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Ultrastructure and serotonin immunocytochemistry of the parietal-pineal complex in the Japanese grass lizard, *Takydromus tachydromoides*

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Abstract. The fine structure and immunocytochemical localization of serotonin in the cells of the receptor line were studied in the parietal eye and pineal organ proper of the Japanese grass lizard, *Takydromus tachydromoides*. Typical photoreceptor cells (PC) were the predominant cell type in the receptor line of the parietal eye, the outer segments of which had regular stacks of numerous disks similar to those of cones. The pineal organ contained relatively few PCs, which showed less well-developed outer segments than those of the parietal eye. In contrast, secretory rudimentary photoreceptor cells (SRPC) accounted for the majority of receptor cells in the pineal organ. These cells were structurally characterized by whorl-like lamellar outer segments and numerous dense-cored vesicles (80–280 nm in diameter). A small number of SRPC were also found in the parietal retina, which were similar to those in the pineal organ. In the parietal–pineal complex, numerous mitochondria located in the PC were larger and rounder than those in the SRPC. In the PC, basal processes prossessed only synaptic ribbons, whereas in the SRPC some of these processes contained synaptic ribbons and others contained dense-cored vesicles, rarely having both. Serotonin-immunoreactive cells were found not only in the pineal organ but also in the parietal eye, which closely resembled the cells of the receptor line in their size and shape. Furthermore, on immunoelectron microscopy for serotonin using the protein A-gold technique, gold particles indicating serotonin-immunoreactive sites were restricted in the core of dense-cored vesicles in the SRPC of the pineal organ. Regional differences in the distributions of the PC, SRPC and serotonin-immunoreactivity were found in the parietal-pineal complex.

Keywords: Parietal eye, pineal organ, photoreceptor cells, secretory rudimentary photoreceptor cells, serotonin immunocytochemistry, Japanese grass lizard

Introduction

It has been clearly demonstrated that the pineal organ of poikilothermic vertebrates is basically a photoreceptive organ possessing neuroendocrine capabilities to produce melatonin. During the course of evolution, the pineal organ gradually lost in its photoreceptive capabilities, and consequently in mammals it appears to be a gland involved in relaying photic information conducted via the lateral eye and sympathetic nervous system (Collin, 1971; Oksche, 1971; Vollrath, 1981). Thus, phylogenetically, the form and function of the vertebrate pineal organ have considerable diversity.

Among reptilian species, the pineal organ varies widely in size and structure: it is sac-like in turtles and gland-like in snakes, while in crocodiles it appears to be completely missing (Quay, 1979). According to Gundy & Wurst (1976), approximately 60% of all lizard genera possess a parietal eye, the so-called third eye, which consists of a lens and a retina. Therefore, the parietal–pineal complex has

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been studied microscopically in more genera and species of lizards than in any other groups of reptiles.

Electrophysiological studies have clearly shown functional photoreceptive capabilities not only in the parietal eye but also in the pineal organ of lizards (Hamasaki & Dodt, 1969; Dodt, 1973; Hamasaki & Eder, 1977).

Moreover, electron microscopic studies have demonstrated that photoreceptor cells (PC) in the parietal eye display a number of structural features similar to those in the retinal cones of the lateral eyes, especially in the outer segment, the inner segment, and the presynaptic pedicles (Steyn, 1960; Oksche & Kirschstein, 1968; Wartenberg & Baumgarten, 1968). In contrast, the chief cells in the receptor line of the lacertilian pineal organ have been termed secretory rudimentary photoreceptor cells (SRPC) (Collin, 1971), which possess a whorl-like lamellar outer segment showing distinct signs of regression in photoreceptive capability.

A large number of dense-cored vesicles, measuring between 50 and 340 nm in diameter, observed in these SRPC suggest activated secretory function in the lizard pineal organ (Collin, 1971; Collin & Oksche, 1981). These ultrastructural indications of secretory capabilities of the SRPC confirm previous demonstrations of yellow fluorescence due to the presence of serotonin in the lizard pineal parenchyma (Quay et al., 1967; Collin, 1968; Wartenberg & Baumgarten, 1969; Collin & Meiniel, 1972; Meiniel et al., 1973). Furthermore, autoradiographic and cytochemical investigations at the electron microscopic level, combined with pharmacological experiments have shown that indoleamines are primarily localized within the dense-cored vesicles in the SRPC of the lizard pineal organ (Collin & Meiniel, 1973; Collin et al., 1977).

In contrast, Meiniel et al. (1973) were unable to demonstrate the presence of serotonin fluorescence in the parietal eye of *lacerta vivipara*. In addition, hydroxyindoe-O-methyltransferase (HIOMT) activity was observed in the pineal organ and the lateral eyes of *Lampropholas guichenoti*, but not in the parietal eye (Joss, 1978). Therefore, the lizard parietal eye may be hardly engaged in indoleamine metabolism. However, to our knowledge, immunohistochemical analysis using antisera against serotonin has not yet been performed in the lizard parietal–pineal complex.

The present investigation was undertaken to compare the ultrastructural features and immunocytochemical localization of serotonin in the cells of the receptor line in both the parietal eye and the pineal organ of the Japanese grass lizard, *Takydromus tachydromoides*.

Materials and methods

Nineteen adult Japanese grass lizards (*Takydromus tachydromoides*) of both sexes weighing an average of 3.7 g were collected in our campus during early April. The lizards were acclimatized to a LD 12:12 lighting regime (lights on at 6.00 am). They were sacrificed by decapitation between 2.00 pm and 3.00 pm in June–August, and the parietal eye and the pineal organ were removed with some of the surrounding cranial tissues.

For light microscopy, six tissue samples were fixed with Bouin's fluid for 18–24 h, dehydrated through a graded ethanol series and embedded in paraffin after decalcification for 3 days with 5% ethylenediaminetetraacetic acid (EDTA) solution. They were sectioned serially at a thickness of 5 μm in the sagittal plane. Some sections were stained with hematoxylin and eosin, and neighboring sections were treated immunohistochemically with antisera against serotonin (Incstar Corporation, USA). Binding of the antisera was visualized by the streptavidin biotin method (Guesdon et al., 1979). Specimens were pretreated with 10% normal goat serum (Vector Laboratories, USA), and subsequently incubated overnight at room temperature with the primary antiserum diluted 1:1000 in 0.01 M phosphate buffered saline (PBS) containing 0.25% Triton X-100. They were incubated with biotinated goat-antirabbit IgG (E-Y Laboratories, USA) diluted 1:1000 in PBS for 20 min, and then with peroxidase-conjugated streptavidin (Zymed Laboratories, USA) diluted 1:100 in PBS for 20 min at room temperature. The sections were washed with PBS three times for 5 min each time after each incubation step. Staining for peroxidase was performed using 0.05 M Tris-HCl buffer (pH7.6) containing 0.05% 3,3′-diaminobenzidine (DAB) and 0.006% H_2O_2. As controls, some sections were treated with antiserotonin antiserum preabsorbed with serotonin sulfate (10–50 μg/ml) or non-immune rabbit serum, and consequently no specific immunoreactivity was observed.

For electron microscopy, parietal eyes and pineal organs of 10 lizards were fixed in a mixture of equal amounts of 2% osmium tetroxide and 3% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) containing 3.5% sucrose at 4°C for 2 h. Following dehydration through a graded ethanol series, they were embedded in Quetol 812. Ultrathin sections were cut on an ultra-microtome (Super Nova, Reihert-Jung) and stained with uranyl acetate and lead compounds. They were examined with a JEM-100SX electron microscope.

For electron microscopic demonstration of serotonin, the protein A-gold (pAg) technique was performed according to Roth et al. (1981). Pineal organs of three lizards were fixed by immersion in 0.1 M cacodylate-buffered 4% paraformaldehyde and 0.1% glutaraldehyde containing 0.2% picric acid (pH 7.4) for 4 h at 4°C. After several rinses, they were dehydrated through a graded ethanol series and embedded in Quetol 812. Ultrathin sections were cut on an ultramicrotome and mounted on 200-mesh nickel grids. The sections were etched on a drop of saturated sodium metaperiodate ($NaIO_4$) solution or 10% potassium hydroxide (KOH) methanol solution for 1–2 min, and washed throughly in distilled water. The sections were incubated for 10 min in a drop of PBS containing 1% bovine serum albumin, and then immunostained in a drop of antiserum against serotonin (diluted 1:1000; Incstar Corporation, USA) for 5 h at room temperature. The

sections were washed with several drops of PBS for 10 min and labeled with the pAg (15 nm) complex (diluted to 1:20; EY Laboratories, USA) for 60 min at room temperature. They were washed in PBS for 10–15 min and finally for 5 min in distilled water. Some sections were only contrasted with 4% aqueous solution of uranyl acetate for 3 min. They were examined with a JEM-100SX electron microscope.

Results

General structure of the parietal – pineal complex

In addition to the pineal organ proper, Japanese grass lizards were found to possess a parietal eye located within the parietal foramen (Fig. 1). The parietal eye was highly differentiated, closely resembling the lateral eyes. The dorsal and ventral walls of the parietal eye were considered to correspond to the lens and retina of the lateral eye, respectively (Fig. 2). The pineal organ proper showed a saccular structure with convoluted walls, and its lumen did not communicate with the third ventricle. Melanin pigment granules were accumulated in the dura mater beneath the parietal eye and were also scattered in the parietal retina and pineal parenchyma (Figs 1 and 2). The paraphysis and dorsal sac were anterior to the pineal organ proper (Fig. 1).

Ultrastructure of the parietal retina

The parietal retina consisted mostly of a photoreceptor layer at the luminal side and a ganglion layer at the basal side. The PC in the peripheral region of the parietal retina were long and slender in shape with a well-developed outer segment (8–10 μm in length) containing regular stacks of from 150 to 250 disks and an elongated inner segment containing large roundish mitochondria horizontally protruding into the parietal lumen (Figs 3 and 4). Whereas in the fundus region of the parietal retina, the PC were less differentiated than those in the peripheral region. Their outer segments ranged from 2 to 4 μm in length, protruding perpendicularly into the parietal lumen, and contained from 50 to 100 disks. Their inner segments were smaller and contained only a few mitochondria (Fig. 5). Usually, the PC had an oval to spherical pale nucleus with a prominent nucleolus, and their supranuclear cytoplasm possessed a large number of mitochondria, well-developed Golgi complex, rough-surfaced endoplasmic reticulum, and numerous free ribosomes (Fig. 6). Their basal processes contained several synaptic ribbons associated with small clear and cored synaptic vesicles (about 50 nm in diameter), and formed neuropil with dendrites of nerve cells between the two layers (Figs 7 and 8).

In some sections, a few cells containing a large number of dense-cored vesicles (80–220 nm in diameter), which had a clear halo surrounding the fine granular core, were mainly demonstrated in the fundus region of the parietal retina, the outer segments of which showed whorl-like lamellar structures (Figs 9–12). Consequently, these cells were considered to be the SRPCs of the receptor line, according to the classification of Collin (1971).

The nerve cells were found in the basal layer, with a spherical to oval pale nucleus and a prominent nucleolus. They also contained well-developed rough-surfaced endoplasmic reticulum and numerous free ribosomes in their perinuclear cytoplasm (Fig. 7).

In addition, supporting cells were also observed between the PC in the parietal retina. They were long and slender in shape, extending from the basal layer to the parietal lumen. They had an oval or triangular dark nucleus in their basal region and numerous microvilli on their luminal surface (Figs 4, 5 and 7). They contained a few organelles and were occasionally seen to contain numerous melanin pigment granules (Fig. 7). Macrophages with an irregularly shaped dark nucleus were found in the parietal lumen, closely associated with the parietal lens cells or the outer segments of the PC and SRPC (Figs 2, 5 and 12).

Ultrastructure of the pineal organ proper

The lizard pineal organ contained relatively few typical PC, and SRPC were the predominant cell type of the receptor line (Figs 13 and 14). These cells were similar to those of the parietal eye. However, the outer segments of the pineal PC were less well-developed than those of the parietal PC, which did not exceed 2 μm in length and contained regular stacks of about 50 disks (Fig. 15). The outer segments of the SRPC were irregularly organized and showed whorl-like lamellar structures (Fig. 16). PC were frequently observed in the dorsal wall of the pineal organ. Both of these types of receptor cells had a spherical to oval pale nucleus with a distinct nucleolus and broad supranuclear cytoplasm containing an aggregation of mitochondria, well-developed Golgi complex, rough-surfaced endoplasmic reticulum, free ribosomes, lysosomes (0.4–1.0 μm in diameter) and occasionally melanin pigment granules (Fig. 17). However, numerous mitochondria in the inner segment and supranuclear cytoplasm of the PC were larger and rounder than those of the SRPC (Fig. 17). Furthermore, a large number of dense-cored vesicles (80–280 nm in diameter) were found only in the SRPC, which were a little larger than those in the parietal PC (Figs 17 and 18).

The pineal PC possessed basal processes containing several synaptic ribbons. In contrast, some of the basal processes of SRPC contained synaptic ribbons, while others contained dense-cored vesicles, SRPC containing both were rarely seen (Figs 18 and 19). Therefore, the SRPC of the pineal organ may project both types of basal processes. Rarely, we found synaptic connections between a nerve cell and a basal process of these cells of the receptor line containing synaptic ribbons (Fig. 20a, b). Also, the nerve cells and supporting cells observed in the pineal wall closely resembled those in the parietal retina.

In the pineal lumen, macrophages containing amorphous dense bodies were in contact with the outer segments of the PC and SRPC, which were a little larger than those of the parietal eye (Fig. 21).

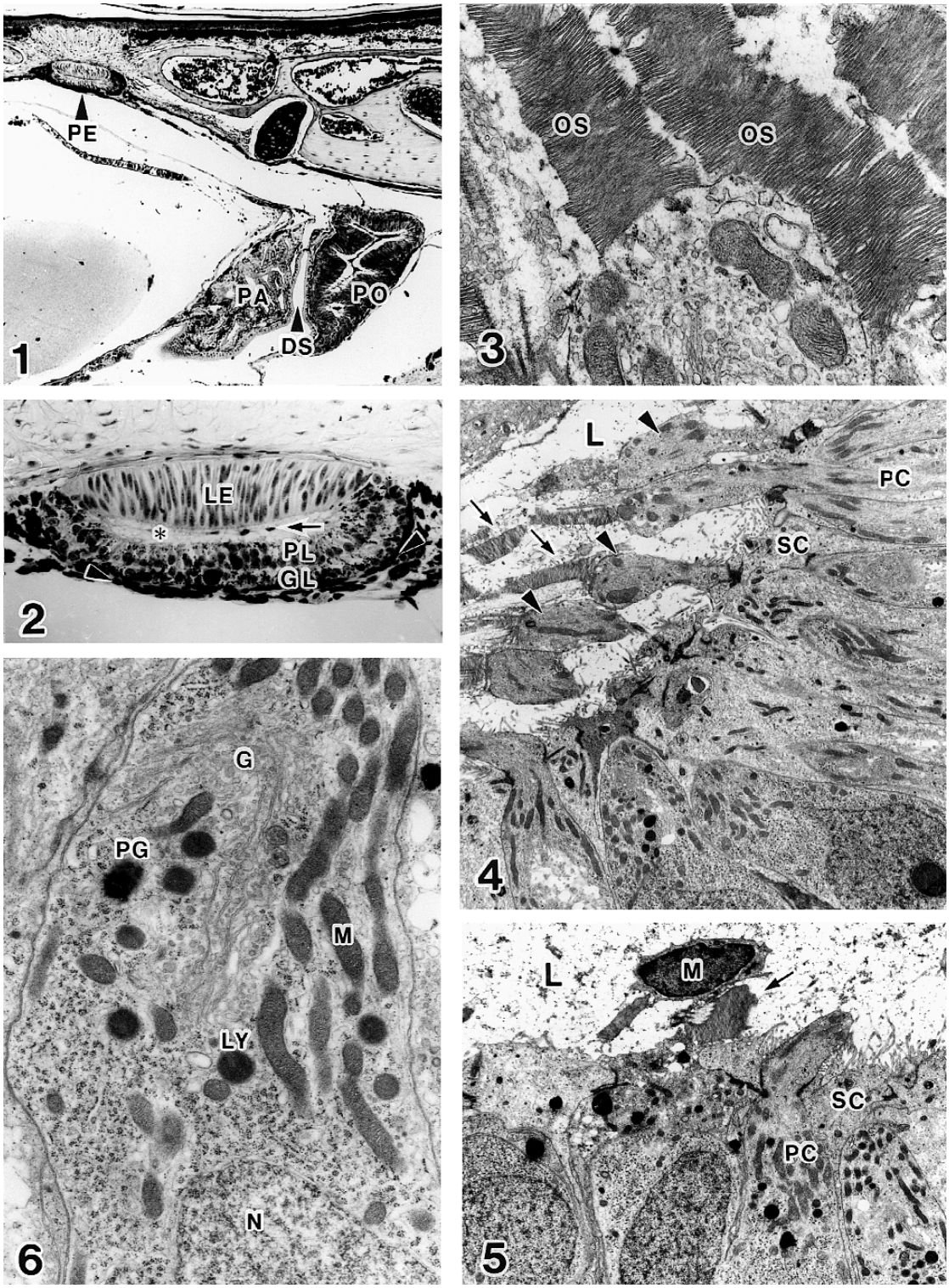


Fig. 1 Light micrograph of a mid-sagittal section through a parietal-pineal complex of *Takydromus tachydromoides*. Hematoxylin and eosin (H-E) staining. PE, parietal eye; PO, pineal organ; PA, paraphysis; DS, dorsal sac.×60.

Fig. 2 Light micrograph of a mid-sagittal section through a parietal eye of *Takydromus tachydromoides*. H-E staining. The parietal retina consists of a photoreceptor layer (PL) and a ganglion layer (GL). Note the macrophage (arrow) in the parietal lumen (asterisk) and numerous melanin pigment granules in the dura mater (arrowheads) beneath the parietal eye. LE, lens. ×260.

Fig. 3 Electron micrograph of well-developed outer segments (OS) of typical photoreceptor cells in *Takydromus tachydromoides*. Note regular stacks of numerous disks in the outer segments. ×16000.

Fig. 4 Electron micrograph of photoreceptor cells in the peripheral region of the parietal retina of *Takydromus tachydromoides*. Note well-developed outer segments (arrows) and elongated inner segments (arrowheads) containing large mitochondria of photoreceptor cells (PC). L, parietal lumen; SC, supporting cell. ×3800.

Fig. 5 Electron micrograph of photoreceptor cells in the fundus region of the parietal retina of *Takydromus tachydromoides*. Note the macrophage (M) in the parietal lumen (L), in contact with the outer segment (arrow) of a photoreceptor cell (PC). SC, supporting cell. ×3800.

Fig. 6 Electron micrograph of photoreceptor cells in the parietal retina of *Takydromus tachydromoides*. Note numerous mitochondria (M) and well developed Golgi complex (G) in the supranuclear cytoplasm. N, nucleus; LY, lysosome; PG, pigment granule. ×12700.

Light microscopic examination of serotonin immunoreactivity

Intense serotonin immunoreactivity was observed in the parietal eye and the pineal organ of the Japanese grass lizard (Figs 22 and 23a, b). The serotonin-immunoreactive cells were found more frequently in the pineal organ than in the parietal eye. The parietal eye contained few of these cells, mainly located in the retinal fundus region (Fig. 22), whereas in the pineal organ proper they were found throughout the parenchyma with relatively few in the dorsal wall (Fig. 23a). Most of these cells in both the parietal and the pineal organ resembled those of the receptor line in their size and shape. Serotonin immunoreactivity was observed throughout the cytoplasm of these cells but not in the outer segment or the nucleus (Figs 22 and 23b). A small number of oval cells showing strong serotonin immunoreactivity were observed in the basal region of the parietal retina and pineal parenchymal wall (Figs 22 and 23b). They had short processes which made no contact with the lumen.

Electron microscopic demonstration of serotonin immunoreactivity

At electron microscope level, serotonin immunoreactivity was confirmed by the pAg technique for anti-serotonin serum. Gold particles indicating serotonin-immunoreactive sites were restricted in the core of dense-cored vesicles in the pineal SRPC (Fig. 24a, b), but not in the PC, supporting cells or nerve cells. The gold particles appeared to be more frequent in large dense-cored vesicles than in small vesicles. Small dense-cored vesicles occasionally showed very weak immunoreactivity.

Discussion

According to Gundy & Wurst (1976), approximately 60% of all lizard genera possess a third eye, consisting of a well-developed lens and retina that are similar to those of lateral eyes. Their parietal PC are more differentiated in sensory structures than those of the pineal organ proper. However, electrophysiological studies have clearly shown functional photosensory capabilities not only in the parietal eyes, but also in the pineal organs of the lizards (Hamasaki & Dodt, 1969; Dodt, 1973; Hamasaki & Eder, 1977). In the present ultrastructural study, the typical PC in the parietal-pineal complex of *Takydromus tachydromoides* closely resembled the cones of the lateral eyes. They were common in the parietal eye, but there were relatively few of these cells in the pineal organ. According to Jenison & Nolte (1979), the parietal PC of *Anolis* and *Iguana* are cytologically indistinguishable from those of other reptiles (Eakin & Westfall, 1959; 1960; Oksche & Kirschstein, 1968; Petit, 1968). In five species of lizards, Oksche & Kirschstein (1968) found that the outer segments of the pineal PC did not exceed 2 μm in length and contained 50–60 disks: in contrast, the outer segments of the parietal PC measured 15 μm in length and contained about 400 disks. Also, in *Takydromus tachydromoides* the outer segments of the parietal PC were longer (8–10 μm in length) and contained a greater number of disks (150–250) than those of the pineal PC (about 2 μm in length and 50 disks).

In the present study, the SRPC were the predominant cell type in the receptor line of the pineal organ, the outer segments of which were less regularly organized than those of the typical PC, showing whorl-like lamellar structures in the pineal lumen. The irregular whorl-like lamellar structures found in the pineal lumen of birds and reptiles are considered to be rudimentary by most authors. In some lizards, the outer segments of the SRPC may be completely lacking (Wartenberg & Baumgarten, 1968). Vigh & Vigh-Teichmann (1981) demonstrated opsin immunoreactivity in the outer segments of the pineal SRPC of some turtles and birds, but not in those of the lacertilian parietal-pineal complex. Some iodopsin-immunopositive outer segments are present in the pineal organ of the Japanese grass lizard, but not in the parietal eye (Masuda et al. 1994). Thus, further investigations are necessary to clarify whether the SRPC in the lizard parietal-pineal complex retain functional photoreceptive capability.

The outermost disks detached from the photoreceptor outer segment of the lateral eye retina are engulfed by the pigment epithelial cells and are then broken down (Young & Bok, 1969). In the parietal eye lacking the pigment epithelium, shedding of disks and subsequent uptake by luminal macrophages has been described by Petit (1968). More recently, Ahmed & Engbretson (1993) obtained static evidence that the PC of the parietal eye synthesize new outer segment material and rhythmically shed their distal tips, and this shed material is then engulfed by luminal macrophages. In the present ultrastructural study, macrophages were frequently seen to be in contact with the outer segments of the PC and the SRPC in the parietal and pineal lumina. Consequently, it was suggested that these luminal macrophages are involved in the process of outer

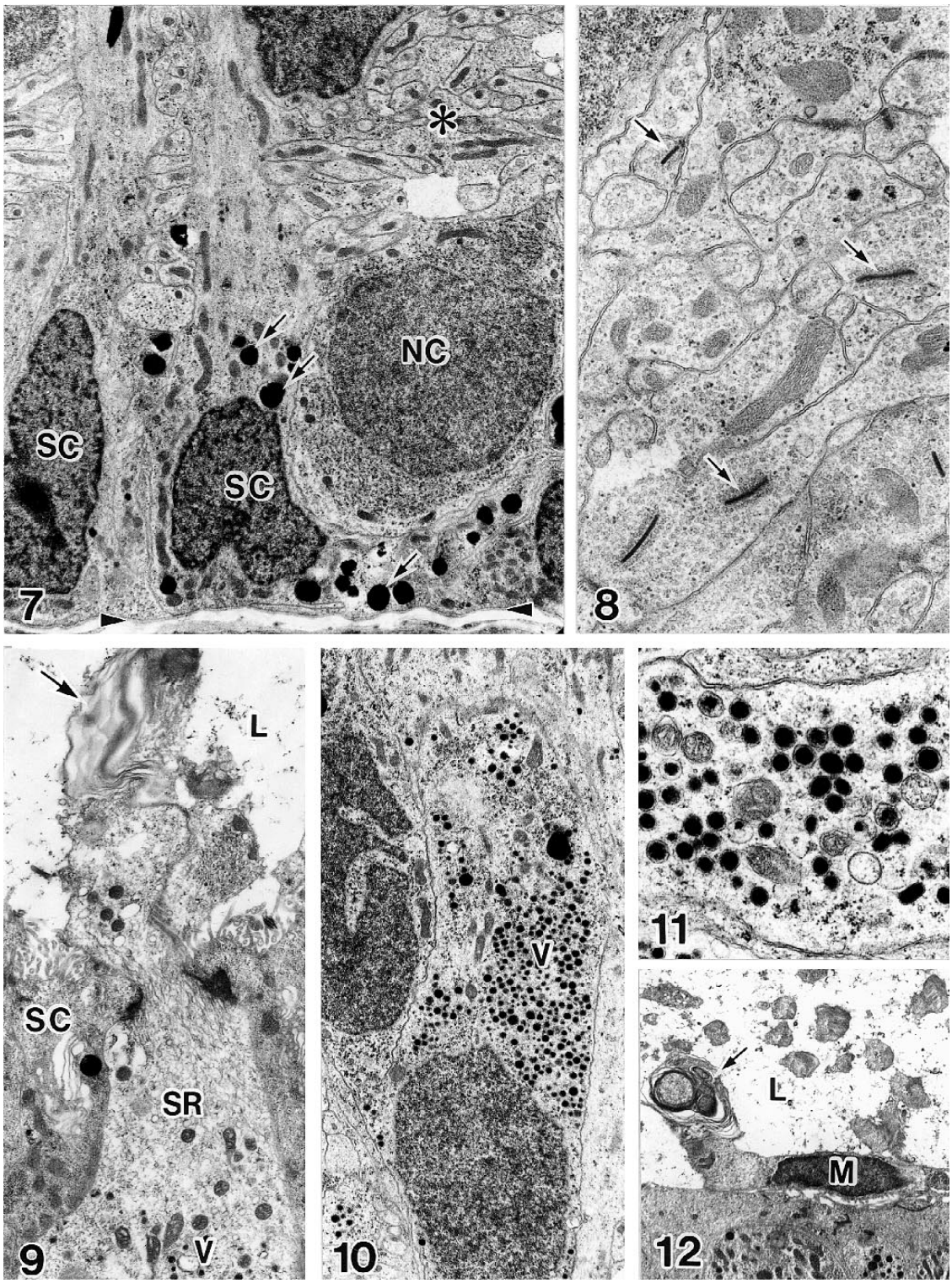


Fig. 7 Electron micrograph of the basal region in the parietal retina of *Takydromus tachydromoides*. Note supporting cells (SC) containing numerous pigment granules (arrows) and neuropil (asterisk) consisting of basal processes of photoreceptor cells and dendrites of nerve cells (NC). Arrowheads show basement membrane. ×6400.

Fig. 8 Electron micrograph of basal processes of photoreceptor cells in the parietal retina of *Takydromus tachydromoides*. Note synaptic ribbons (arrows). ×18000.

Fig. 9 Electron micrograph of the fundus region of the parietal retina of *Takydromus tachydromoides*. Note the whorl-like lamellar outer segment (arrow) of a secretory rudimentary photoreceptor cell (SR) containing dense-cored vesicles (V). SC, supporting cell; L, parietal lumen. ×6300.

Fig. 10 Electron micrograph of a secretory rudimentary photoreceptor cell in the parietal retina of *Takydromus tachydromoides*. Note the large number of dense-cored vesicles (V) in the supranuclear cytoplasm. ×18000.

Fig. 11 Electron micrograph of the basal process of a secretory rudimentary photoreceptor cell of the parietal retina of *Takydromus tachydromoides*. Note numerous dense-cored vesicles with a clear halo surrounding a fine granular core. ×18000.

Fig. 12 Electron micrograph of the parietal lumen (L) of *Takydromus tachydromoides*. Note the macrophage (M), in contact with a whorl-like lamellar outer segment (arrow). ×3800.

segment renewal of the cells of the receptor line in the parietal–pineal complex.

An other characteristic feature of the sensory elements of the parietal–pineal complex is accumulation of mitochondria forming an ellipsoid in the inner segments of the cells of the receptor line. In this study, these mitochondria in the inner segment of the PC were larger and rounder than those of the SRPC, and were especially large in the parietal PC. Such accumulation of mitochondria has been suggested to be a source of energy required for the transduction process in the photoreceptor outer segments (Collin & Oksche, 1981). Therefore, the size and form of the ellipsoid mitochondria may be correlated with the photoreceptive capability in the cells of the receptor line of the parietal–pineal complex; more photosensitive cells may have larger round mitochondria.

The most distinct feature of the secretory elements in this study was the presence of numerous dense-cored vesicles in the SRPC. According to Vollrath (1981), the SRPC of the reptilian pineal organ differ from their counterparts in other vertebrate classes in that they contain an abundance of secretory granules measuring between 50 and 340 nm in diameter. These ultrastructural indications of the secretory capability in the SRPC confirm to the previous reports of yellow fluorescence due to the presence of serotonin in the lizard pineal parenchyma (Quay et al., 1967; Collin, 1968; Wartenberg & Baumgarten, 1969; Collin & Meiniel, 1972; Meiniel et al., 1973). Moreover, autoradiographic and cytochemical investigations at the electron microscopic level combined with pharmacological experiments have shown that indoleamines are primarily localized within the dense-cored vesicles in the SRPC of the lizard pineal organ (Collin & Meiniel, 1973; Collin et al., 1977).

The present investigation using the pAg technique is the first to demonstrate the immunocytochemical localization of serotonin in the dense-cored vesicles of the SRPC of the lizard pineal organ. Tamotsu et al. (1990) observed serotonin-immunoreactive cells in the pineal complex of the river lamprey, which resembled photoreceptors in their size and shape at the light microscopic level. Wartenberg & Baumgarten (1969) observed an extravesicular pool of serotonin throughout the cytoplasm of the SRPC in the pineal organ of *Lacerta viridis* and *L. muralis*. Thus, the exact sites of indoleamine metabolism in the SRPC may vary among different species of lizards, although there is no doubt that the pineal SRPC are actively engaged in serotonin metabolism.

Serotonin fluorescence has been observed in the pineal organs of snakes (Quay et al., 1968; Collin & Meiniel, 1972) and turtles (Collin & Meiniel, 1972; Vivien-Roels & Arendt, 1979) as well as the above-mentioned lizards, but it has not been demonstrated in the lizard parietal eye (Quay et al., 1967; Meiniel et al., 1973). Also, HIOMT activity was found in the pineal organ and the lateral eye of *Lampropholas guichenoti*, but not in its parietal eye (Joss, 1978). Jenison & Nolte (1979) found no obvious criteria for subdividing the ultrastructures in the parietal PC of *Anolis carolinensis* and *Iguana iguana*. However, the present ultrastructural and immunohistological study demonstrated the presence of serotonin-immunoreactive cells in the parietal eye as well as the pineal organ, which were considered to be equivalent to SRPC exhibiting the ultrastructural characteristics described above. Furthermore, the appearance of round cells with distinct serotonin immunoreactivity in the basal region of the parietal retina and the pineal wall suggested that SRPC lacking the outer and inner segments, and making no contact with the lumen exist in the lizard parietal-pineal complex.

The present study demonstrated that the basal processes of the PC in both the parietal eye and pineal organ contained synaptic ribbons, and were in contact with nerve cells. The synapses between photoreceptor terminals and nerve cell bodies or dendrites in the saurian parietal eye have been characterized by the presence of the synaptic ribbons (Quay, 1979; Engbretson, 1992). It has been suggested that the pineal SRPC become independent of the neurons because of distinct signs of regression in their sensory structures with no ribbon synapses or contact with secondary neurons (Collin & Kappers, 1968; Wartenberg & Baumgarten, 1968; Collin & Oksche, 1981). In the mammalian pineal gland, the synaptic ribbons may be involved in intercellular communications between adjacent pinealocytes (Vollrath, 1973; Vollrath & Huss, 1973). However, the functional significance of these synaptic ribbons is not yet clear. We found that the pineal SRPC contained not only numerous dense-cored vesicles, but also synaptic ribbons in separate processes. The SRPC of the lizard pineal organ may serve both hormonal and neural output functions in separate processes at the same time.

Trost (1953) demonstrated regional differences in the pineal organs of *Anguis fragilis* and *Chalcides ocellatus*; sensory cells are much less abundant in the dorsal region of the pineal vesicles than in their ventral and lateral areas. In the river lamprey pineal organ, serotonin immunoreactivity

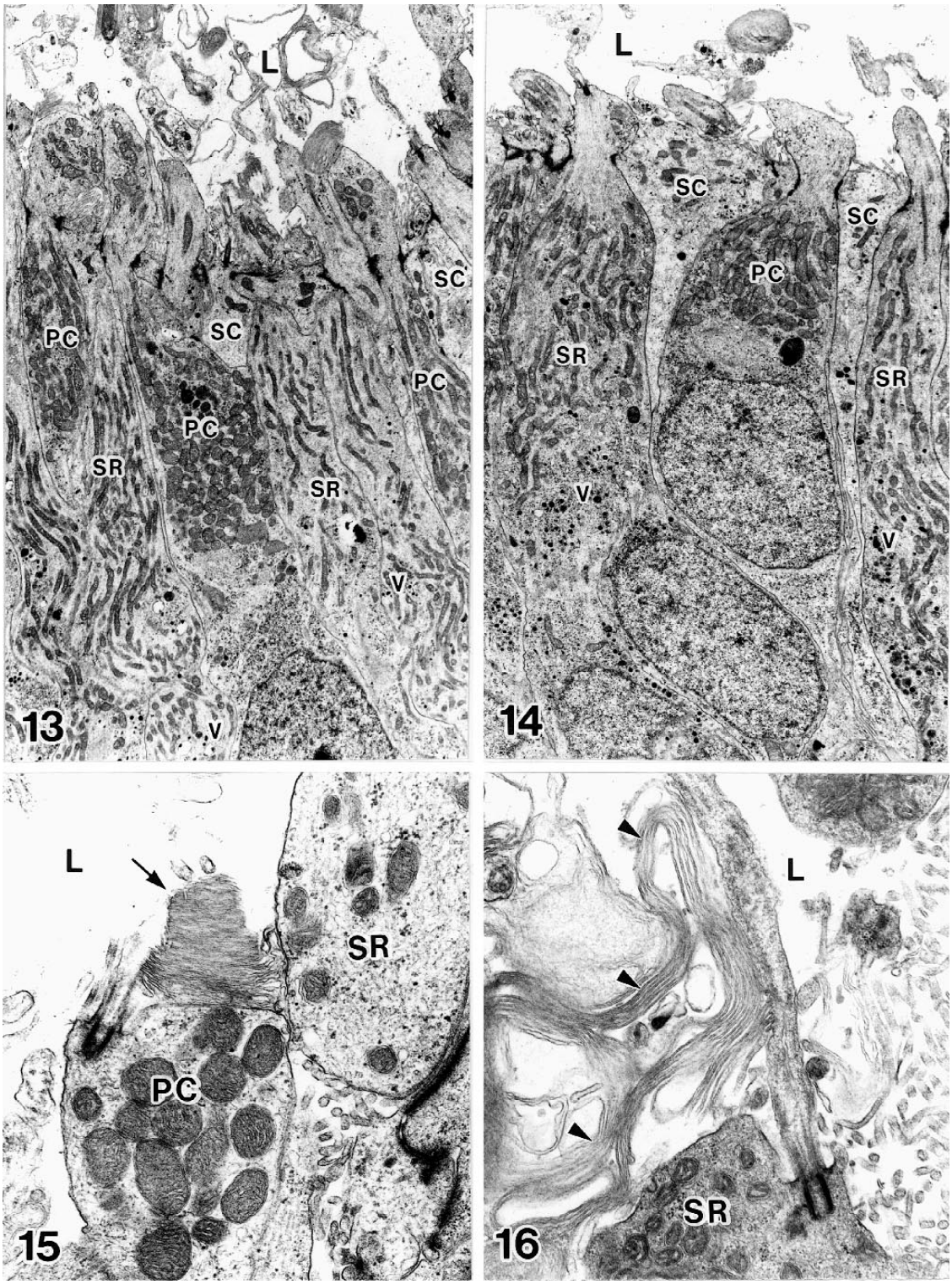
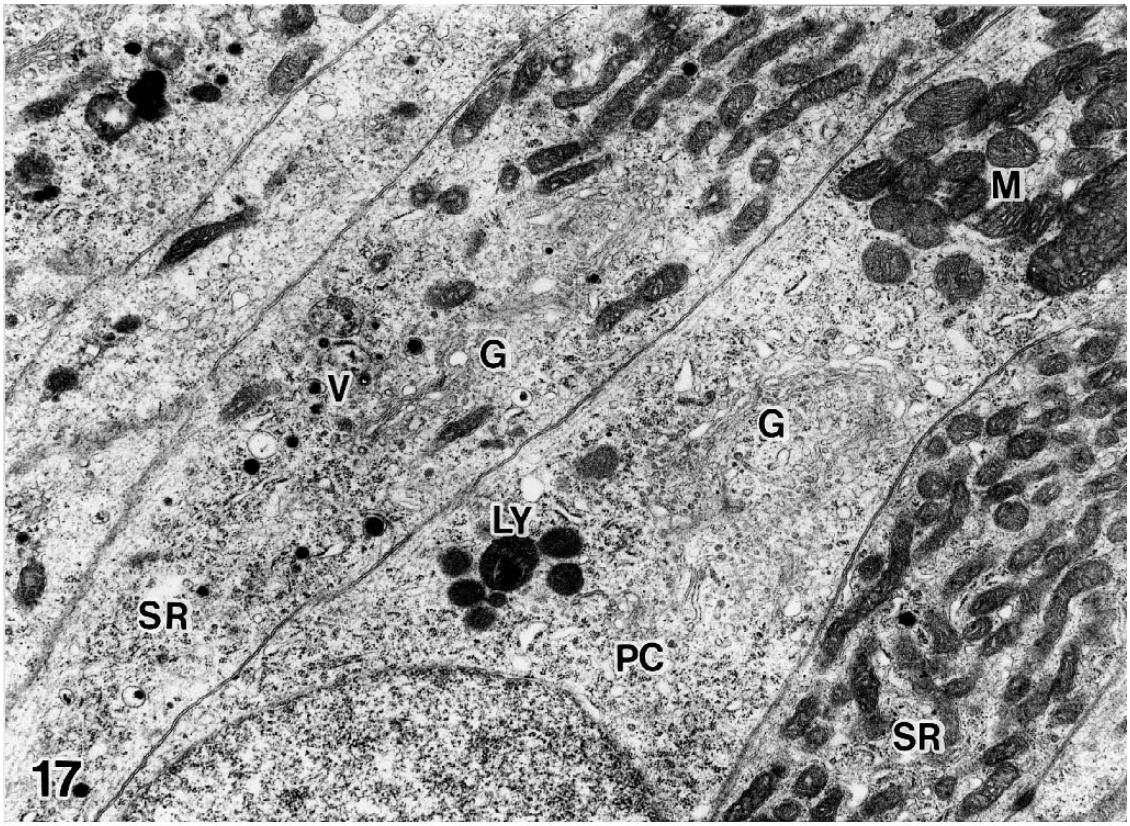
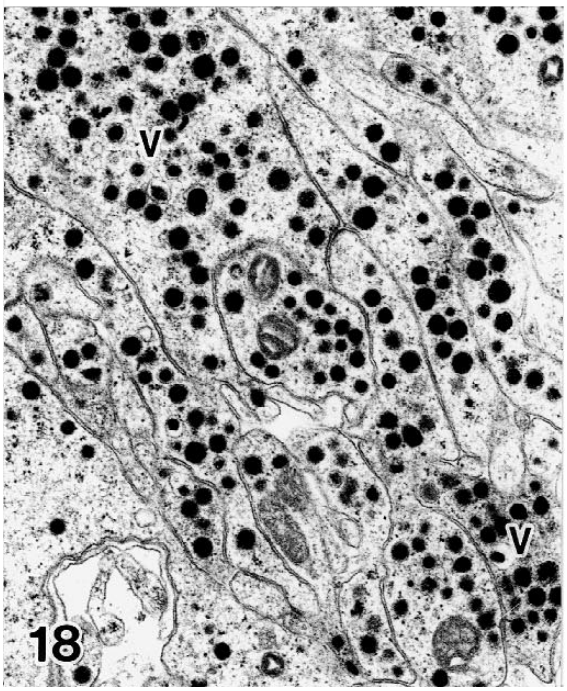


Fig. 13 and 14 Electron micrographs of dorsal (Fig. 13) and lateral (Fig. 14) walls of the pineal organ of *Takydromus tachydromoides*. Note the more numerous photoreceptor cells in the dorsal than in the lateral wall, and larger and rounder mitochondria in photoreceptor cells (PC) than in secretory rudimentary photoreceptor cells (SR). V, dense-cored vesicles in the secretory rudimentary photoreceptor cells; SC, supporting cells; L, pineal lumen. ×3800.

Fig. 15 Electron micrograph of outer and inner segments of a photoreceptor cell in the pineal organ of *Takydromus tachydromoides*. Note the regularly arranged outer segment (arrow) and inner segment of a photoreceptor cell (PC) containing larger and rounder mitochondria than the secretory rudimentary photoreceptor cells (SR). L, pineal lumen. ×6000.

Fig. 16 Electron micrograph of outer and inner segments of a secretory rudimentary photoreceptor cell in the pineal organ of *Takydromus tachydromoides*. Note the whorl-like lamellar outer segment (arrowheads) and inner segment containing small mitochondria of the secretory rudimentary photoreceptor cells (SR). L, pineal lumen. ×16000.





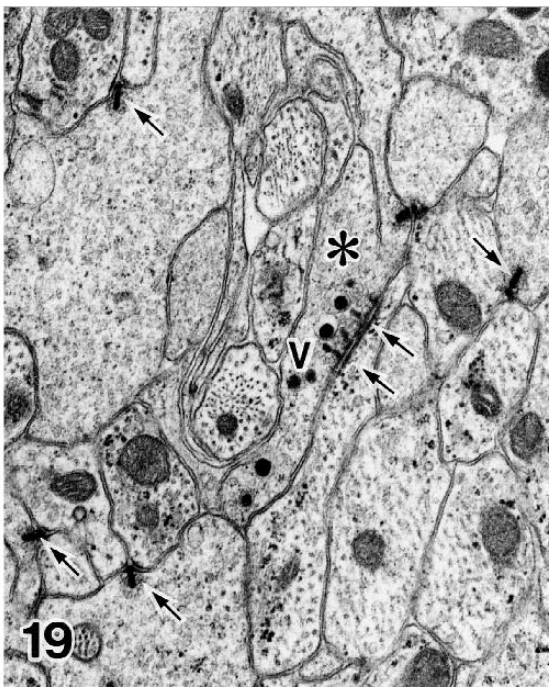


Fig. 17 Electron micrograph of supranuclear cytoplasm of cells of the receptor line in the pineal organ of *Takydromus tachydromoides*. Note larger and rounder mitochondria (M) in the photoreceptor cell (PC) than in secretory rudimentary photoreceptor cells (SR) containing dense-cored vesicles (V). G, Golgi complex; LY, lysosomes. ×12700.

Fig. 18 Electron micrograph of basal processes of secretory rudimentary photoreceptor cells in the pineal organ of *Takydromus tachydromoides*. Note numerous dense-cored vesicles (V). ×18000.

Fig. 19 Electron micrograph of basal processes of secretory rudimentary photoreceptor cells in the pineal organ of *Takydromus tachydromoides*. Note the coexistence of synaptic ribbons (arrows) and dense-cored vesicles (V) in the basal processes (asterisk). ×18000.

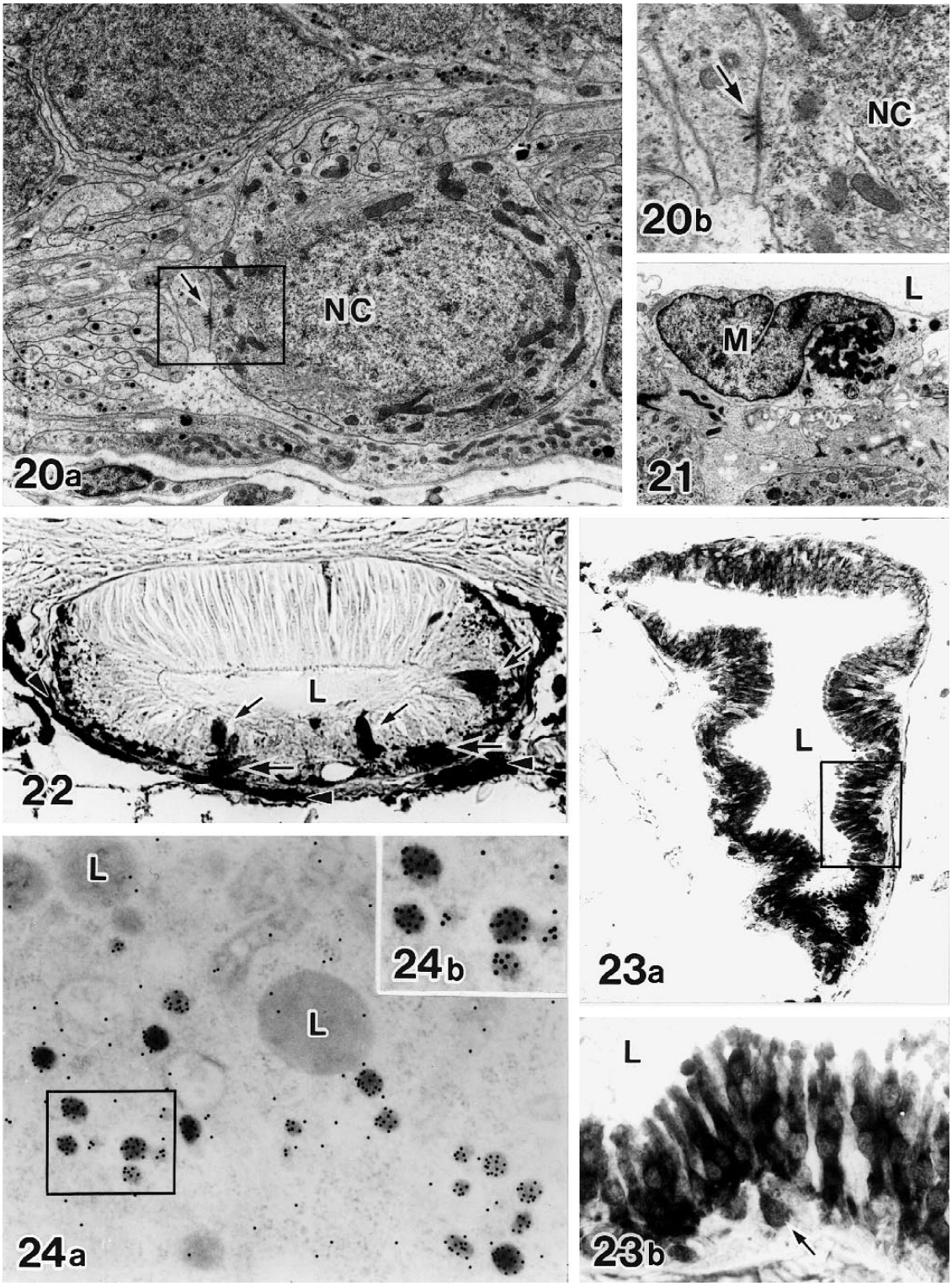


Fig. 20a, b Electron micrographs of a nerve cell in the basal region of the pineal organ of *Takydromus tachydromoides*. **(b)** A higher magnification view of the framed area in **(a)** shows a synaptic connection between a basal process of the photoreceptor cell containing synaptic ribbons (arrow) and a nerve cell (NC). (a) ×6400, (b) ×18000.

Fig. 21 Electron micrograph of a macrophage (M) in the pineal lumen (L) of *Takydromus tachydromoides*. Note the irregularly shaped nucleus and amorphous dense bodies in the macrophage. ×3800.

Fig. 22 Light microscopic demonstration of serotonin immunoreactivity in the parietal eye of *Takydromus tachydromoides*. Note five serotonin-immunoreactive cells (arrows) in the parietal retina. Large arrows show two oval immunoreactive cells in the basal layer, and arrowheads show aggregations of melanin pigment granules in the dura mater. L, parietal lumen. ×370.

Fig. 23a, b Light micrographs indicating serotonin immunoreactivity in the pineal organ of *Takydromus tachydromoides*. **(b)** A higher magnification view of the framed area in **(a)** shows numerous serotonin-immunoreactive cells resembling the cells of the receptor line in their size and shape. Note the oval immunoreactive cell in the basal region (arrow). L, pineal lumen. (a) ×140, (b) ×540.

Fig. 24a, b Electron micrographs showing serotonin immunoreactivity in a secretory rudimentary photoreceptor cell of the pineal gland of *Takydromus tachydromoides*. **(b)** A higher magnification view of the framed area in **(a)** shows a number of gold particles indicating serotonin-immunoreactive sites restricted in the core of dense cored-vesicles. Gold particles are more frequent in large dense-cored vesicles than in small vesicles. L, lysosomes. (a) ×30 000, (b) ×47000.

was restricted to scattered cells in the end-vesicle, but was characteristic of numerous cells in the atrium (Tamotsu et al., 1990). In the present study, the PC were more abundant in the peripheral region of the parietal eye and in the dorsal wall of the pineal organ than the other region, being approximately reciprocal to the distribution of the SRPC and serotonin immunoreactivity. Therefore, it seems likely that regional differences in the sensory and secretory functions exist in both the parietal eye and pineal organ proper of the lizard parietal–pineal complex.

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