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The ultrastructure of the pineal ganglion in the ferret

G. F. X. DAVID, J. HERBERT AND G. D. S. WRIGHT*

*Departments of Anatomy, University of Cambridge and *University of Birmingham*

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INTRODUCTION

The pineal gland of the ferret is essential for changes in environmental light to alter the secretion of gonadotrophin from the pituitary. Pinealectomized ferrets kept in natural daylight no longer continue to breed at the normal time of year, and, if they are exposed to artificial light, their breeding season is not activated prematurely, as it can be in the intact animal (Herbert, 1969, 1972). It seems likely that there is a neural link between retina and pineal (Moore, Heller, Wurtman & Axelrod, 1967), so that studying the nervous elements in the gland has particular importance. Nerve fibres enter the ferret's pineal from at least two sources – the superior cervical ganglia, which supply autonomic fibres via the *nervi conarii*, and from the epithalamus (Trueman & Herbert, 1970). Furthermore, within the caudal part of the pineal itself is a collection of nerve cells which we have called the 'pineal ganglion' (Trueman & Herbert, 1970; Herbert, 1971). Studies of the pineal ganglion under the light microscope have shown that it contains acetylcholinesterase but no monoamines, although the latter are present in high concentrations in the rest of the gland (that is, the pineal parenchyma) (Trueman & Herbert, 1970). Nerve cells have also been described in the pineal glands of other species, including the monkey and rabbit (Levin, 1938; Clark, 1940; Romijn, 1972) although no structure comparable to the pineal ganglion has, so far, been observed in any species other than the ferret.

We have now extended our work to include examination of the pineal ganglion under the electron microscope to establish the ultrastructural characteristics of this unusual structure.

MATERIALS AND METHODS

Animals

Both albino and 'polecat' (i.e. pigmented) adult female ferrets were used. They were kept, two per cage, in a room illuminated by daylight, and fed a mixture of MRC diet 41B, commercial dog food, fish and dried milk. Water was available *ad libitum*. The ultrastructure of the pineal ganglion was examined in ten ferrets, and the distribution of cholinesterase in a further six. In addition, the pineal ganglia from two ferrets that had been given 5-OH DOPA were also studied.

Reprint requests to J. Herbert, Department of Anatomy, University of Cambridge, Cambridge CB2 3DY.

Preparation for electron microscopy

Animals were anaesthetized with pentobarbitone sodium (Nembutal, Abbott, 36 mg/kg body weight). The chest was opened, the descending aorta clipped, and the ascending aorta cannulated with a polythene catheter (approximately 1 mm internal diameter) through an incision in the left ventricle. About 200 ml of fixative was then perfused (without preliminary washout) at room temperature from a transfusion apparatus suspended approximately 150 cm above the animal.

Ferrets in which the normal ultrastructure of the gland was to be examined were perfused with a modified Karnovsky's (1965) fluid, containing 4 % paraformaldehyde and 1 % glutaraldehyde in 0·1 M sodium cacodylate. 2·5 mg of anhydrous calcium chloride was added per 100 ml fixative, and pH adjusted to 7·4. After perfusion had been completed, the pineal gland was removed and its caudal half (which contains the pineal ganglion) further fixed in modified Karnovsky's solution at 4 °C for 2 hours, washed in 0·1 M cacodylate buffer, post-fixed in 1 % osmium tetroxide, dehydrated and embedded in Araldite.

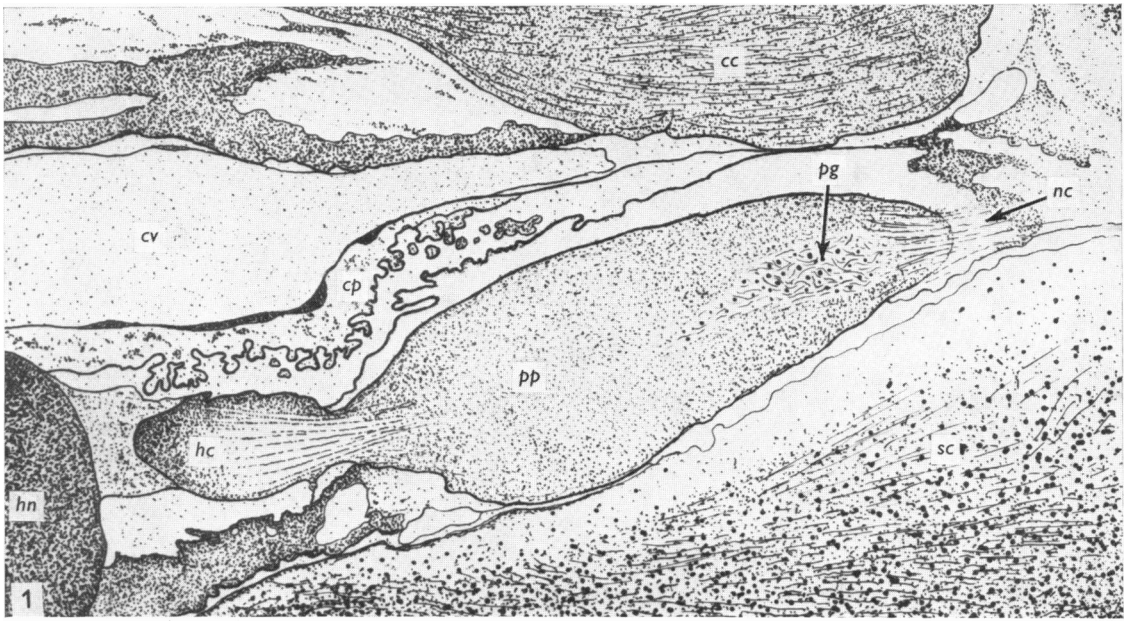
Ferrets in which the distribution of cholinesterase was studied were perfused with a mixture of 4 % paraformaldehyde and 1 % glutaraldehyde in sodium cacodylate, with 2 ml 0·2 M calcium acetate added per 100 ml, and adjusted to pH 7·3. The caudal half of the pineal was fixed in the same fluid for a further 2 hours, and then transferred to a second fixative consisting of 3·2 % paraformaldehyde, 33 % 0·2 M sodium cacodylate, 2 ml % 0·2 M calcium acetate and 5 ml % 0·2 M cacodylic acid. After this it was washed in cacodylate buffer overnight, and then treated according to the procedure described by Lewis & Shute (1969). Acetylcholinesterase was demonstrated by incubation with a mixture of acetylthiocholine iodide and ethopropazine hydrochloride, whereas pseudocholinesterase was demonstrated by using butyrylthiocholine iodide as substrate at pH 5·5. Incubation lasted 4 hours at 4 °C to demonstrate acetylcholinesterase, or 6 hours for pseudocholinesterase; the tissue was then post-fixed in Dalton's (1955) fluid at room temperature, dehydrated and embedded in Araldite.

Animals in which catecholamine storage sites were to be demonstrated were injected with 200 mg/kg body weight 5-OH DOPA (3,4,5 trihydroxy-phenylalanine) intraperitoneally 90–120 minutes before perfusion. They were perfused with modified Karnovsky fluid, and the pineal was fixed for 30 minutes in the same fluid after removal, and then post-fixed in Dalton's fluid.

Sections 0·5–1·0 μm thick were taken, stained with methylene blue, and examined under the light microscope. Sections for electron microscopy were mounted on uncoated grids, stained with uranyl acetate and/or lead citrate and examined with a Philips EM 300.

Measurements

Dimensions of cells were measured in thick sections under light microscopy and those of vesicles on electron micrographs, only from profiles clearly showing a synaptic apparatus; care was taken not to measure the same cell twice on different sections. Data which seemed to represent a reasonably widely distributed sample of the tissue are shown as means and standard deviations, and the number on which each mean is based is indicated in the text. Other measurements are shown as ranges.



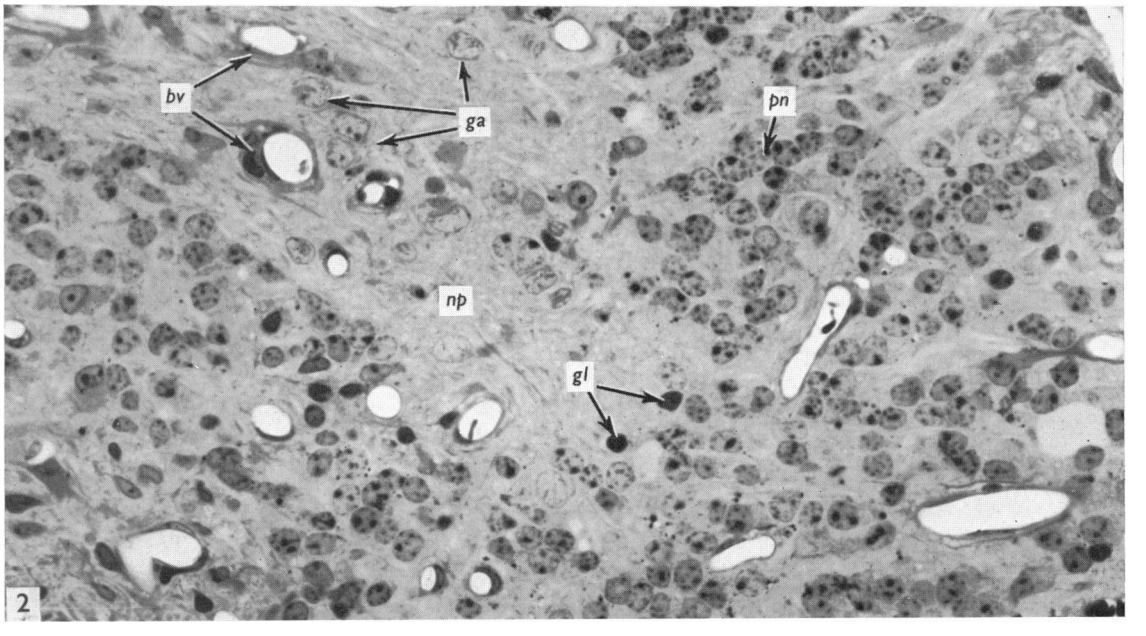


Fig. 1. Diagram of a sagittal section through the pineal gland of the ferret to show its relationship with the neighbouring structures. Corpus callosum (*cc*); choroid plexus (*cp*); great cerebral vein (*cv*); habenular commissure (*hc*); median habenular nucleus (*hn*); nervi conarii (*nc*) pineal ganglion (*pg*); pineal parenchyma (*pp*); superior colliculus (*sc*).

Fig. 2. The appearance of the pineal ganglion and adjacent pineal parenchyma in a thick section stained with methylene blue. Blood vessels (*bv*); nuclei of ganglion cells (*ga*); nucleus of glial cell (*gl*); neuropil of pineal ganglion (*np*); nucleus of pinealocyte lying in parenchyma (*pn*). × 880.

RESULTS

Light microscopy

Gross morphology

The pineal ganglion is approximately spherical and is located in the caudal half of the ferret's pineal (Fig. 1). It is clearly differentiated from the rest of the gland as a structure in which many nerve fibres mingle with rather few cells (the ganglion cells) resembling neurons. Its position is somewhat variable; it is usually situated centrally, though it may be found adjacent to the gland's superior surface. The size of the ganglion is also variable, though usually between 75 and 150 μm in diameter. No precise boundary separates the pineal ganglion from the surrounding parenchyma; although pinealocytes are absent from its centre, at its periphery neurons mingle with parenchymal cells (Fig. 2). Both myelinated and non-myelinated nerve fibres are present within the ganglion, and from it a band of fibres courses towards the rostral part of the gland. Glial cells are scattered between the neurons and capillaries; the latter seem less frequent in this part of the pineal than in the surrounding parenchyma.

Ganglion cells

These are easily distinguished from both pinealocytes and glial cells by being much larger and staining less deeply (Fig. 2). They are usually found lying singly, surrounded by neuropil, but may occur in groups of two or three. Ganglion cells measure from 12 to 25 μm in diameter ($\bar{x} = 17{\cdot}8 \pm 3{\cdot}35$; $n = 50$). Their nuclei are characteristically centrally placed and often deeply indented. The diameter of the nucleus is 10–15 μm ($\bar{x} = 12{\cdot}4 \pm 1{\cdot}58$; $n = 50$). There is a prominent nucleolus, usually situated close to the nuclear membrane.

Glial cells

These are oval or polyhedral, and much smaller than ganglion cells. They measure from 9 to 19 μm in diameter ($\bar{x} = 11{\cdot}9 \pm 2{\cdot}05$; $n = 30$). They are present in other regions of the gland as well as in the pineal ganglion. In the latter they are particularly frequent near ganglion cells and blood vessels (Fig. 2).

Electron microscopy

Ganglion cells

There seems to be only one type of ganglion cell, though individual cells differ appreciably in cytoplasmic density and in the configuration of the rough endoplasmic reticulum. They are approximately circular, and in this they differ from the darker, elongated, asymmetrical pinealocytes (Fig. 3). Moreover, ganglion cells receive axosomatic synapses (see below) and these have never been observed on pinealocytes.

The indented nucleus is centrally placed and is surrounded by a double membrane system about 20–30 nm thick, the inner membrane being the more regular. The outer nuclear membrane may be seen to project into the surrounding cytoplasm (Fig. 5) and is, in places, apparently continuous with the cisternae of the endoplasmic reticulum. Most of the nucleus is comparatively electron-lucent though small areas

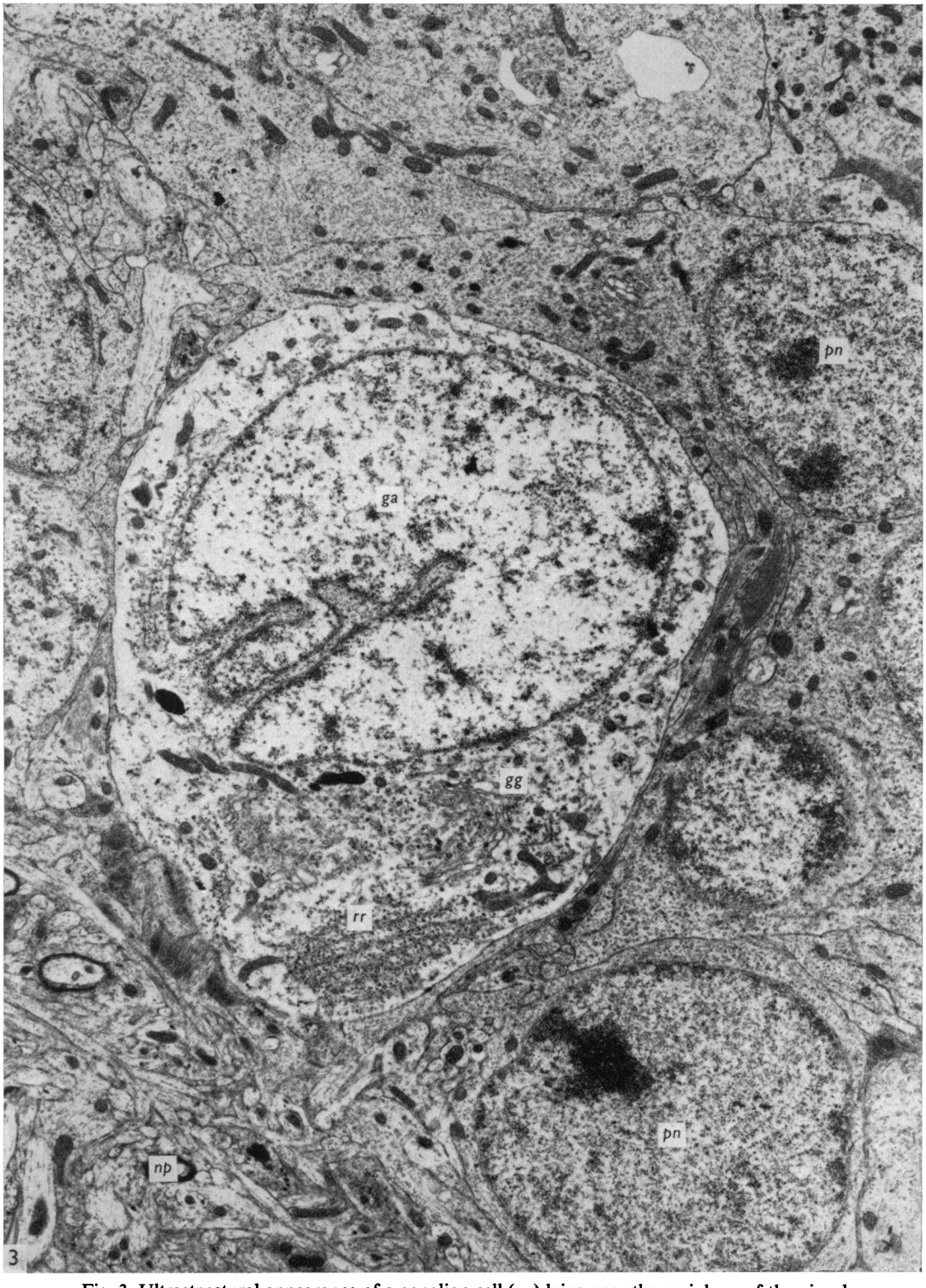


Fig. 3. Ultrastructural appearance of a ganglion cell (*ga*) lying near the periphery of the pineal ganglion. Near it lie pinealocytes (*pn*) and neuropil (*np*). Golgi apparatus (*gg*); rough endoplasmic reticulum (*rr*). ×8730.

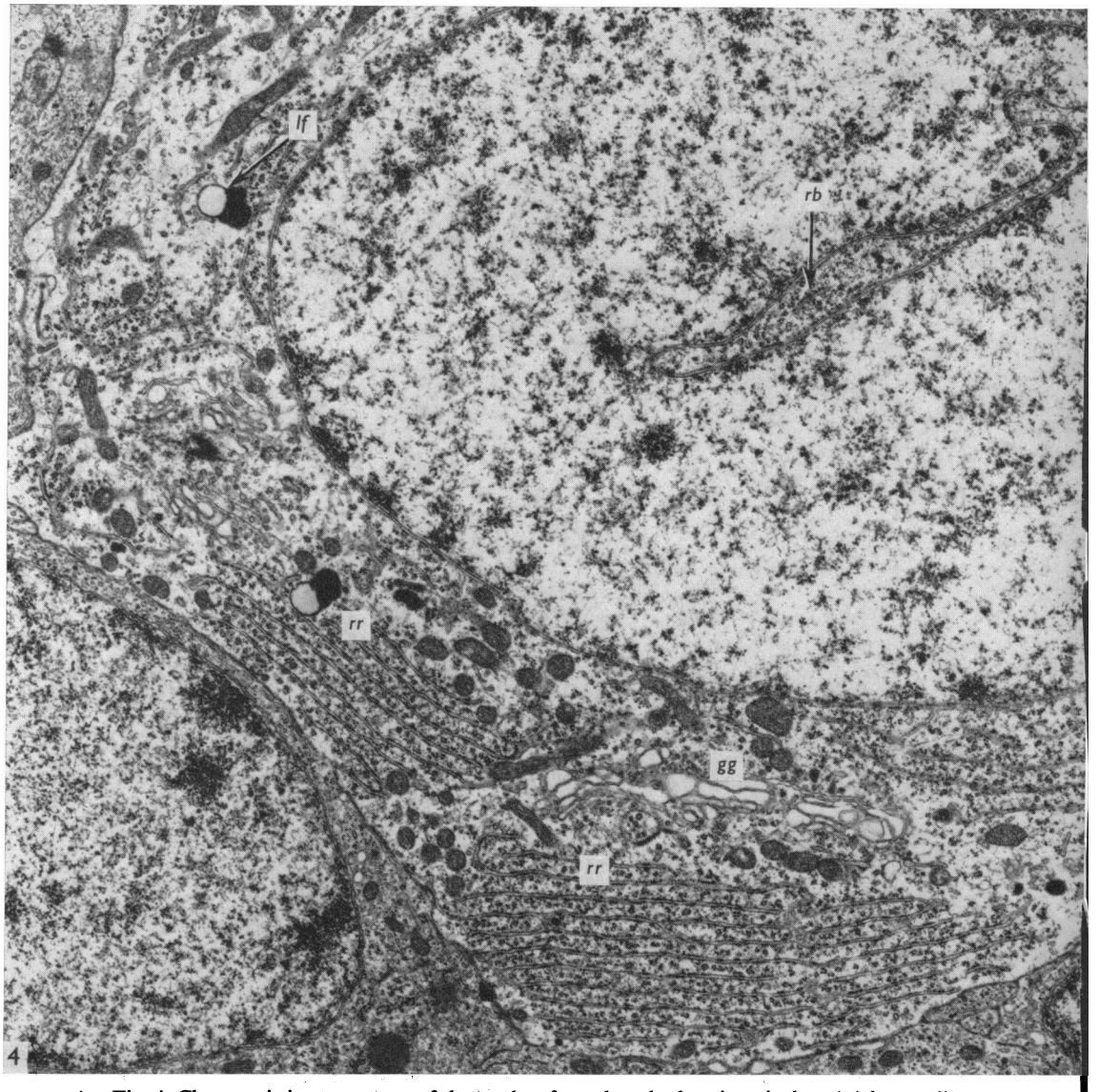
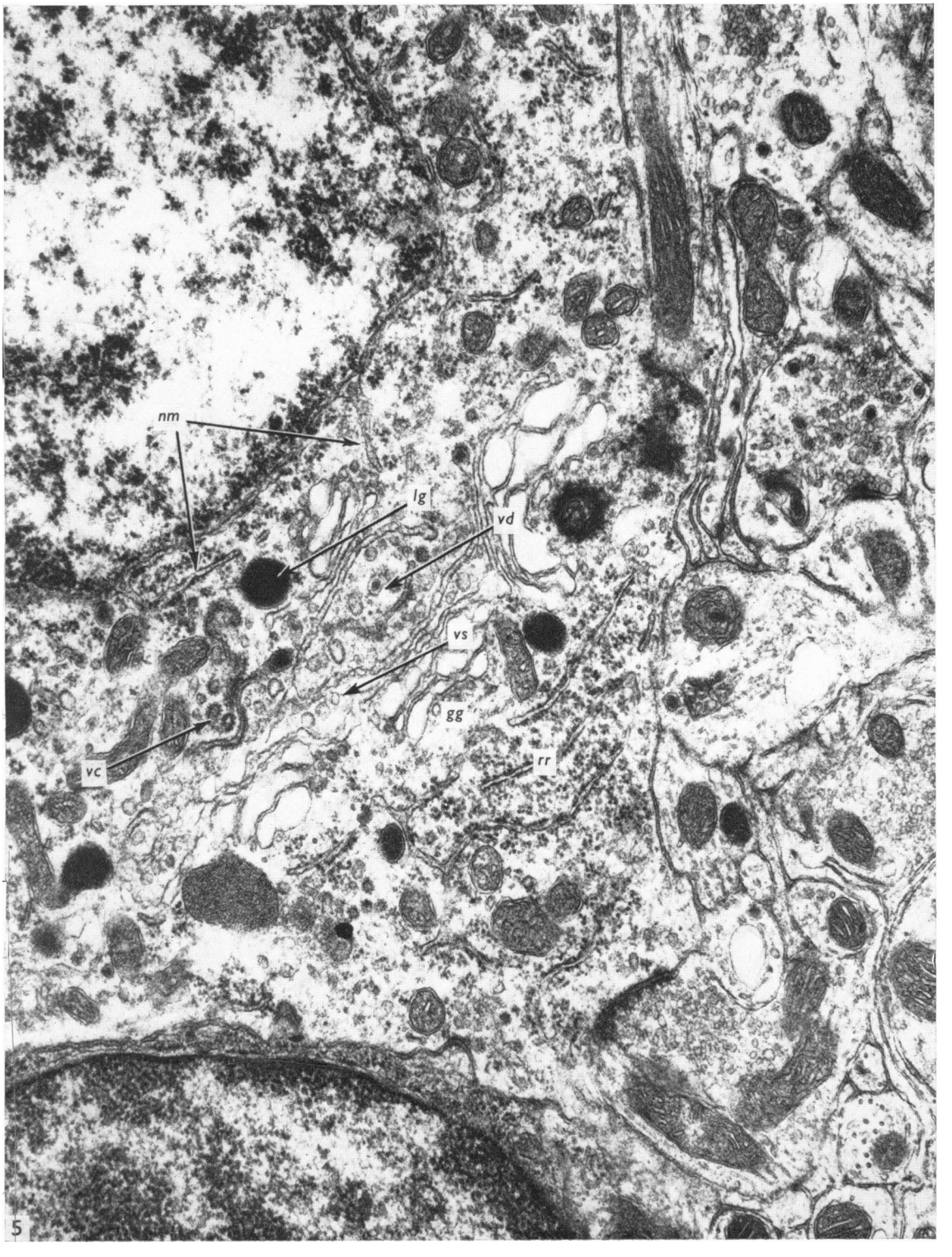


Fig. 4. Characteristic appearance of the stacks of rough endoplasmic reticulum (*rr*) in ganglion cells. Also shown are lipofuscin bodies (*lf*) and free ribosomes (*rb*) in the indentation of the nucleus. Golgi apparatus (*gg*). ×12960.

Fig. 5. Golgi apparatus (*gg*) and surrounding region of a ganglion cell to show the variety of vesicles found in this area. Large membrane-bound granule (*lg*); nuclear membrane projecting into the cytoplasm (*nm*); rough endoplasmic reticulum (*rr*); coated vesicle (*vc*); clear vesicles (*vs*); dense-cored vesicle (*vd*). ×35000.



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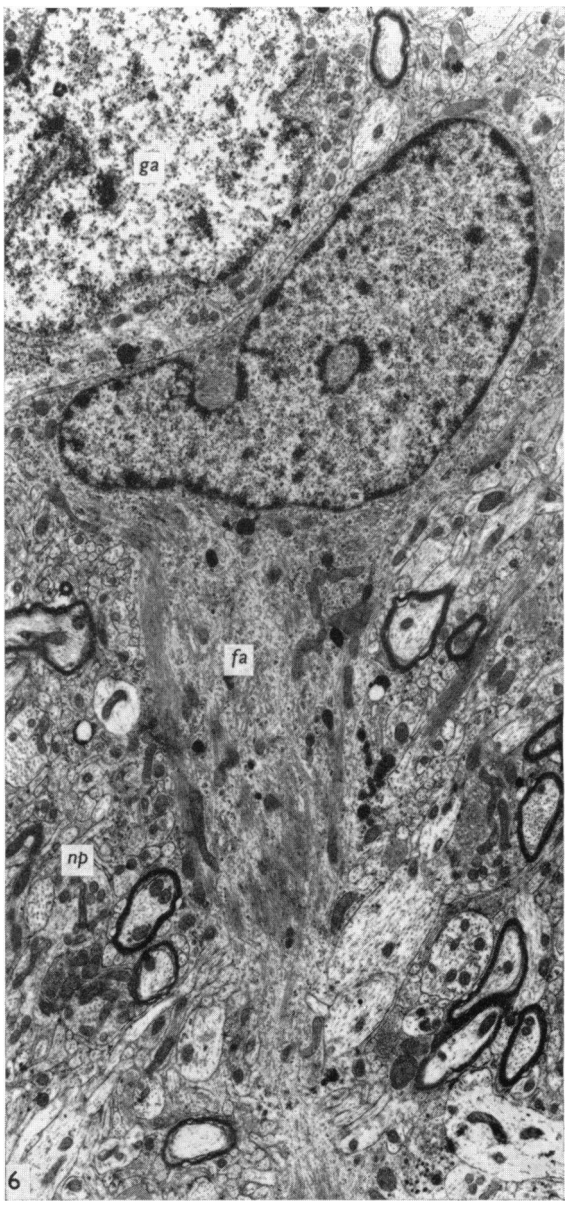


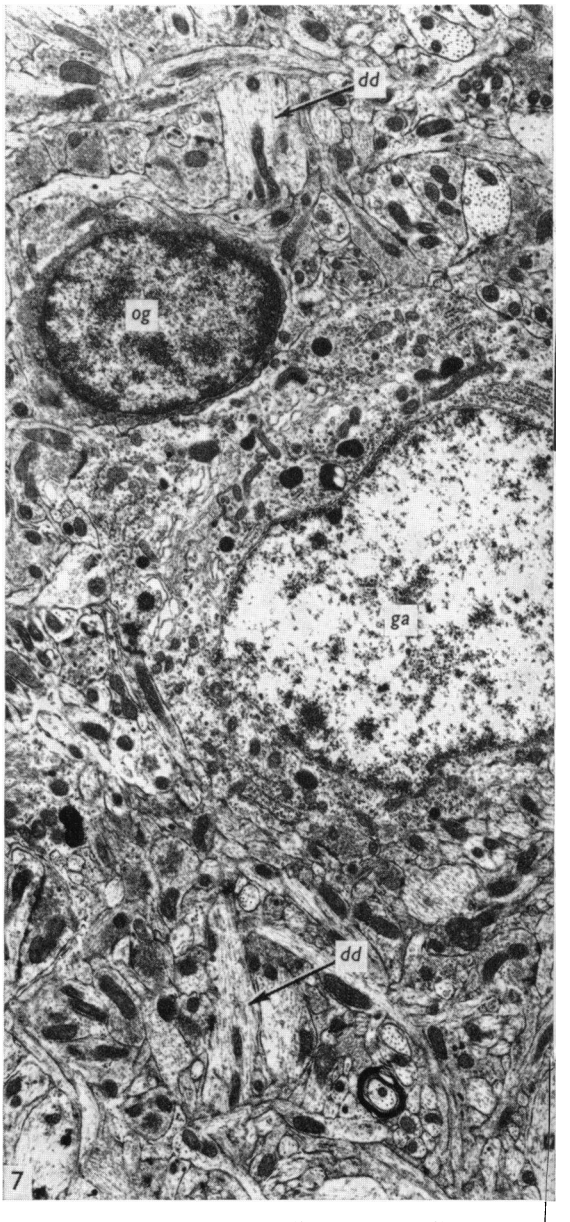


Fig. 6. An astrocyte whose perikaryon contains many fibrils (*fa*). Ganglion cell (*ga*); neuropil (*np*). × 7400.

Fig. 7. Oligodendrocyte (*og*) lying near a ganglion cell (*ga*) and surrounded by neuropil containing dendrites (*dd*). × 7400.

of more dense appearance are visible within the nucleoplasm. The nucleolus is the only electron-dense structure and measures about 1–2 μm in diameter.

The cytoplasm contains both smooth and granular endoplasmic reticulum.

Rough endoplasmic reticulum is a characteristic feature of ganglion cells. It may be present as a single strand, but is more often arranged in stacks to form 'Nissl' substance (Fig. 4). There may be two or three such stacks visible in the same cell, and each may contain up to 12 parallel lamellae, though most have fewer. Stacks of rough endoplasmic reticulum can occur throughout the cytoplasm, but seem rather more frequent near the cell membrane than near the nucleus. The parallel membranes of the rough endoplasmic reticulum lie about 12–20 nm apart, and individual membranes are about 6–8 nm thick. The lamellae are separated from each other by about 150–250 nm and the space between them is rich in free ribosomes. Single strands of rough endoplasmic reticulum are distributed throughout the cytoplasm.

Smooth endoplasmic reticulum is scanty, though occasional dilated cisternae have been observed.

Ribosomes are distributed both in association with the endoplasmic reticulum (Fig. 4) (see above) and as free structures throughout the cytoplasm, being particularly abundant near the nucleus and within its indentation (Figs. 4, 5). Free ribosomes, however, are much less frequent in ganglion cells than in pinealocytes.

Ganglion cells contain a well defined Golgi apparatus, near which different types of vesicles (see below) are observed.

Mitochondria, measuring about 0·2–0·3 μm in diameter, are distributed throughout the perikaryon. In transverse section their cristae are seen to run transversely. No giant mitochondria, such as those seen in pinealocytes, were observed in ganglion cells.

Various vesicles have been observed within the cytoplasm of ganglion cells (Fig. 5). Electron-lucent vesicles are often coated, measure about 50–80 nm in diameter, and are distributed either singly or in groups. Rather larger dense-cored vesicles (60–120 nm) are also found, particularly near the Golgi apparatus. Much larger (200–300 nm) electron-dense membrane-bound granules are also seen in the vicinity of the Golgi apparatus (Fig. 5). In addition, multi-vesicular bodies about 300–400 nm in diameter, lysosomes, lipofuscin bodies and, occasionally, lipid droplets have been observed.

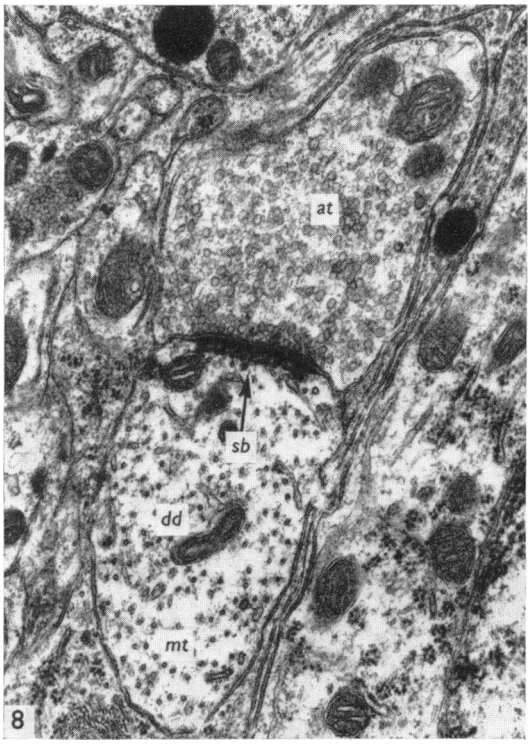
Microtubules (about 20–22 nm in diameter) and neurofilaments (about 7–12 nm in diameter) are seen running haphazardly within the cytoplasm; they become particularly prominent at the origin of a cell process and pass into it as parallel bundles.

Cilia are occasionally seen.

Glial cells

Within the pineal ganglion, glial cells occur singly in the neuropil, either close to a ganglion cell or near the capillaries.

Their nuclei are spherical or indented, and appear denser than those of the ganglion cells because they contain larger patches of chromatin material. Two types of glial cells can be distinguished on the basis of their appearance. The first resembles a 'protoplasmic' astrocyte, and has a rather variable appearance: sometimes the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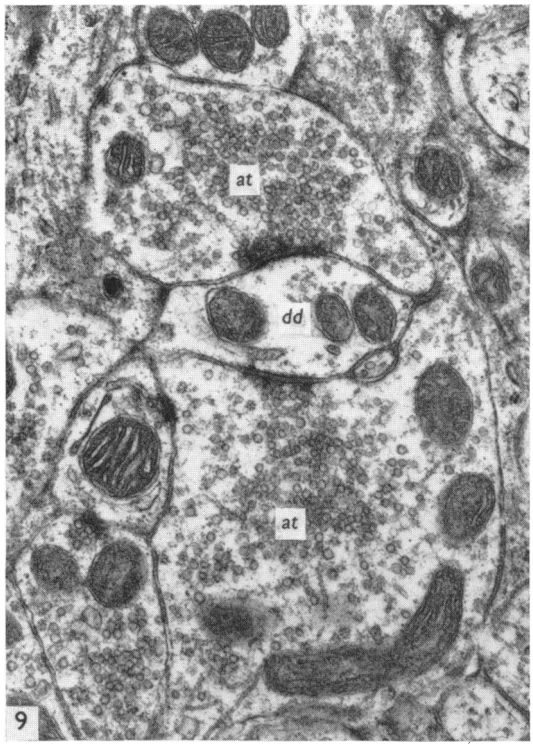
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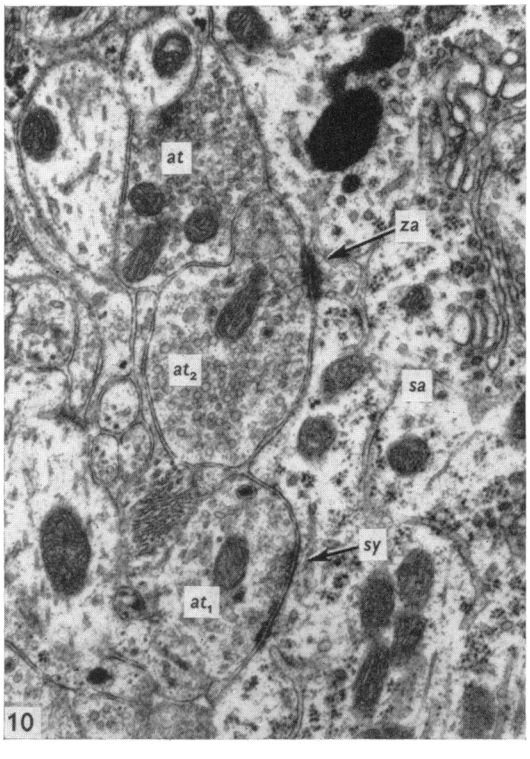
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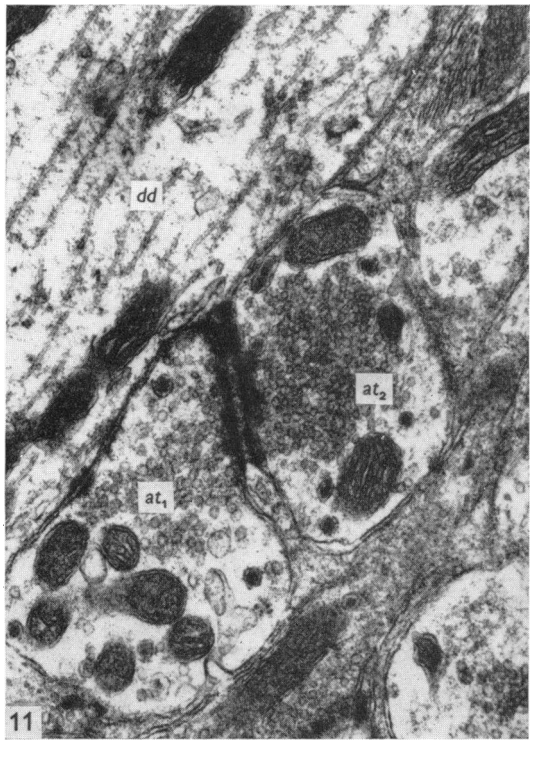
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cytoplasm appears electron-lucent and rather 'empty' (though it may contain a few small vesicles and some fibrils), and sometimes it resembles that of a fibrous astrocyte, and is filled with dense bundles of fibrils (Fig. 6). All astrocytes display the usual cell inclusions, for example, mitochondria, Golgi apparatus, and short strands of rough endoplasmic reticulum. The second type of glial cell is probably an oligodendrocyte. This cell has a rounded nucleus surrounded by a thin film of cytoplasm, and is found close to ganglion cells (Fig. 7).

Neuropil

The major part of the pineal ganglion is formed by neuropil. This consists of dendrites, axon terminals, unmyelinated and myelinated fibres, and contains many synapses (see Fig. 7).

Dendrites, presumably originating from ganglion cells, appear as pale profiles of varying diameter (Fig. 7). Some are observed to branch, and dendritic spines are occasionally visible. A dendrite near its origin from a ganglion cell contains the same structures as the latter, including rough endoplasmic reticulum, elongated mitochondria, microtubules, neurofilaments and free ribosomes. Rough endoplasmic reticulum may be arranged in stacks, though this is not seen in the smaller dendritic profiles, which contain little of it. Mitochondria are elongated, and lie parallel to the dendrite's long axis. Microtubules are prominent, and run the length of the dendrite; their diameter is about 20–30 nm, and they are usually separated by an interval of about 60–150 nm (Fig. 8). Neurofilaments, about 6–8 nm in diameter, are also seen. Occasionally dense-cored vesicles 75–90 nm in diameter lie within the dendrite.

Small unmyelinated fibres (about 0·5–1·0 μm in diameter) are very numerous and are present both in bundles or as single units. Myelinated fibres are less common; no process from a ganglion cell has been seen to develop a myelin sheath, so whether myelinated processes originate, at least in part, from these cells or wholly from outside the ganglion is unresolved. Axons contain parallel rows of microtubules about 26–28 nm in diameter and separated by about 50–80 nm; these structures are thus more compactly arranged in axons than in dendrites.

Many axon terminals are present in the neuropil (Figs. 8, 9, 10 and 11). Mostly they contain membrane-bound electron-lucent vesicles measuring 32–60 nm in diameter ($\bar{x} = 43{\cdot}8 \pm 6{\cdot}37$; $n = 99$) which tend to aggregate near the site of synaptic thickening (Figs. 8, 9). A lesser number of larger vesicles, 60–120 nm in diameter

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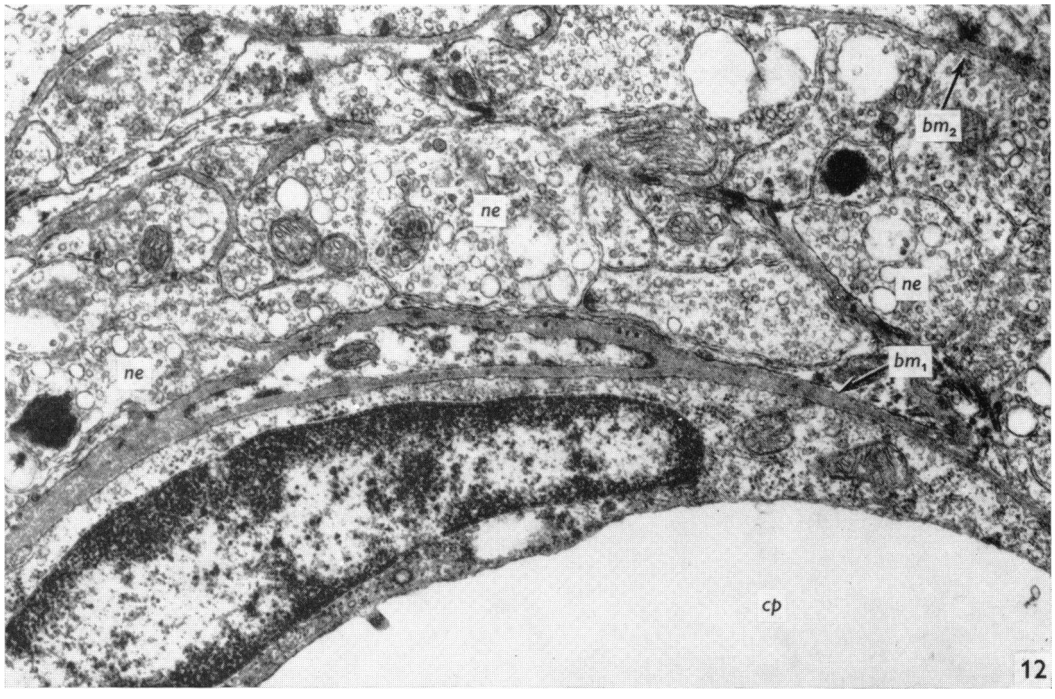
Fig. 8. Axodendritic synapse in the pineal ganglion in which electron-dense substance is asymmetrically disposed on either side of the synaptic cleft. Axon terminal (*at*) containing electron-lucent vesicles; dendrite (*dd*) containing microtubules (*mt*). 'Subjunctional bodies' (*sb*). × 27000.

Fig. 9. Axodendritic synapses in which electron-dense substance is arranged symmetrically on either side of the synaptic clefts. Axon terminal (*at*); dendritic spine (*dd*). × 27000.

Fig. 10. Synapse (*sy*) between an axon terminal (at_1) and soma (*sa*) of a ganglion cell. Immediately above another axon terminal (at_2) makes a zona adhaerens (*za*). × 27000.

Fig. 11. Synapse between two axon terminals (at_1 and at_2), lying adjacent to a dendritic shaft (*dd*). × 27000.



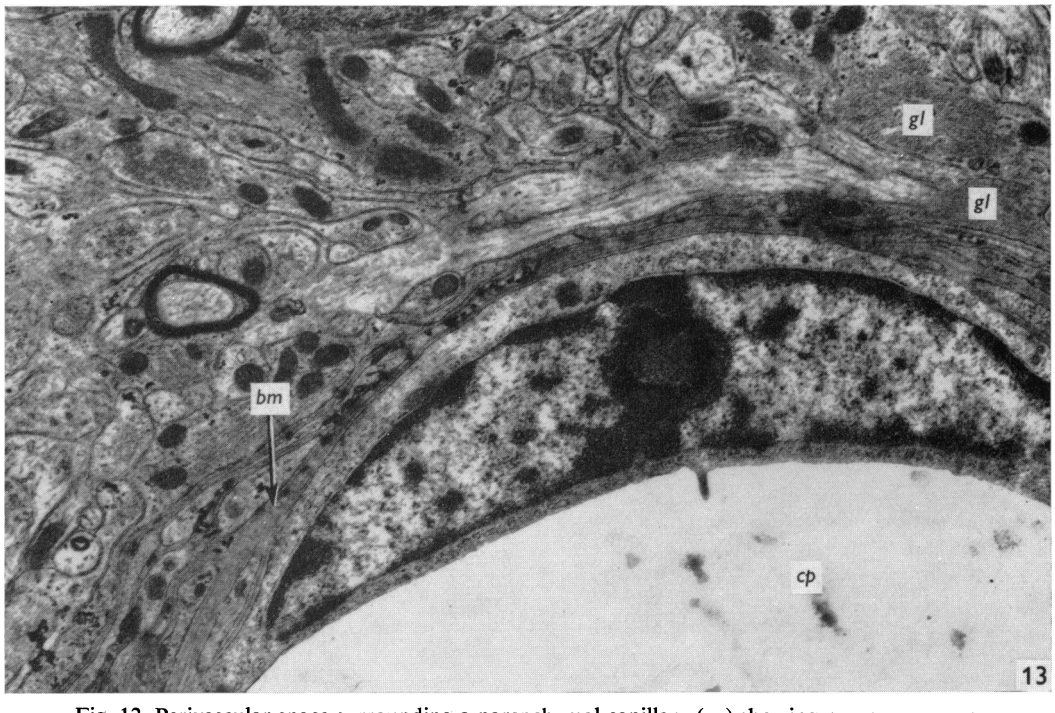


Fig. 12. Perivascular space surrounding a parenchymal capillary (*cp*) showing numerous nerve endings (*ne*) enclosed by the basement membranes (bm_1 and bm_2). ×25000.

Fig. 13. Similar view of capillary from the pineal ganglion showing the absence of nerve endings in the perivascular space. Glial process (*gl*) surround the capillary (*cp*) and basement membrane (*bm*). ×25000.

($\bar{x} = 88{\cdot}9 \pm 15{\cdot}0$; $n = 99$), with an electron-dense core, have also been observed intermingled with the electron-lucent variety. No endings contained flattened vesicles. Mitochondria are commonly present in axon terminals, but microtubules and neurofilaments are generally absent. Presynaptic terminals are found making contact with both the dendrites and the somata of ganglion cells (Figs. 8, 9, 10). Axodendritic synapses constitute the majority, and end on both dendritic shafts and spines, some of which contain a spine apparatus. At the point of contact, the plasma membranes of the two components are separated by a cleft 12–26 nm wide ($\bar{x} = 15{\cdot}4 \pm 2{\cdot}14$; $n = 50$). Electron-dense substance accumulates in the cytoplasm and may be disposed symmetrically on either side of the cleft (Fig. 10) or asymmetrically (Figs. 8, 9), but axodendritic or axosomatic synapses cannot be distinguished by this criterion. Rays of dense material, perpendicular to the plasma membrane, are also often visible near the presynaptic membrane, and structures resembling the 'subjunctional bodies' described by Milhaud & Pappas (1966) are occasionally seen beneath the post-synaptic membrane (Fig. 8). Axo-axonal synapses are uncommon, though they have been observed occasionally (Fig. 11). In such cases the two terminals involved have differed from one another in that the synaptic vesicles in one were rather larger than those of the other. Moreover, the width of the synaptic cleft between them is rather greater (about 29 nm) than in other types of synaptic contacts; the dense substance which is visible lying adjacent to the plasma membrane at the point of contact is symmetrically arranged (Fig. 11).

Other non-synaptic contacts, which may be *zonulae adhaerentes*, are also present (Fig. 10). They are distinguished from synapses by having no accumulation of vesicles near them, and by thin rays which extend from the symmetrically arranged dense material into the cytoplasm.

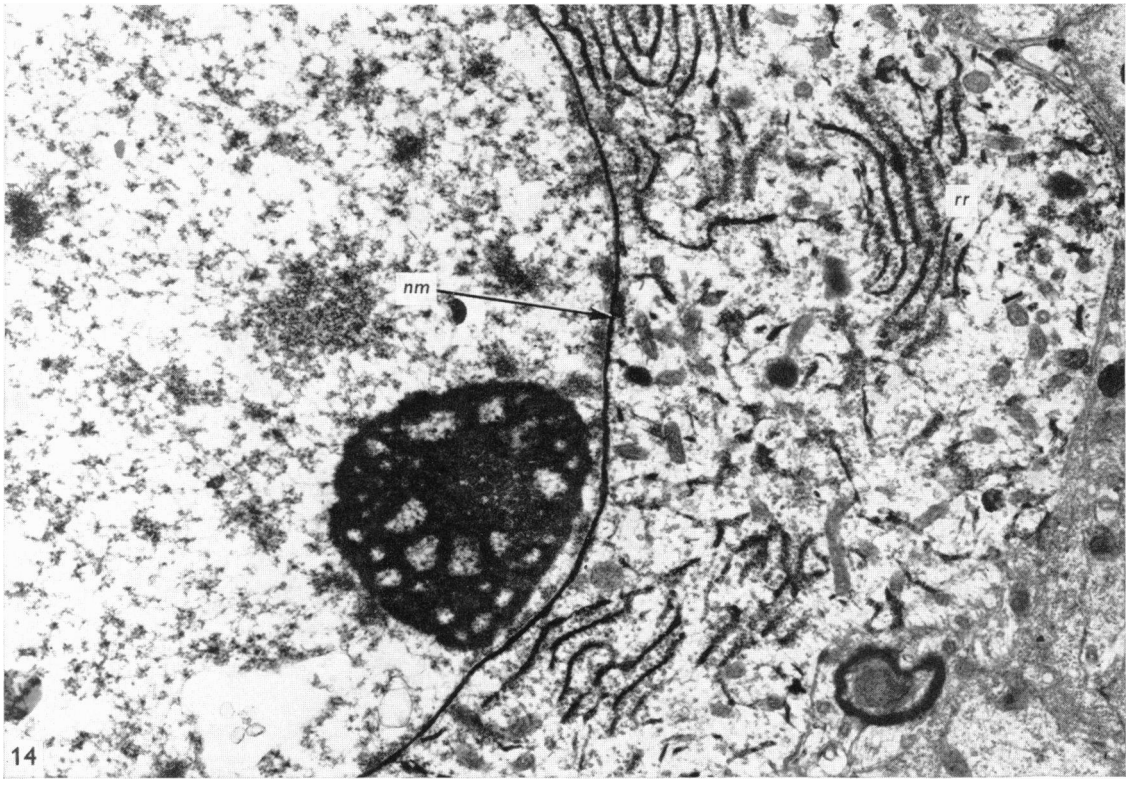
Capillaries

Since the pineal parenchyma and the ganglion are not absolutely demarcated from each other, comparisons have been made between capillaries in the centre of the ganglion and those in the parenchyma proper. In the parenchyma, the capillary endothelial cells are surrounded by a wide perivascular space, enclosed by basement membranes and filled with numerous processes and terminals from both pinealocytes and noradrenergic nerves (Fig. 12) (Johnson, Meyer, Westaby & Herbert, 1972). Glial cell processes are absent or scanty. The space around the capillaries of the ganglion is also bounded by two basement membranes, but this is narrow compared with that surrounding the parenchymal capillaries and rarely contains nerves or nerve endings. Moreover, glial processes filled with microfilaments are often present within the pericapillary space, partly or entirely encircling the endothelial cells (Fig. 13). Fenestrations are not seen in capillaries from either part of the gland.

Acetylcholinesterase

Both ganglion cells and their dendrites contain specific acetylcholinesterase, which is concentrated mainly in the cisternae of the rough endoplasmic reticulum and within the nuclear membranes (Fig. 14). The nucleus itself, the Golgi apparatus, mitochondria, ribosomes, and lysosomes are devoid of enzyme activity.

Within the neuropil, the enzyme is present mainly in the dendrites and within these



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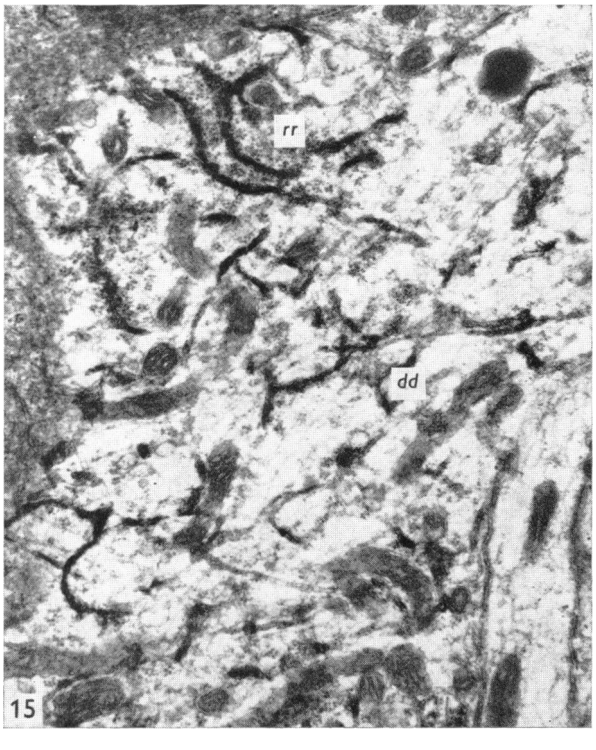

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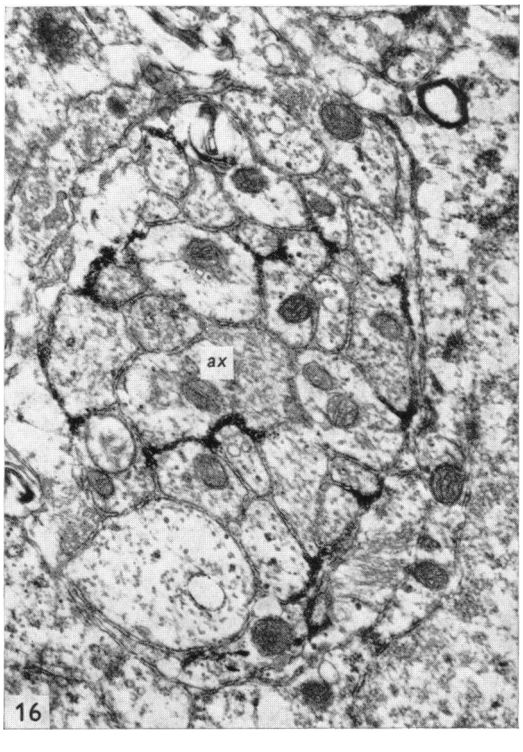

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in the cisternae of the rough endoplasmic reticulum (Fig. 15). The axolemma of unmyelinated axons also, in places, shows the presence of acetylcholinesterase (Fig. 16) but it could not be demonstrated in axon terminals, in synapses, or in myelinated nerve fibres. Neither do glial cells or the endothelial cells lying round the capillaries contain acetylcholinesterase.

Preliminary results also suggest that acetylcholinesterase is not demonstrable in the endings surrounding the parenchymal perivascular space.

Non-specific cholinesterase was not found in any structure within the pineal ganglion.

5-OH DOPA

None of the synapses within the pineal ganglion showed the presence of small dense-cored vesicles after the animal had received 5-OH DOPA. This is in contrast to the pineal parenchyma, in which a proportion of endings within the perivascular space (the autonomic endings) was prominently 'labelled' after this treatment (Fig. 17, 18) (Johnson *et al.* 1972).

DISCUSSION

These findings establish that the pineal ganglion contains the elements found in other parts of the nervous system and is therefore undoubtedly composed of nervous tissue.

The neurons of the pineal ganglion contain parallel stacks of endoplasmic reticulum (Nissl substance) as elsewhere in the nervous system. There is no consistent morphological evidence that the ganglion cells of the ferret's pineal are neurosecretory, though membrane-bound granules (200–300 nm) reminiscent of those found in the supraoptic and paraventricular nuclei have been observed occasionally (Fig. 5), and octapeptides related to those produced in the hypothalamic neurosecretory system have been isolated from the pineal (Pavel, 1965). The distribution of acetylcholinesterase in the cytoplasm is also characteristic of cholinergic cells elsewhere in the CNS, in which this enzyme is localized in the rough endoplasmic reticulum and within the perinuclear membranes (Lewis & Shute, 1966, 1969; Navaratnam & Lewis, 1970). Finally, the occurrence of axosomatic synapses unquestionably demonstrates that the ganglion cells are, in fact, neurons.

The profusion of synapses found in the pineal ganglion has not been found before in the mammalian pineal though Romijn (1972) described occasional synapses, which he considered to be preganglionic parasympathetic fibres, on neurons in the rabbit's pineal. Those in the ferret cannot be subdivided according to the vesicles contained within them; all synapses seem to contain at least two types. The more frequent, smaller, electron-lucent type compares in size to that observed elsewhere in the CNS (Peters, Palay & Webster, 1970). Neither synapses nor ganglion cells accumulate dense-cored vesicles after 5-OH DOPA treatment, or show fluorescence after the

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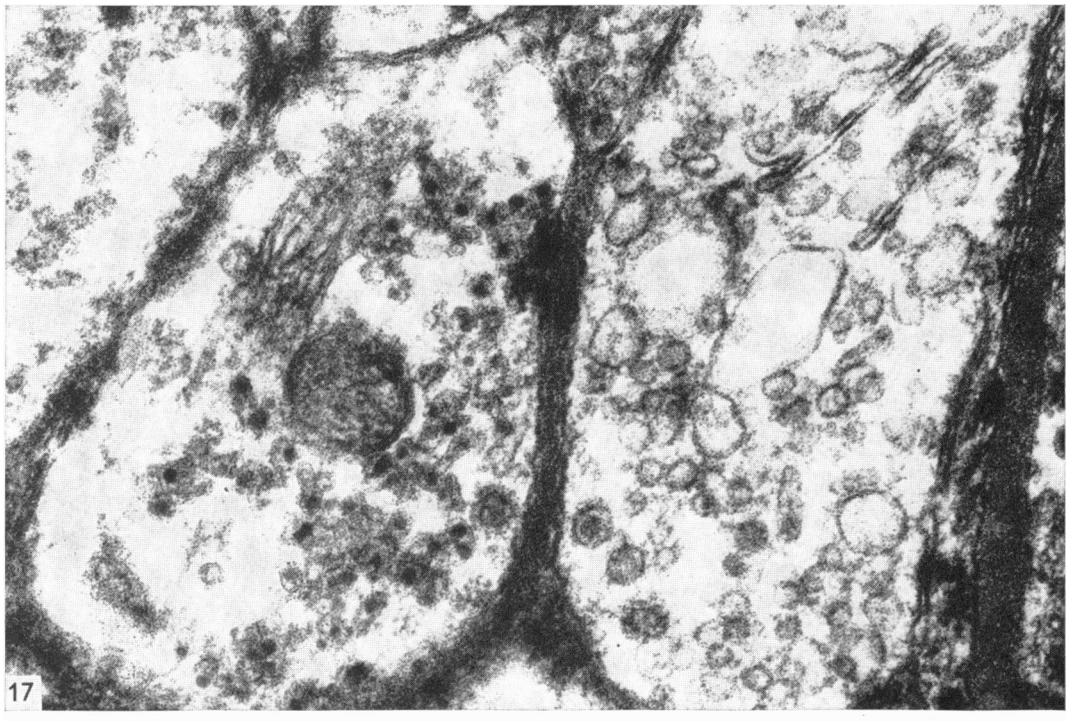
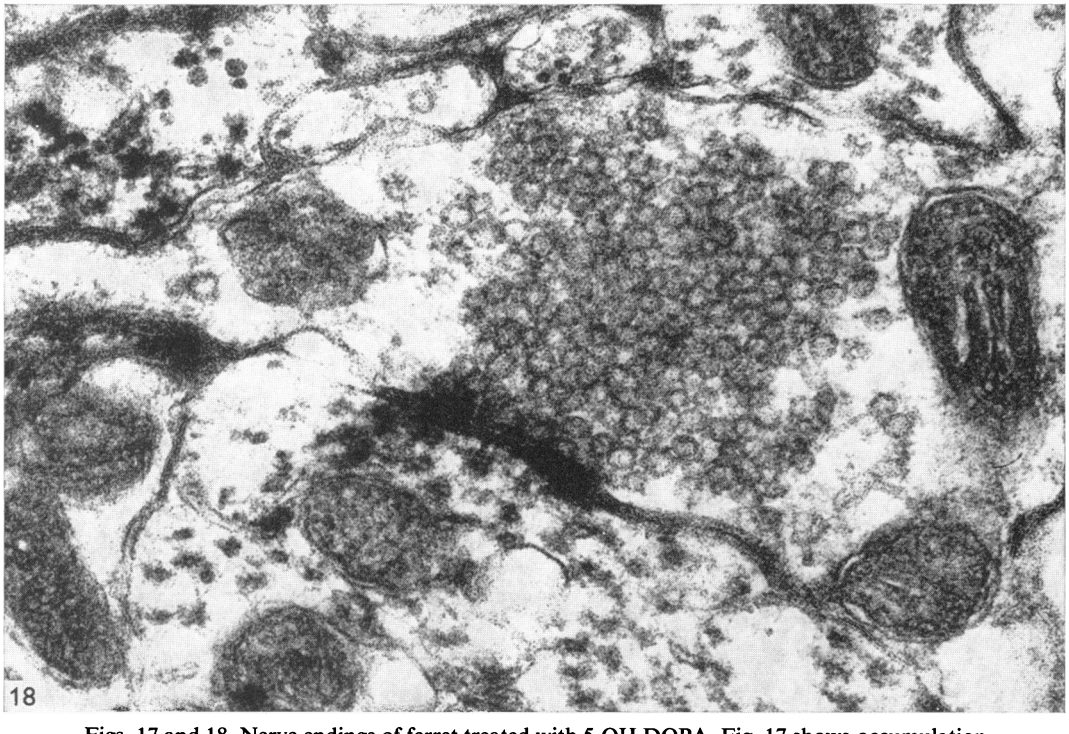
Fig. 14. Acetylcholinesterase activity in nuclear membrane (*nm*) and the cisternae of rough endoplasmic reticulum (*rr*). × 9000.

Fig. 15. Acetylcholinesterase in the rough endoplasmic reticulum (*rr*) of a dendrite (*dd*). × 25000.

Fig. 16. Acetylcholinesterase in the inter-axonal space and axolemma of non-myelinated nerve bundles (*ax*). × 25000.





Figs. 17 and 18. Nerve endings of ferret treated with 5-OH DOPA. Fig. 17 shows accumulation of electron-dense cores within vesicles of an ending in the pineal parenchyma. Fig. 18 shows the absence of dense cores in the vesicles of an ending within the pineal ganglion. $\times 83200$.

Falck procedure (Trueman & Herbert, 1970), and this argues against the larger dense-cored vesicles also observed being necessarily associated with monoamines. The electron-dense substance lining the clefts of synapses was sometimes symmetrically and sometimes asymmetrically arranged, and it did not seem as if either arrangement was particularly associated with dendritic shafts or spines.

As in other areas of the CNS, axodendritic synapses were most commonly seen in the pineal ganglion, though both axosomatic and axo-axonal contacts have also been observed. It is too early to speculate fruitfully on the relative roles of the different categories of synapses present in the pineal ganglion. But it is interesting that we were unable to demonstrate cholinesterase activity in synapses. This argues against such synapses originating from the ganglion cells themselves, though it is difficult to believe that intramural connexions between the ganglionic neurons do not exist.

Acetylcholinesterase was also found in some nerve fibres. These fibres might have originated from the ganglion cells or be autonomic fibres *en passage* to the pineal parenchyma, since acetylcholinesterase is present in the noradrenergic fibres of the pineal (see Eranko, Rechardt, Eranko & Cunningham, 1970; Lores Arnaiz & Pellegino de Iraldi, 1972); but the complete absence of catecholamine fluorescence in the ganglion is against the second interpretation. If cholinesterase-containing fibres are destined to end on ganglion cells it is curious that we were unable to demonstrate cholinergic synapses, and they may be running out of the pineal and towards the habenula as part of the band of fibres stretching between the two structures.

Although there is no clear boundary between the ganglion and the rest of the pineal, structural differences between the two regions are very marked. The ferret's pinealocytes, besides being smaller than the ganglion cells, do not contain stacks of rough endoplasmic reticulum, though they contain many more free ribosomes. The pinealocyte process projects to the perivascular space and ends close by autonomic endings, and the vesicle populations of pinealocyte and autonomic endings differ from those found in the pineal ganglion (Johnson *et al.* 1972). No synaptic contacts have been found in the parenchyma, whereas vesicle-crowned rodlets ('synaptic ribbons') present in pinealocytes (Johnson *et al.* 1972), are not demonstrable in the ganglion. The dense neuropil in which the scattered ganglion cells lie cannot be observed in the rest of the pineal, and dendritic profiles may be confined to the ganglion or the area immediately surrounding it. The capillaries of the two areas also show considerable differences. Those in the pineal parenchyma are surrounded by a wide perivascular space filled with terminals. These are not seen in the pineal ganglion, in which the capillaries are characteristically sheathed by glial processes containing bundles of microfibrils. Glial processes, when they do occur around the parenchymal capillaries, are inconspicuous. However, both ganglion and parenchyma are within the 'blood brain barrier' in the ferret, since (i) intraperitoneally administered trypan blue does not enter the ferret's pineal; (ii) 5-OH dopamine is not taken up into the autonomic perivascular plexus of the pineal, though it 'labels' such endings in the iris, and (iii) orally administered silver nitrate does not penetrate the pineal, though it can be seen in the median eminence and area postrema (Malcolmson, Johnson, Wright & Herbert, unpublished observations).

The observations reported here are preliminary to attempts to answer some

interesting questions about the pineal ganglion: Whence does it originate? What are its connexions? How does it relate to the pineal parenchyma? What is its function? There is no evidence, so far, that processes from the pinealocytes make contact with the ganglion cells, though vesicle-crowned rodlets in pinealocytes have been seen to be adjacent to processes whose nature is not clear at the moment (Johnson *et al.* 1972). Neither is it clear whether the ferret's ganglion cells are homologous with the neurons found in the pineal glands of reptiles and amphibians (Kelly, 1971; Collin, 1971), in which pineal photoreceptors synapse with nerve cells and then project centrally to the habenula. Experimental studies on the ferret have shown that degenerating synapses are demonstrable in the pineal ganglion following habenular lesions (David & Herbert, 1973), a finding which is against the ganglion being a parasympathetic nucleus. The possible functional significance of this direct connexion between part of the pineal and the CNS should not be overlooked.

SUMMARY

The ultrastructure of the pineal ganglion, a group of nerve cells found in the caudal part of the ferret's pineal, has been studied. The ganglion is composed of neurons and glial cells set in a matrix of neuropil; it blends with the surrounding parenchyma. In the neurons, acetylcholinesterase was demonstrated in the stacks of rough endoplasmic reticulum and in the outer nuclear membrane. The neuropil contains many dendrites, unmyelinated and myelinated nerve fibres, and axon terminals. Axodendritic, axosomatic and axo-axonal synapses were seen, the first being the most common. All axon terminals contained electron-lucent spherical vesicles 32–60 nm in diameter; some also contained larger (60–120 nm) dense-cored vesicles as well. No endings containing flattened vesicles were found. Electron-dense material was disposed both symmetrically and asymmetrically on pre- and post-synaptic membranes. No acetylcholinesterase activity could be demonstrated on either side of the synaptic cleft. Capillaries in the ganglion were not surrounded by the closely packed endings which characterized those of the parenchyma. Neither ganglionic nor parenchymal capillaries were fenestrated. In ferrets given 5-OH DOPA, no 'labelled' endings could be observed in the ganglion area, though dense cored vesicles were profuse in the terminals of the perivascular space in the parenchyma. Both fibrous and protoplasmic astrocytes, as well as oligodendrocytes, were observed in the pineal ganglion.

Synaptic structures such as those observed in the present investigation have not been described before in the mammalian pineal. Although the neurons of the pineal ganglion are cholinergic, the endings on them are neither cholinergic nor adrenergic. It is unlikely that the ganglion forms part of the parasympathetic system.

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