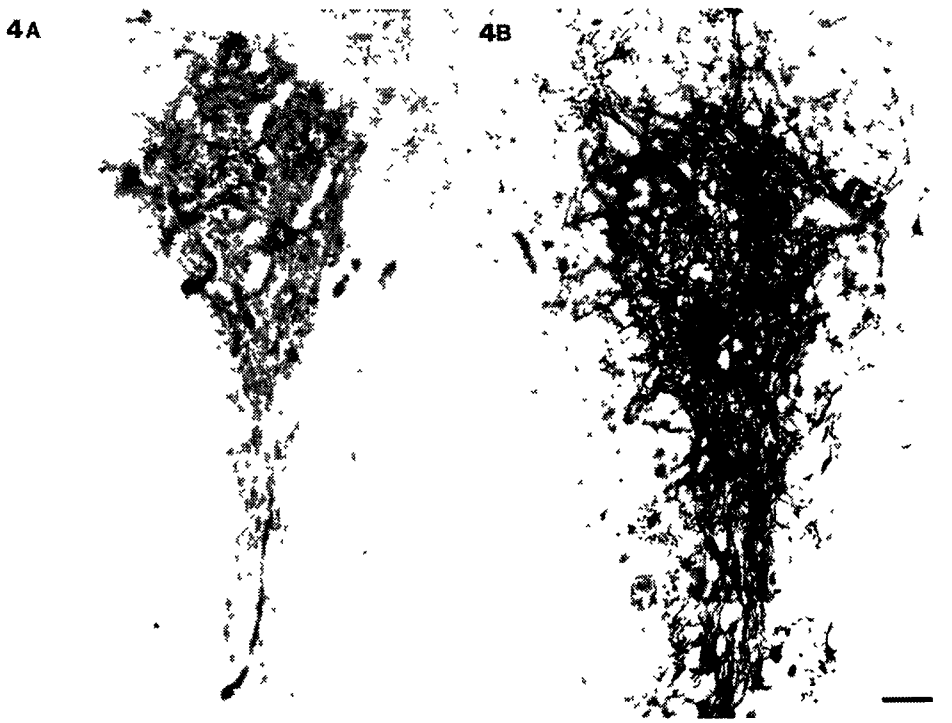

Figure 4. Distribution of neurocalcin (*A*) and S-100 (*B*) in neuronal ganglia Note the neurocalcin immunopositive neural processes The network of S-100 processes appears extremely dense Scale bar = 40 μm

compact follicles. They had large pale cytoplasm with a secretory polarity. Their nuclei were basally located and showed dark motted chromatin with two prominent nucleoli (Fig. 1). Pinealocytes were easily detected using anti-calbindinD-28k and anti-calretinin antibodies (Fig. 2*A* and *B*). Approximately half of pinealocytes per follicle were immunoreactive, but presenting different staining intensities. Although overlapping immunoreactivity could not be excluded the majority of pinealocytes were either calbindin-D28k-Ir or calretinin-Ir. Calbindin-D28k allowed a detailed morphological analysis such as short running processes inside follicles (Fig. 2*B*). Most nuclei were also labelled. Synaptophysin was labelling pinealocytes without specific localisation, about one third displayed immunostaining with medium to strong intensity (Fig. 2*C*). Calmodulin and recoverin were not detected in *Cricetomys gambianus* pineal gland.

Interstitial cells

One main type of interstitial cells was detected using hematoxylin-eosin staining

They were distributed in crown at the periphery of the pineal follicles, in the vicinity of capillaries (Fig. 1). They exhibited a small fusiform shape with a centrally located dark nucleus. The cytoplasm appeared uniformly stained and no chromatin or nucleolus were visible. These cells were calbindin-D28k negative, and poorly S-100β positive. The calretinin and calbindin-D28k positive ones were found homogeneously throughout the superficial part of the pineal gland. The S-100β positive cells were scarce (Fig. 2*D*) and not directly observed in contact with the follicles except in the ganglia where they formed a dense network (Fig. 4*B*).

Neurons

Two distinct neuronal nuclei were detected in the pineal gland, one localized in the deepest part near the pineal stalk and the other one in the most superficial parts of the gland. Immunoreactive neurons were medium sized (15–20μm) (Fig. 3*A* and *B*) and had long thick processes running towards the stalk. Calretinin and calbindin-D28k positive neurons were staining with different intensities. Thin processes were detected by anti-calretinin whereas thick processes were prefentially calbindin-D28K positive. Neurocalcin labelled few smaller neurons and many thin processes whithin the whole ganglia (Fig. 4*A*). Within the ganglia many astrocytic processes were S-100β positive (Fig. 4*B*).

Discussion

The presence of neurons in the rat pineal is not yet commonly recognized (see page 399 of Paxinos 1995). Only recently a few data provided morphological evidence strongly suggesting that neurons were indeed present in the rat pineal gland (Pochet et al. 1994; Bastianelli et al. 1995; Novier et al. 1996). The present work provides further evidence for the existence of true neurons in the rodent pineal. *Cricetomys gambianus,* an African rodent rarely studied but presenting many anatomical similarities with the albino rat (Codjia et al. 1990), allowed us to detect true neurons in its pineal. This was made possible because first, in contrast to rat pineal, *Cricetomys gambianus* pineal gland contains neural ganglia and second, most of the neurons and processes clustered in the ganglia could be seen upon immunostaining using anti-calbindin-D28K and anti-calretinin. Furthermore, the use of immunostaining using neurocalcin, another intracellular calcium-binding protein (Hidaka and Okazaki 1993), present in different neuronal subpopulations (Bastianelli and Pochet 1993; Bastianelli et al. 1995) allowed the labelling of many processes and a few small neurons in the ganglia, and gave a complementary aspect reinforcing data obtained with the two previous antibodies. Finally, S-100β, an astroglial cell marker (Celio 1996) gave an immunohistochemical picture which perfectly fits the ganglia architecture as detected with the 3 other neuronal antigens. Electron microscopy can be expected to provide further evidence of the neuronal structure of those gan-

glion cells. At this stage, we may consider what could be the possible physiological significance of neurons in the mammalian pineal gland. The vertebrate pineal organ rhythmically synthesizes and secretes melatonin during nighttime and forms an essential component of the photoneuroendocrine system which allows humans and animals to measure and keep time. In mammals, the pineal organ has lost both the direct light sensitivity and the capacity to generate circadian rhythms, and melatonin biosynthesis is regulated by retinal photoreceptors and a circadian oscillator located in the suprachiasmatic nucleus of the hypothalamus. Due to this spatial separation, it is thus understandable that the photoneuroendocrine system of mammals comprises neuronal and neuroendocrine pathways which interconnect its components. Recent data (Larsen et al. 1998) provide further evidence on the synaptology of the neural circuitry controlling the rhythmic secretion of melatonin by the rat pineal whose neurons seemed to be connected to each other through gap junctions (Condorelli et al. 1998). Although this study does not provide data on the connections between these ganglia and the brain, preliminary data indicated that neurons from these two ganglia seem to project through the pineal stalk toward the cerebellum. If this is true, it could be an entirely new situation as previous studies have shown that the pineal stalk connects the pineal gland to the diencephalon in many species.

Acknowledgements. This research was supported by grant from the FRSM and the C U D (Coopération Universitaire au Développement)

References

Andressen C , Blumcke I , Celio M R (1993) Calcium-binding proteins selective markers of nerve cells Cell Tissue Res **271**, 181—208

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

Bastianelli E , Pochet R (1993) Sexual dimorphism among calbindin-D28k immunoreactive cells in the rat pineal body Histochemistry **100**, 449—455

Bastianelli E , Pochet R (1994) Calbindin-D28k, calretinin and recoverin immunoreactivities in developing chick pineal gland J Pineal Res **17**, 103—111

Bastianelli E , Pochet R (1995) Calbindin-D28k, calretinin, and S-100 immunoreactivities in rat pineal gland during postnatal development J Pineal Res **18**, 127—134

Bastianelli E , Okazaki K , Hidaka H , Pochet R (1993) Neurocalcin immunoreactivity in rat olfactory bulb Neurosci Lett **161**, 165—168

Bastianelli E , Polans A S , Hidaka H , Pochet R (1995) Differential distribution of six calcium-binding proteins in the rat olfactory epithelium during postnatal development and adulthood J Comp Neurol **354**, 395—409

Baudier J , Mandel P , Gerard D (1983) Bovine brain S100 proteins Separation and characterization of a new S100 protein species J Neurochem **40**, 145—152

Bhattacharya A , Frampton J , Stephan H (1990) The megachiropteran pineal organ A comparative morphological and volumetric investigation with special emphasis on the remarkably large pineal of *Dobsonia praedatrix* J Anat **168**, 143—166

Borregón A , Boya J , Calvo J L , López-Muñoz F (1993) Immunohistochemical study of the pineal glial cells in the postnatal development of the rat pineal gland J Pineal Res **14**, 78—83

Braak E , Braak H (1993) The new monodendritic neuronal type within the adult human cerebellar granule cell layer shows calretinin-immunoreactivity Neurosci Lett **154**, 199—202

Celio M R (1990) Calbindin D-28k and parvalbumin in the rat nervous system Neuroscience **35**, 375—475

Celio M R (1996) Guidebook to the Calcium-binding Proteins Sambrook & Tooze, Oxford

Chakravarty N (1992) The roles of calmodulin and protein kinase C in histamine secretion from mast cells Agents Actions **36**, 183—191

Cocchia D (1981) Immunocytochemical localization of S100 protein in the brain of adult rat An ultrastructural study Cell Tissue Res **214**, 529—540

Codjia J T C , Heymans J C , Adjallala H F (1990) Description du comportement de coprophagie chez les cricétomes (Cricetomys gambianus, Waterhouse), rongeurs africains Cah Ethol Appl **10 (2)**, 143—150

Condorelli D F Parenti R , Spinella F , Salinaro A T , Belluardo N Cardile V , Cicirata F (1998) Cloning of a new gap junction gene (Cx36) highly expressed in mammalian brain neurons Eur J Neurosci **10**, 1202—1208

David G F X , Herbert J , Wright G D S (1973) The ultrastructure of the pineal ganglion in the ferret J Anat **115**, 79—97

Floris A , Diño M Jacobowitz D M Mugnaini E (1994) The unipolar brush cells of the rat cerebellar cortex and cochlear nucleus are calretinin-positive A study by light and electron microscopic immunocytochemistry Anat Embryol (Berlin) **189** 495 520

Hidaka H Okazaki K (1993) Neurocalcin family A novel calcium-binding protein abundant in bovine central nervous system Neurosci Res **16**, 73—77

Huang S -K , Klein D C Korf H -W (1992) Immunocytochemical demonstration of rhodopsin S-antigen, and neuron-specific proteins in the human pineal gland Cell Tissue Res **267** 493—498

Jacobowitz D M Winsky L (1991) Immunocytochemical localization of calretinin in the forebrain of the rat J Comp Neurol **304**, 198—218

Karasek M , King T S , Hansen J T , Reiter R J (1982) Ulstrastructure of the pineal gland of the eastern chipmunk (*Tamias striatus*) J Morphol **173**, 73—86

Korf H , Moller M , Gery I , Zigler J S , Klein D C (1985) Immunocytochemical demonstration of retinal S-antigen in the pineal organ of four mammalian species Cell Tissue Res **239**, 81—85

Korf H -W , White B H , Schaad N C , Klein D C (1992) Recoverin in pineal organs and retinae of various vertebrate species including man Brain Res **595**, 57—66

Larsen P J , Enquist L W , Card J P (1998) Characterization of the multisynaptic neuronal control of the rat pineal gland using viral transneuronal tracing Eur J Neurosci **10**, 128—145

Matsushima S , Reiter R J (1978) Electron microscopic observations on neuron-like cells in the ground squirrel pineal gland J Neural Transm **42**, 223—237

Mockett B G , Lapwood K R (1994) Changes in ovine pineal gland neuron-specific enolase immunoreactivity following bilateral, but not unilateral, superior cervical ganglionectomy J Pineal Res **16**, 202—209

Moller M , Ingild A , Bock E (1978) Immunohistochemical demonstration of S-100 protein and GFA protein in interstitial cells of rat pineal gland Brain Res **140**, 1—13

Moutairou K , Hayez N , Pohl V , Pattyn G , Pochet R (1996) Calbindin localization in African giant rat kidney (*Cricetomys gambianus*) Biochim Biophys Acta **1313**, 187—193

Mugnaini E , Floris A (1994) The unipolar brush cell a neglected neuron of the mammalian cerebellar cortex J Comp Neurol **339**, 174—180

Mugnaini E , Diño M , Jaarsma D (1997) The unipolar brush cells of the mammalian cerebellum and cochlear nucleus cytology and microcircuitry Progr Brain Res **114**, 131—150

Nakano A , Terasawa M , Watanabe M , Usuda N , Morita T , Hidaka H (1992) Neurocalcin, a novel calcium binding protein with three EF-hand domains, expressed in retinal amacrine cells and ganglion cells Biochem Biophys Res Commun **186**, 1207—1211

Novier A , Nicolas D , Krstic R (1996) Calretinin immunoreactivity in pineal gland of different mammals including man J Pineal Res **21**, 121—130

Pasteels B , Rogers J H , Blachier F , Pochet R (1990) Calbindin and calretinin localization in retina from different species Visual Neurosci **5**, 1—16

Paxinos G (1995) The Rat Nervous System 2nd ed Academic Press, San Diego

Pochet R , Pasteels B , Seto-Ohshima A , Bastianelli E , Kitajima S , Van Eldik L J (1991) Calmodulin and calbindin localization in retina from six vertebrate species J Comp Neurol **314**, 750—762

Pochet R , Van Rampelbergh J , Bastianelli E , Van Eldik L J (1994) Calmodulin, calbindin-D28k and calretinin in rat and chicken pineal glands immunohistochemical and immunoblotting analysis Biochim Biophys Acta **1223**, 318—324

Polans A S , Burton M D , Haley T L , Crabb J W , Palczewski K (1993) Recoverin, but not visinin, is an autoantigen in the human retina identified with a cancer-associated retinopathy Invest Ophthalmol Visual Sci **34**, 81—90

Redecker P , Bargsten G (1993) Synaptophysin–A common constituent of presumptive secretory microvesicles in the mammalian pinealocyte A study of rat and gerbil pineal glands J Neurosci Res **34**, 79—96

Redecker P , Cetin Y , Korf H W (1996) Differential immunocytochemical localization of calretinin in the pineal gland of three mammalian species J Neurocytol **25**, 9—18

Reiter R J (1981) The mammalian pineal gland structure and function Amer J Anat **162**, 287—313

Résibois A , Rogers J H (1992) Calretinin in rat brain An immunohistochemical study Neuroscience **46**, 101—134

Romijn H J (1975) Structure and innervation of the pineal gland of the rabbit, *Orycto lagus cuniculus* (L) III An electron microscopic investigation of the innervation Cell Tissue Res **157**, 25—51

Schwaller B , Buchwald P , Blumcke I , Celio M R , Hunziker W (1993) Characterization of a polyclonal antiserum against the purified human recombinant calcium binding protein calretinin Cell Calcium **14**, 639—648

Seto-Ohshima A (1994) Review Calcium-binding proteins in the central nervous system Acta Histochem Cytochem **27**, 93—106

Spencer R , Charman M , Emtage J S , Lawson D E M (1976) Production and properties of vitamin-D-induced mRNA for chick calcium-binding protein Eur J Bio-

chem **71**, 399—409

Van Eldik L J, Watterson D M (1981) Reproducible production of antiserum against vertebrate calmodulin and determination of the immunoreactive site J Biol Chem **256**, 4205—4210

Van Eldik L J, Fok K-F, Erickson B W, Watterson D M (1983) Engineering of site-directed antisera against vertebrate calmodulin by using synthetic peptide immunogens containing an immunoreactive site Proc Natl Acad Sci USA **80**, 6775—6779

Vollrath, L (1981) The pineal organ In Handbuch der Mikroskopischen Anatomie des Menschen Springer, Berlin

Final version accepted February 27, 1999