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Structure of the Parapineal Organ of the Adult Rainbow Trout, *Salmo gairdneri* Richardson

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Summary. The parapineal organ of the teleost *Salmo gairdneri* Richardson[1] was investigated with the light and electron microscopes. It is a small cell mass, 0.1—0.3 mm in diameter, containing a narrow lumen and consistently situated to the left of the pineal stalk and dorsal to the left habenular nucleus. It is connected with the habenular nucleus through a conspicuous parapineal tract. The parapineal organ continues to grow at least until the fish reaches sexual maturity and shows no sign of cellular degeneration at the age of two years.

The parapineal tissue consists of supporting cells and nerve cells; the latter give rise to the axons of the parapineal tract. Furthermore, a small number of receptor cells of the type existing in the pineal organ is present. No morphological evidence was obtained to suggest a sensory or secretory function of the parapineal organ.

The existence of the parapineal organ in the adult pike, *Esox lucius*, L., and of a connection between the pineal tract and the habenular commissure in *Salmo gairdneri* is briefly reported. The results are discussed in the light of existing literature.

Introduction

The earliest studies of the development of the pineal organ of teleosts were made by Rabl-Rückhard (1882, 1883) and Hoffmann (1884) on the same two species, *Salmo salar* L. and *S. trutta fario* L. These authors found the pineal organ to originate in front of the posterior commissure as a broad sack-like evagination, but did not observe any other evagination from the diencephalic roof.

It is the merit of Hill (1891, 1894) to have discovered a second epiphyseal evagination, appearing during the ontogeny of *Amia calva* L. (Holostei) and the teleost species *Coregonus albus* Günth., *Lepomis pallidus* Mitch., *Stizostedion vitreum* Mitch., *Castomus teres* Mitch., *Salmo trutta fario* L., *S. purpuratus* Pallas, and *S. fontinalis* Mitch. Hill used the terms posterior epiphysis and anterior epiphysis for the pineal organ and the newly discovered evagination, respectively. He described them to originate, in close proximity to each other, from the roof of the third ventricle, the posterior epiphysis in the median plane and the anterior epi-

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[1] The rainbow trout is better known under the name of *Salmo irideus* Gibbons. However, according to Ladiges and Vogt (1965), *S. gairdneri* Richardson is more correct.

physis in an asymmetrical position rostral to and a little to the left of the former. Both organs originally had a central lumen in connection with the third ventricle but, whereas the posterior epiphysis maintained its connection with the roof of the brain and its lumen remained continuous with the third ventricle, the anterior epiphysis lost the above-mentioned connection at a body length of 13 mm (*Salmo* sp.) and assumed the form of a small, thick-walled vesicle, the lumen of which was nearly obliterated. At a body length of 25 mm, the lumen of the anterior epiphysis had completely disappeared and the remaining compact cell mass was completely free from surrounding structures and was situated immediately to the left of the stalk of the pineal organ. In individuals with a body length of 160 mm (aged 2 yr) HILL was able to find the anterior epiphysis only on one occasion. In this case it was reduced to $^2/_3$ of the size of the preceding stage, but was structurally unchanged. HILL therefore considered the anterior epiphysis to be rudimentary.

STUDNIČKA, in his important review of 1905, suggested a homology between the parapineal organ of *Lampetra* (Petromyzontidae) and the anterior epiphysis of teleosts and adopted the use of the former name also for this group. He did not contribute any personal observations to this question.

In 1910 TERRY described the development of a rudimentary parapineal organ in the teleost species *Batrachus* (*Opsanus*) *tau* L. This author reported the parapineal organ to be compact from the very beginning and to originate closely to the left and a little in front of the pineal organ, however, without ever being directly connected to it. In adult forms he did not observe the organ.

Very few modern studies deal with the parapineal organ of teleosts. In 1932 FRIEDRICH-FREKSA published a report on *Coregonus macrophthalmus* NÜSSLIN. Studying living animals, he found the parapineal organ to evaginate medially, closely rostral to the pineal evagination. After losing the connection with its point of evagination, the small cell mass was displaced obliquely forwards and established a secondary nervous connection with the habenular commissure above the left habenular nucleus. FRIEDRICH-FREKSA reported degenerative changes to occur in the parapineal organ already before hatching and did not observe the organ in any adult forms.

U. HOLMGREN (1959, 1965) described the parapineal organs of *Salmo trutta* L., *S. salar* L., and *Salvinellus fontinalis* L. He observed the parapineal vesicle to be connected to the roof of the brain on the right side of the pineal organ, a position that he assumed not to be the original one. The parapineal organ in *Salvinellus* could be observed to lose its connection with the brain already at 10 mm body length. It did not establish any secondary nervous connection and finally degenerated. U. HOLMGREN, on the basis of his results and those of earlier authors, concluded that adult teleosts generally do not have a parapineal organ.

The present author has followed the development of the parapineal organ of the pike, *Esox lucius* L., from pre-hatching stages to 82 mm body length. In this species the establishment of a secondary connection with the left habenular nucleus was clearly seen and still existed in the oldest stage examined. The parapineal organ originally had a distinct lumen but relatively soon (above 20 mm

body length) this was obliterated, or at least very much reduced in size. The question regarding a symmetrical or asymmetrical *Anlage* of the parapineal organ could not be solved because of the lack of certain early stages (RÜDEBERG, unpublished observations).

Recently, the author has found the parapineal organ to be present in three adult individuals of the pike, *Esox lucius* L. (weight approx. 2 kg), and in 35 adult rainbow trouts, *Salmo gairdneri* RICHARDSON (weight 250—450 g). Since the latter were abundantly available, this species was chosen for a light and electron microscopic investigation. It was considered of special interest to clarify if a nervous connection still exists between the parapineal organ and the habenular commissure in adult forms, if the cell types of the pineal organ are also present in the parapineal organ, and if any degenerative changes occur in the parapineal organ of the sexually mature fish. Finally it was hoped that the functional significance of the organ would be revealed through its ultrastructural characteristics.

Materials and Methods

Light Microscopy. A collection of brains from seventeen 1-yr-old and one 2-yr-old rainbow trouts, *Salmo gairdneri* RICHARDSON, (Table 1, Nos. 1—18) from the collection of Prof. OKSCHE, was at my disposal. These had been fixed in 10% formalin, Bodian's fluid, or Bouin's fluid (Table 1), dehydrated in alcohol, embedded in Paraplast, sectioned at 10 μ, and stained according to Table 1. Exact information regarding the size of each individual was lacking and the values listed in Table 1 were based on notes of DR. MORITA (then of the W. G. Kerckhoff-Institut, Max-Planck Gesellschaft, Bad-Nauheim), who originally killed and fixed the fishes. Single individuals may have been larger than the lengths given. Individual No. 18 may also have been an exceptionally large 1-yr-old fish.

To extend the investigation further 10 brains of 2-yr-old rainbow trouts were fixed in Bouin's fixative. They were dehydrated in alcohol, embedded in paraffin, cut and stained according to Table 1, Nos. 19—28. All these fishes were 27—33 cm long and weighed 250—450 gm. Both females and males were sexually mature.

The following histological and histochemical methods were used (see Table 1):

General morphology and histology: nuclear fast red, toluidine blue, iron haematoxylin (Heidenhain), trichrome stain (Jerusalem). *Nerve cells* and *nerve fibres*: gallocyanin, toluidine blue, luxol fast blue MBS-cresyl violet (Klüver-Barrera), gallocyanin-luxol fast blue MBS (MAUTNER, 1965), silver protargol (Bodian-Ziesmer). *Glycogen*: periodic acid-Schiff (PAS), lead tetraacetate (SHIMIZU and KUMAMOTO, 1952), both methods including diastase controls. *(Neuro)secretory substances*: chrome alum-haematoxylin-phloxin (Gomori-Bargmann), paraldehyde fuchsin (Dawson), paraldehyde fuchsin (Gabe), paraldehyde fuchsin (Halmi, as modified by U. HOLMGREN, 1958).

Electron Microscopy. Seven sexually mature rainbow trouts, 27—33 cm long, were used. They were killed by decapitation and the braincases were rapidly opened. When the tissues were fixed only in osmium tetroxide, a relatively large part of the brain was immediately dissected out, immersed in the fixing fluid, and further dissected during the dehydration. Since the parapineal organ is not covered by any other brain parts and measures only 0.1—0.3 mm in diameter, this method was quite adequate.

When glutaraldehyde was used as a fixative, it was dripped on the brain *in situ* and the relevant part was then accurately dissected out and immersed in the fixative. However, due to the small size of the organ, it could not be isolated, but had to be further prepared together with surrounding tissue.

As an aid in preparing the fixatives the osmotic pressures of blood and cerebrospinal fluid of the rainbow trout were measured with a Knauer osmometer and found to be 320 mOsM for both fluids.

Table 1. *Survey of material and methods for light microscopy*

Animal number	Age (year)	Body length (cm)	Fixation	Section thickness (μ)	Section plane[a]	Staining method
1	1	10—15	formalin	10	sag.	nuclear fast red
2	1	10—15	formalin	10	trans.	nuclear fast red
3	1	10—15	formalin	10	sag.	nuclear fast red
4	1	10—15	formalin	10	sag.	nuclear fast red/gallocyanin[b]
5	1	10—15	formalin	10	sag.	gallocyanin
6	1	10—15	formalin	10	sag.	nuclear fast red
7	1	10—15	formalin	10	sag.	nuclear fast red
8	1	10—15	formalin	10	sag.	nuclear fast red
9	1	15—20	Bodian	10	sag.	silver protargol (Bodian-Ziesmer)
10	1	15—20	Bodian	10	sag.	silver protargol (Bodian-Ziesmer)
11	1	10—15	Bouin	10	sag.	luxol fast blue MBS-cresyl violet (Klüver-Barrera)
12	1	10—15	Bouin	10	sag.	iron haematoxylin (Heidenhain)
13	1	10—15	Bouin	10	sag.	gallocyanin-luxol fast blue (Mautner)
14	1	10—15	Bouin	10	sag.	gallocyanin-luxol fast blue (Mautner)
15	1	10—15	Bouin	10	trans.	gallocyanin-luxol fast blue (Mautner)
16	1	10—15	Bouin	10	hor.	gallocyanin-luxol fast blue (Mautner)
17	1	10—15	Bouin	10	hor.	iron haematoxylin (Heidenhain)
18	2	25—30	Bouin	10	sag.	silver protargol (Bodian-Ziesmer)
19	2	27—33	Bouin	7	trans.	chrome alum-haematoxylin-phloxin (Gomori-Bargmann)
20	2	27—33	Bouin	7	trans.	silver protargol-paraldehyde fuchsin (Bodian-Ziesmer-Dawson)
21	2	27—33	Bouin	7	hor.	silver protargol (Bodian-Ziesmer)
22	2	27—33	Bouin	7	sag.	luxol fast blue MBS-cresyl violet (Klüver-Barrera)
23	2	27—33	Bouin	7	sag.	silver protargol (Bodian-Ziesmer)
24	2	27—33	Bouin	7	sag.	lead tetraacetate (Shimizu-Kumamoto)/periodic acid-Schiff[b]
25	2	27—33	Bouin	7	hor.	paraldehyde fuchsin (Gabe)/paraldehyde fuchsin (Halmi-Holmgren)[b]
26	2	27—33	Bouin	7	hor.	silver protargol (Bodian-Ziesmer)
27	2	27—33	Bouin	7	trans.	toluidine blue/gallocyanin[b]
28	2	27—33	Bouin	8	sag.	trichrome stain (Jerusalem)

[a] sag. = sagittal, trans. = transversal, hor. = horizontal.
[b] Where divided through the sign /, the methods were not combined.

The following procedures were used:

1. 1% OsO_4 in 440 mOsM phosphate buffer (1.80 g $NaH_2PO_4 . H_2O$, 23.25 g $Na_2HPO_4 . 7H_2O$, 5.00 g NaCl per 1 H_2O; ZAMBONI and DE MARTINO, personal communication). Fixation time 1 hr.

2. 1% OsO_4 in 0.13M phosphate buffer (Millonig) + 0.075M NaCl (buffer + NaCl: 375 mOsM). Fixation time 1 hr.

3. 2.5% glutaraldehyde (Schuchardt: 295 mOsM, or Merck: 270 mOsM) in 0.13M phosphate buffer (Millonig) + 0.075M NaCl (buffer + NaCl: 375 mOsM). Fixation time 1 hr. Postfixation 1 hr in fixative 2.

4. 2.5% glutaraldehyde (Merck: 270 mOsM) in 0.065M phosphate buffer (Millonig; buffer: 120 mOsM). Fixation time 1 hr. Postfixation 1 hr in fixative 2.

In fixative No. 3 the buffer with the added salt, but excluding the glutaraldehyde, was c. 15% hypertonic compared to the blood. Fixative No. 4, including the glutaraldehyde, was c. 20% hypertonic to the blood. The fixatives were always adjusted to pH 7.3—7.5 and chilled to 0—+ 4° C.

The specimens were dehydrated in alcohol and via styrene embedded in Vestopal W (Kurtz). During dehydration the specimens were treated for 30 to 40 min in a 1% solution of uranyl acetate in 100% alcohol, to improve the contrast.

Due to the extreme smallness of the parapineal organ the specimens had to be cut into semi-fine sections (ca. 1 μ) until the organ was found. These sections were stained with methylene blue-thionin (RÜDEBERG, 1967) for light microscopy. When the parapineal organ had been localized, ultrathin sections (500—1000 Å) were produced with the Porter-Blum MT2 ultramicrotome and placed on copper grids without supporting film. The contrast was further increased with lead citrate (Venable and Coggeshall) and micrographs were prepared with a Siemens Elmiskop 1 at 60 kV.

Results

Light microscopy. The parapineal organ of the rainbow trout is a small (0.1—0.3 mm, Table 2) globular, ovoid, lens-, or spindle-shaped cell mass with a very small central lumen and thick walls. It is situated to the left of the proximal portion of the pineal stalk, dorsocaudal to the entrance of the habenular commissure in the left habenular nucleus, and dorsal to this nucleus. It is located in a narrow space limited rostrally by the *saccus dorsalis*, and caudally, dorsally, and to the right by the *tectum opticum*. This space, however, is not completely closed and its walls represent the outer surface of the brain (Fig. 1a, b).

As can be seen in Table 2 it is not possible to find any constant relationship between length, height, and width of the parapineal organ. Therefore, only the composite of the three figures representing these dimensions was considered an acceptable basis for comparing the mean size of the organs of the 1-year-old fishes with that of the 2-year-old. However, the figures representing the sizes give only a relative value, that of the volume of the smallest box that could possibly hold the respective parapineal organ.

Statistical evaluation according to the "Student's test" shows that there exists a highly significant difference ($p < 0.001$) between the sizes of the parapineal organs of the two age groups. It is thus evident that the organ grows at least until the rainbow trout is sexually mature. Since no sign of cellular degeneration has been observed in any individual, the organ probably persists throughout life.

The walls of the parapineal organ are externally limited by a very thin connective tissue capsule. The parenchyma of the organ contains two well discernible cell types. One of these is characterized by an irregularly formed, mostly elongated, hyperchromatic nucleus, the other by a larger, round to ovoid nucleus.

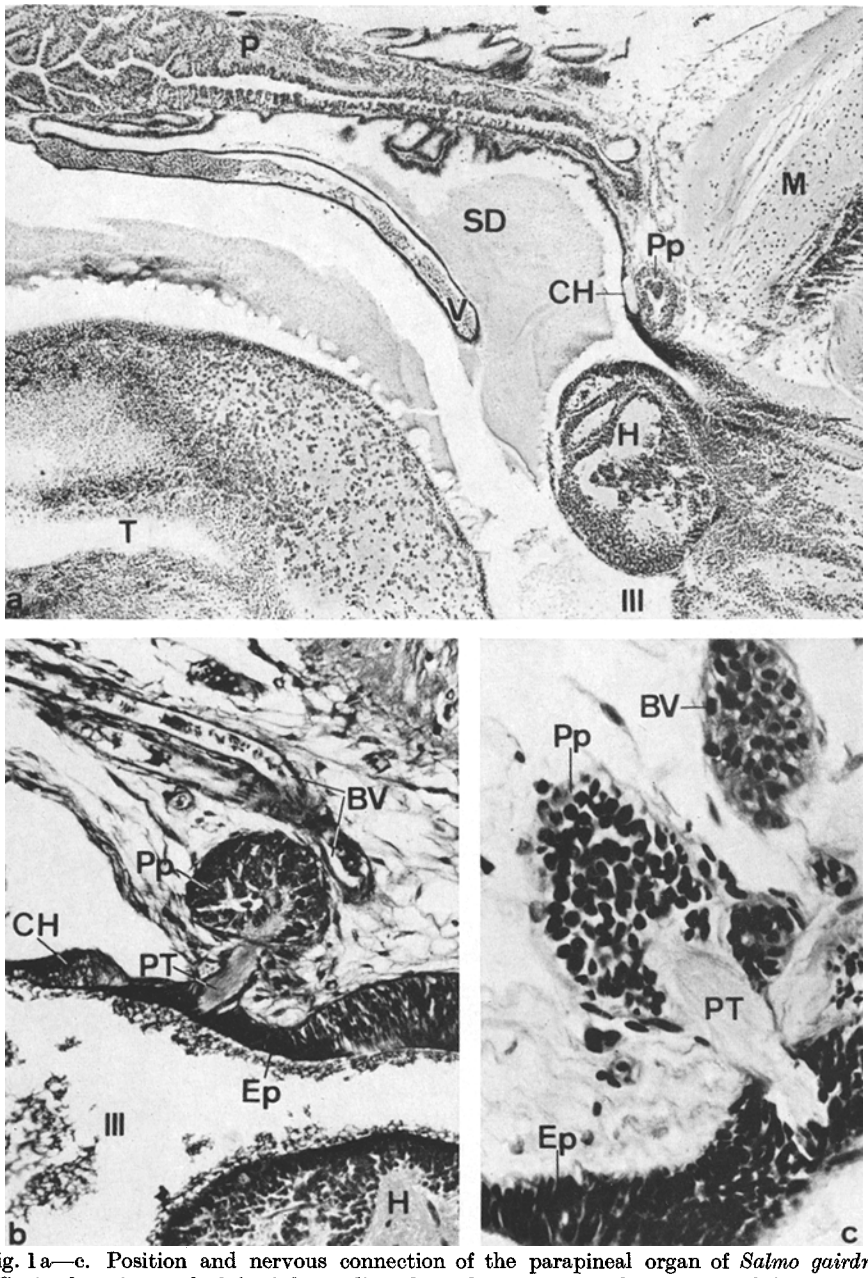

Fig. 1a—c. Position and nervous connection of the parapineal organ of *Salmo gairdneri*. a Sagittal section to the left of the median plane, demonstrating the position of the parapineal organ, *Pp*. The pineal stalk is not seen in this section; it runs in the median plane and joins the roof of the third ventricle closely to the right of the parapineal organ. *CH commissura habenularum; H* left habenular ganglion; *M* mesencephalon (*tectum opticum*); *P* pineal organ (*epiphysis cerebri*); *SD saccus dorsalis; T* telencephalon; *V velum transversum; III* third ventricle. Bodian's fixative. Bodian-Ziesmer. 10 μ. × 58. b Sagittal section through the parapineal tract, *PT*. The tract enters the ependymal roof, *Ep*, of the third ventricle; more laterally it will run together with the habenular commissure into the left habenular nucleus. *BV* blood vessels of the parapineal organ. Bouin. Klüver-Barrera. 7 μ. × 147. c Sagittal section through the parapineal tract. The tract is clearly seen to leave the parapineal organ and to enter the ependymal wall. Bouin. Jerusalem. 8 μ. × 365

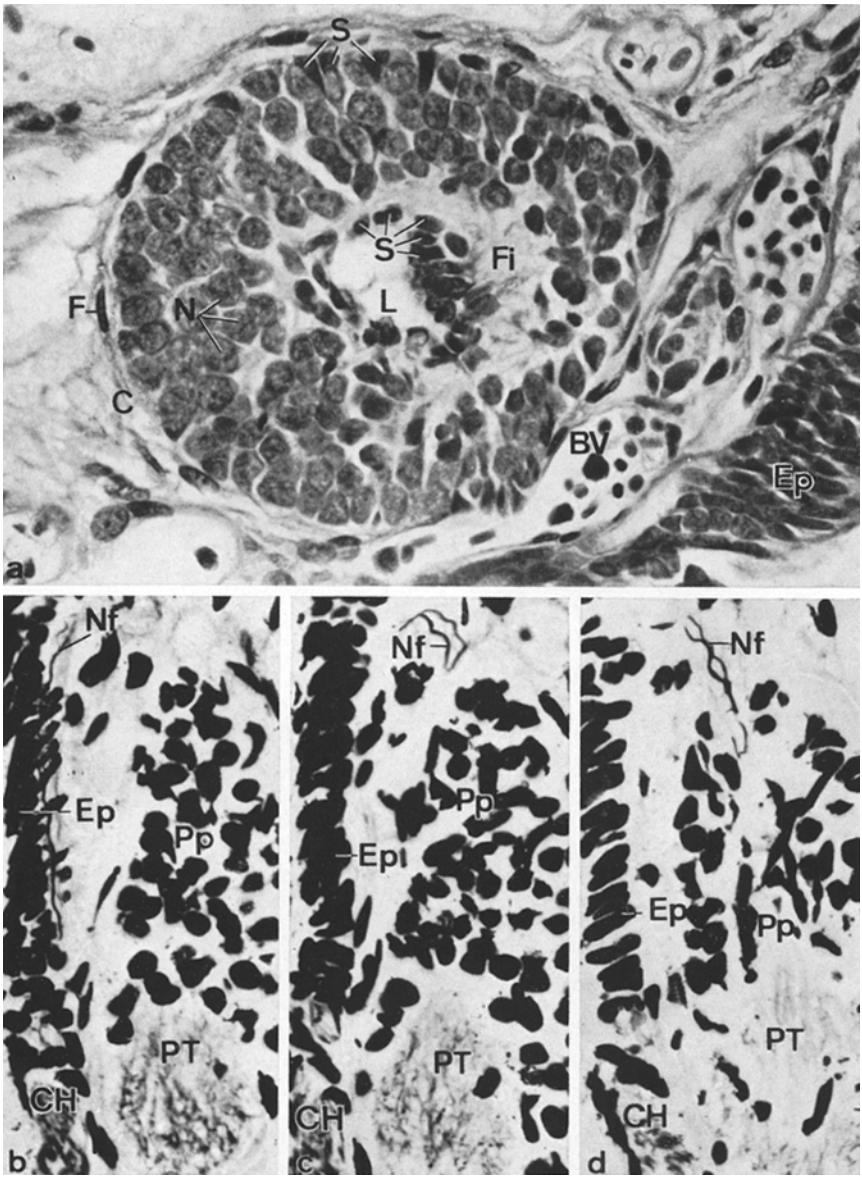

Fig. 2a—d. *Salmo gairdneri.* a Structure of the parapineal organ. Transverse section. *BV* blood vessel; *C* connective tissue capsule; *Ep* ependymal roof of the third ventricle; *F* fibroblast; *Fi* fibrous zone; *L* lumen; *N* nerve cell; *S* supporting cell. Bouin. Gomori-Bargmann. 7 μ. × 585. b—d Horizontally sectioned sequence near the ventral limit of the parapineal organ, *Pp*, demonstrating the entrance of a few thick nerve fibres, *Nf*, into the caudal part of the parapineal organ. Note that the parapineal tract, *PT*, is leaving the parapineal organ in its rostral part, close to the indicated part, *CH*, of the habenular commissure. *Ep* ependymal roof of the third ventricle. Bouin. Bodian-Ziesmer. 7 μ. × 585

Typical for the cells of the parapineal organ is a pronounced scarcity of cytoplasm, giving the impression that the tissue consists almost entirely of nuclei. The hyperchromatic nuclei are found close to the limiting connective tissue and around the

Table 2. *Dimensions of the parapineal organ in the rainbow trout*

Animal number	Body length (cm)	Parapineal dimensions			Length × height × width
		length (μ)	height (μ)	width (μ)	
	Group 1. 1-year-old fishes				
1	10—15	90	100	90	810,000
2	10—15	80	90	85	612,000
3	10—15	90	105	80	756,000
4	10—15	115	130	100	1,495,000
5	10—15	85	100	80	680,000
6	10—15	85	90	90	688,500
7	10—15	90	135	80	972,000
8	10—15	85	120	80	816,000
9	15—20	115	135	90	1,397,250
10	15—20	100	150	125	1,875,000
11	10—15	105	90	90	850,500
12	10—15	105	90	90	850,500
13	10—15	170	105	90	1,606,500
14	10—15	120	90	85	918,000
15	10—15	120	120	100	1,440,000
16	10—15	90	120	65	702,000
17	10—15	140	100	85	1,190,000
	Arithmetic mean and standard error				1,038,800 ± 384,100
	Group 2. 2-year-old fishes				
18	25—30	170	135	160	3,672,000
19	27—33	130	140	100	1,820,000
20	27—33	110	155	80	1,364,000
21	27—33	200	180	110	3,960,000
22	27—33	140	100	120	1,680,000
23	27—33	80	300	110	2,640,000
24	27—33	130	110	120	1,716,000
25	27—33	160	140	140	3,136,000
26	27—33	160	260	90	3,744,000
27	27—33	130	115	160	2,392,000
28	27—33	85	110	140	1,309,000
	Arithmetic mean and standard error				2,493,900 ± 988,650[a]

[a] Statistical evaluation according to the "Student's test". The difference between the mean values of the two groups is highly significant ($p < 0.001$).

small central lumen. The remaining, much larger, space is filled by the other cell type, except for a zone that is mostly occupied by fibres belonging to the parapineal tract (Fig. 2a, *Fi*).

The parapineal organ has a conspicuous connection with the habenular commissure, shortly before the entrance of this commissure into the left habenular nucleus. The fine, apparently unmyelinated fibres of this parapineal tract which is unexpectedly thick in relation to the size of the organ (Fig. 1b, c), run with the habenular commissure into the left habenular nucleus, where it is impossible to trace them further. The parapineal tract may join the *fasciculus retroflexus* of

Meynert but this could not be unequivocally confirmed, nor could the possibility be excluded that the tract divides and reaches different destinations. In no case was the parapineal organ found on the right side of the pineal stalk and consequently no connection of the parapineal tract with the right habenular nucleus was observed.

In order to classify the two cell types of the parapineal organ, a comparison was made with the pineal organ. The pineal parenchyma of the rainbow trout consists of receptor cells and supporting cells (BREUCKER and HORSTMANN, 1965). There is also a third cell type, the ganglion cells. However, these are very difficult to differentiate from the receptor cells, due to the absence of Nissl bodies (OKSCHE according to MORITA, 1966; OKSCHE, personal communication).

Like one (the first type) of the parapineal cell types, the supporting cells of the pineal organ have hyperchromatic and irregularly formed, often elongated nuclei and thin, drawn-out cell bodies. Also the typical arrangement of this cell type in the parapineal organ, namely that one end of the cell rests on the connective tissue capsule and the other penetrates between the cells of the second type, suggest that this cell type should be classified as a parapineal supporting cell (*cf.* BREUCKER and HORSTMANN, 1965).

The receptor cells of the pineal organ have a relatively large cytoplasmic volume, partly due to the cytoplasmic extension that forms the inner segment. The small amount of cytoplasm in the second cell type of the parapineal organ makes it unlikely that it represents a receptor cell type. A further characteristic of the receptor cells of the pineal organ is the projection of the outer segment into the lumen of the organ (*cf.* BREUCKER and HORSTMANN, 1965). In the parapineal organ, however, the minute lumen is lined mainly by supporting cells (Fig. 2a) and only rarely are other cells observed close to the lumen. Structures projecting into the lumen of the parapineal organ are very rarely seen.

The large diameter of the parapineal tract is an indication that this tract is principally afferent, because it is difficult to imagine a cell mass of the smallness of the parapineal organ receiving an efferent tract of such a dimension, without being able to pass the information on to other tracts or known to have an important function. At most, the parapineal tract may in part have efferent functions but this would not change the fact that the cells to all of which or to part of which the axons of the parapineal tract belong have to lie in the parapineal organ. In short, the bulk of the parapineal cells are presumably nerve cells, sending their axons into the parapineal tract. It has not been possible to verify this conclusion with any of the special light microscopical methods used (silver impregnation, staining with gallocyanin, toluidine blue, fast blue MBS, cresyl violet). However, this is merely a reflection of the fact that the nerve cells in question are lacking discrete Nissl bodies and that their axons are unmyelinated and very thin.

In one silver impregnated specimen it was possible to observe a few fibres entering that end of the parapineal organ which is opposite to the parapineal tract (Fig. 2b, c, d). The origin of these fibres which may be efferent is not known.

When studying the silver impregnated series, it was found that the pineal tract of *Salmo gairdneri* always has a ventral, smaller component (Fig. 3), a condition

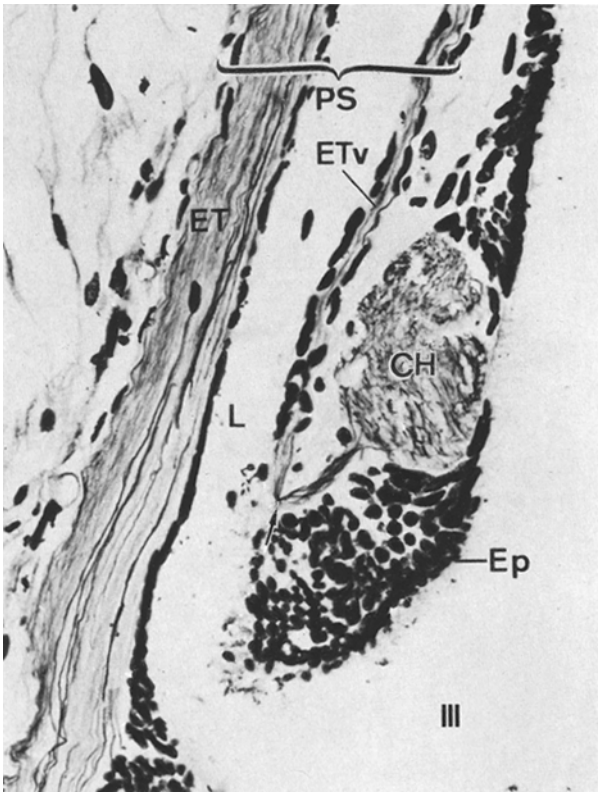

Fig. 3. Median section through the proximal part of the pineal stalk, *PS*, of *Salmo gairdneri*. The epiphyseal tract (= pineal tract) consists of a main dorsal part, *ET*, and a smaller ventral part, *ETv*, which at ↑ is seen to make an abrupt turn to enter the habenular commissure, *CH*. *Ep* ependymal roof of the third ventricle, *III*; *L* lumen, connecting the larger lumen of the pineal vesicle with the third ventricle. Bouin. Bodian-Ziesmer. 7 μ. × 365

which is not typical for teleosts (*cf.* N. Holmgren, 1917, 1918; U. Holmgren, 1959; Rüdeberg, 1966, 1968; Hafeez and Ford, 1967; Oksche and Kirschstein, 1967). On one occasion some of these ventral fibres could be very clearly seen to enter the habenular commissure (Fig. 3). In the other silver impregnated specimens this condition could not be verified with certainty. The connection may correspond to that described by N. Holmgren (1917, in *Osmerus eperlanus* L.) which was considered a variation representing an original, primitive condition.

No trace of glycogen was found in the parapineal organ, nor was it possible to detect (neuro)secretory material.

In the pike, *Esox lucius* L., where the parapineal organ has been observed in three silver impregnated (Bodian-Ziesmer) specimens (brains from adult individuals, each weighing about 2 kg), the presence of two parapineal cell types and a parapineal tract was confirmed. The existence of a lumen, however, could not be verified, since silver impregnated specimens are not ideal for general morphological studies. The dimensions of the organ vary between 0.1 and 0.2 mm diameter.

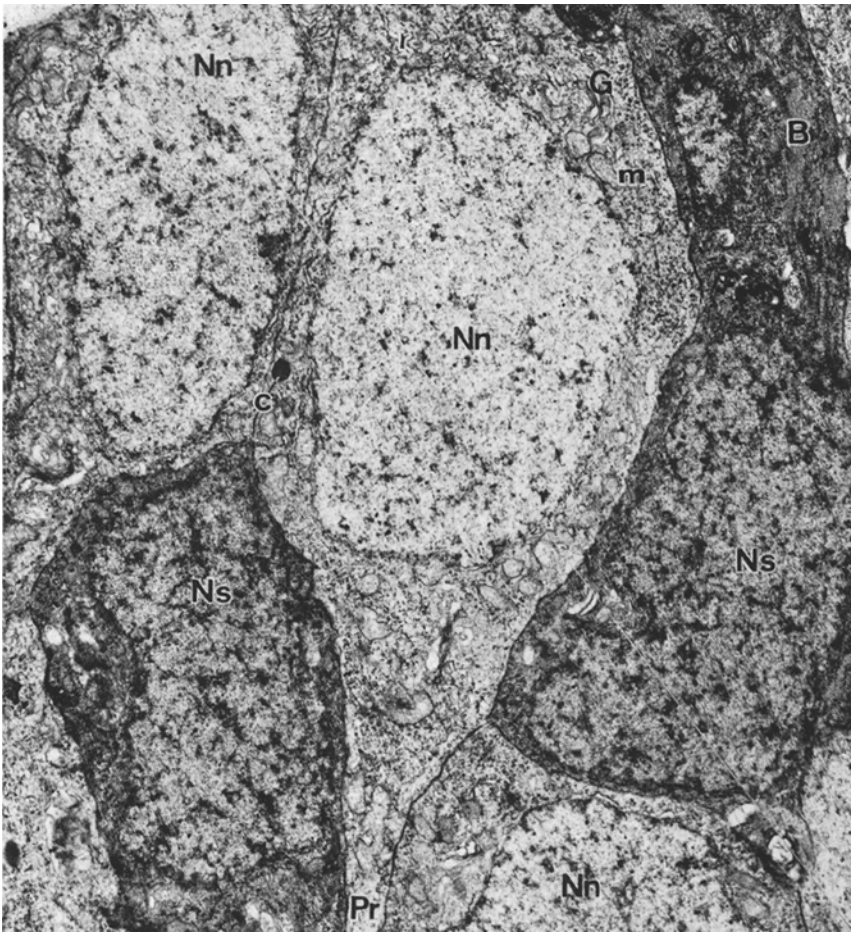

Fig. 4. Parapineal nerve cells and supporting cells of *Salmo gairdneri*. Note that the electron density of the supporting cells is greater than that of the nerve cells. *B* bundle of filaments typically present in the supporting cell cytoplasm; *c* cytosome; *G* Golgi complex; *m* mitochondrion; *Nn* nerve cell nucleus; *Ns* supporting cell nucleus; *Pr* nerve cell prolongation, probably the initial segment of an axon; *r* rough endoplasmic reticulum. Fixative 3 (glutaraldehyde). × 9,600

Electron Microscopy. Electron microscopic investigation of seven parapineal organs has confirmed the light microscopic classification of the parapineal cell types. Further it has been proven that a very small number of receptor cells of the type found in the pineal organ also occur in the parapineal organ.

The supporting cells are characterized by a variably formed nucleus, relatively electron-dense cytoplasm, and the development of conspicuous filamentous bundles which sometimes seem nearly to fill the cytoplasm of the cell (Figs. 4, 8a). The nucleus has a high electron density and a distinct chromatin network. The cytoplasm is scarce and poorly provided with organelles, with the exception of the already mentioned filaments. The few mitochondria are of an elongated form

Fig. 5a—c. Ultrastructure of the parapineal nerve cell in *Salmo gairdneri*. a Longitudinal section, showing the body and axonal process, *ax*, of a nerve cell. *B* supporting cell filament bundle; *C* connective tissue capsule with collagen fibres; *c* cytosome; *G* Golgi complex; *m* mitochondrion; *Nn* nerve cell nucleus; *Ns* supporting cell nucleus; *r* rough endoplasmic reticulum. Fixative 2 (OsO_4). × 6,000. b and c Details of nerve cell cytoplasmic organelles. Abbreviations as above. Fixative 2 (OsO_4). × 24,000

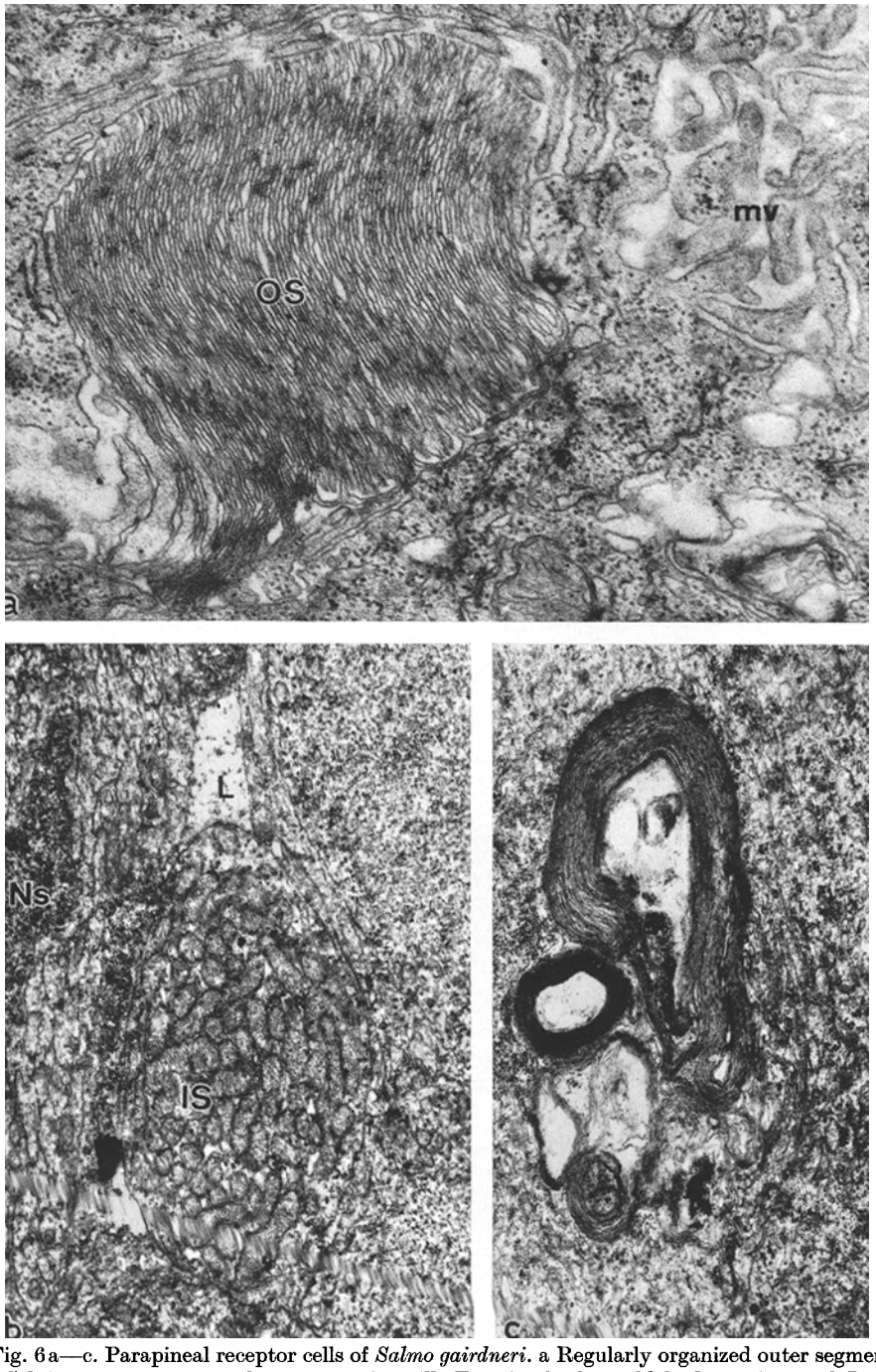

Fig. 6a—c. Parapineal receptor cells of *Salmo gairdneri*. a Regularly organized outer segment, *OS*, lying in a very narrow lumen. *mv* microvilli. Fixative 3 (glutaraldehyde). × 38,400. b Inner segment, *IS*, with a remarkable concentration of mitochondria. *L* lumen; *Ns* supporting cell nucleus. Fixative 1 (OsO_4). × 12,000. c Irregular membrane whorls, probably a degenerating outer segment. Fixative 1 (OsO_4). × 17,500

and have tubular cristae. Sparsely distributed cisternae, belonging to the rough endoplasmic reticulum, may be observed. The filaments have a diameter of 100 Å.

The Golgi complex of the supporting cells is typically formed and does not demonstrate any special activity. Cytosomes occur rarely and elements of the smooth endoplasmic reticulum have not been observed. The supporting cells send long, thin cytoplasmic extensions between nerve cells. Bundles of filaments constitute a prominent part in these prolongations (Figs. 5a, 7a, 8a). Those supporting cells which border the parapineal lumen are generally more rounded than the peripheral ones and have less filaments. They are provided with microvilli, penetrating into the lumen (Fig. 8b).

The lumen has the form of a narrow slit or canal that winds through the central part of the parapineal organ (Fig. 8a). Its relative prominence in light microscopic preparations is the result of tissue shrinkage. No part of the lumen seems to be without microvilli and on occasion some cilia have been observed. These cilia may emerge either from supporting cells or from nerve cells, possibly from both. The outer segments of receptor cells likewise project into the lumen.

The nerve cell nucleus has a rounded to ovoid form, is less electron-dense than that of the supporting cell, and has a more finely dispersed chromatin (Figs. 4, 5a). The cytoplasm of the nerve cell is scarce. There are no filaments in the perinuclear cytoplasm, in the axon hillock, or in the proximal part of the axon.

The Golgi complex is distinct but, like that of the supporting cell, does not seem to be exceptionally active. No secretory products can be observed in the cisternae or the vesicles of the Golgi complex (Fig. 5b, c). The rough endoplasmic reticulum is sparsely but evenly distributed within the cell. The mitochondria are elongate and have tubular cristae. Free ribosomes form small groups throughout the cytoplasm. Cytosomes exist but are not numerous. These probably lysosomic organelles show a finely granulated, electron-dense content, limited by a single membrane. Their maximal size is about 0.7 μ (Fig. 5a, b).

Microtubules, 200 Å in diameter, are observed in the axon hillock, and very rarely in the perinuclear cytoplasm.

Dendrites have not been observed; the nerve cells are apparently unipolar. No synapses are seen along the axon or perikaryon.

Receptor cells are rarely present in the parapineal organ and have been observed only on three occasions. The first of the outer segments recorded is relatively well developed (Fig. 6a) with flattened saccules arranged like those of a retinal cone (Nilsson, 1964). The saccules have an average thickness of 200—250 Å, membranes included. The second outer segment observed is smaller and organized like the afore-mentioned one. The third is only a whorl of membranes (Fig. 6c) as can be seen also in the pineal organ of fishes (*cf* Breucker and Horstmann, 1965). One inner segment was also localized. This is characterized by a dense cluster of mitochondria (Fig. 6b). The cell body of the receptor cell has not been found.

Centrally in the parapineal organ, ventral to the lumen and partly around it (*cf* Fig. 2a), a neuropil-like zone is found. Although some probably synaptic vesicles (diameter 400—600 Å) are observed, no typical synapses have been seen. Most of the existing fibres which are all unmyelinated, are the axons of

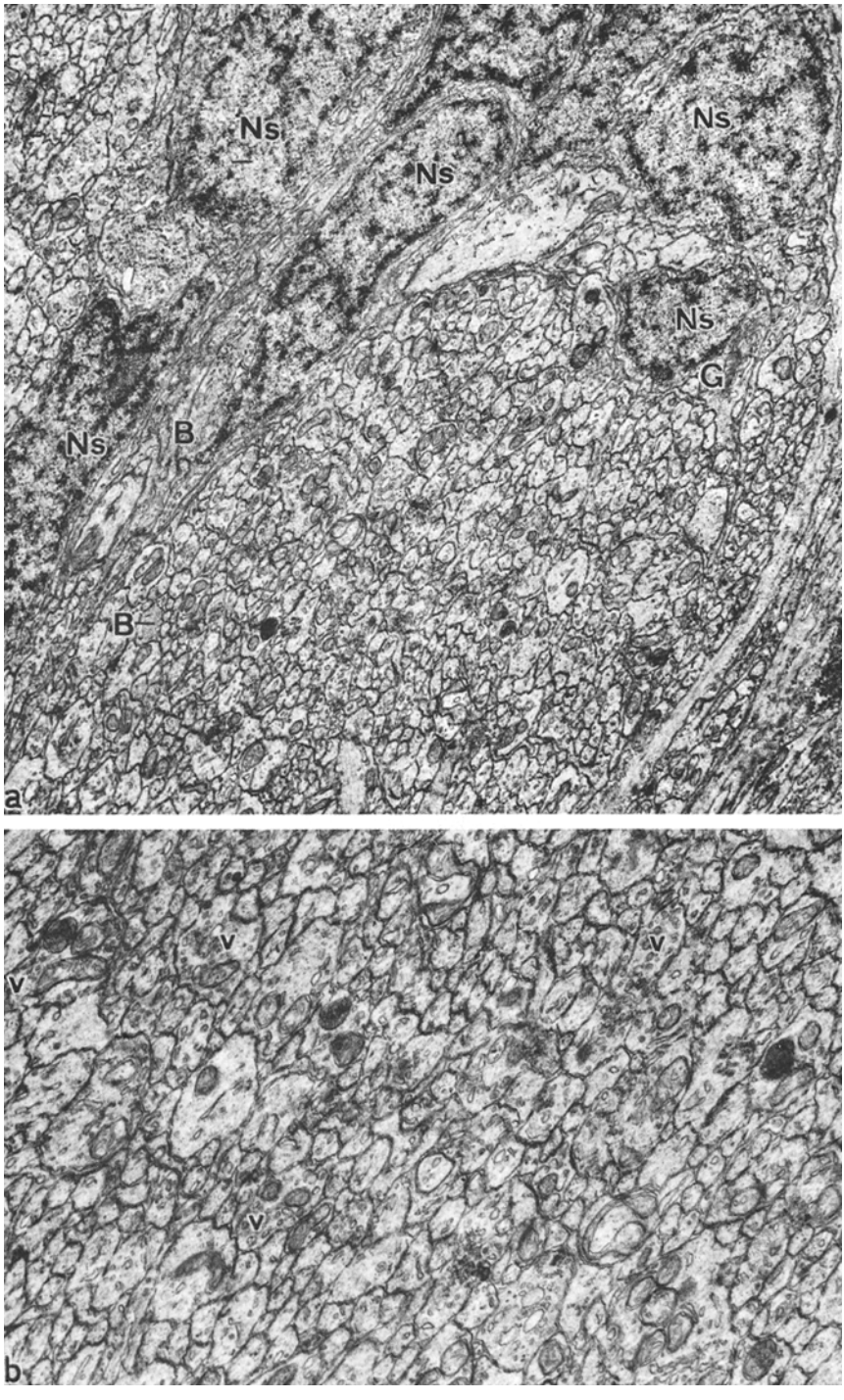

Fig. 7a and b

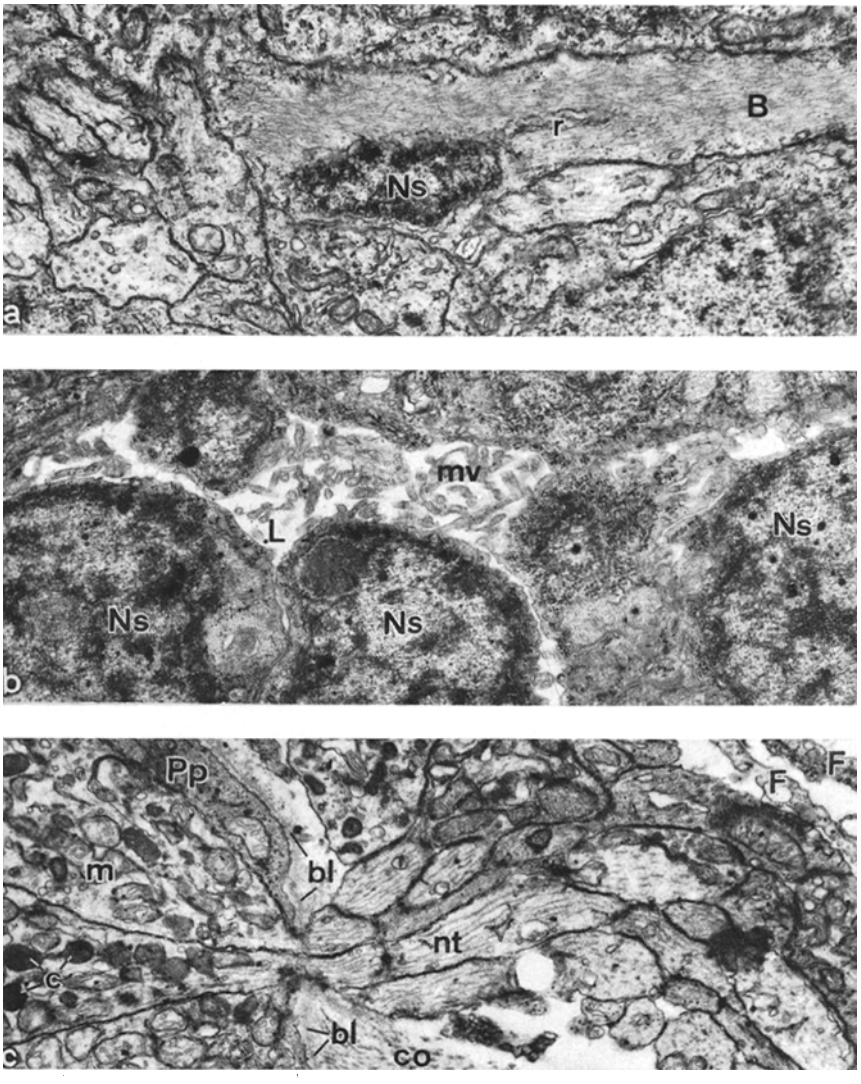

Fig. 8a—c. *Salmo gairdneri.* a Detail of a parapineal supporting cell. Note the thick filament bundle, *B*, with some cisternae of rough endoplasmic reticulum, *r*. *Ns* supporting cell nucleus. Fixative 2 (OsO_4). × 18,000. b Parapineal lumen, *L*, surrounded by supporting cells. *mv* microvilli. Fixative 3 (glutaraldehyde). × 14,400. c Very small bundle of nerve fibres entering the parapineal organ, *Pp*. The fibres, containing distinct neurotubules, *nt*, break through the basal lamina, *bl*, and widen noticeably after their entrance. The wider part contains many mitochondria, *m*, and cytosomes, *c*. The further course of these fibres is not known. *co* collagen fibres; *F* fibroblast. Fixative 3 (glutaraldehyde). × 16,800

Fig. 7a and b. Neuropil-like zone in the parapineal organ of *Salmo gairdneri.* a Proximal part of the parapineal tract; supporting cell elements form the boundary of the parapineal parenchyma. No typical synapses are present. The supporting cells give rise to thin projections characterized by filament bundles, *B*. *G* Golgi complex of a supporting cell; *Ns* supporting cell nucleus. Fixative 2 (OsO_4). × 9,600. b Detail of the zone shown in a. No typical synapses are seen but some vesicles, *v*, possibly of synaptic type, are observed. Fixative 2 (OsO_4), × 19,200

the parapineal tract but probably also some smaller nerves, coming from the outside and breaking through the connective tissue capsule (Figs. 8c, 9), are also found here. The destinations of the latter nerves have not been detected. A few single myelinated fibres have also been observed. These correspond probably to the relatively thick fibres seen in the light microscope (Fig. 2b, c, d).

The fine axons constituting the parapineal tract contain neurotubules and small mitochondria and also a few cytosomes. The smallest nerve fibres have a diameter of only about 0.2 μ. The presence of filaments if doubtful. It is supposed that bundles of filaments seen in the initial part of the parapineal tract belong exclusively to supporting cell offshoots (Fig. 7a, b).

The outer limit of the parapineal organ is always well marked by a basal lamina. Fibroblasts and collagen fibres constitute the thin connective tissue capsule (Fig. 8c). On one side of the organ, a few small blood vessels establish close contact with the capsule for a short distance. These vessels lack in specializations (Fig. 9).

Remarks on the Fixation Methods Used for Electron Microscopy. There are different views in the literature regarding the preparation of fixatives for electron microscopy. When using osmium tetroxide alone, the tonicity of the fixative is not very important but the composition of the buffer plays a great role (ERICSSON *et al.*, 1965; WOOD and LUFT, 1965). In accordance herewith it was found in the present study that the buffer of fixative 1 (*cf. Material and Methods*) gave a less satisfying result than that of fixative 2. The tissue was well fixed and without apparent artifacts in both cases but while the tissue treated with fixative 2 permitted brilliant micrographs with finely detailed structural features (Figs. 5a, b, c, 7a, b), the tissue fixed in fixative 1 gave micrographs lacking in clearness and detail (Fig. 6b, c).

For glutaraldehyde fixatives the situation is very confusing. MAUNSBACH (1966) recommends that the buffer be made isotonic or a little hypertonic to the tissue. SPRUMONT (1967) points out the importance of taking into consideration also the tonicity of the glutaraldehyde itself. The two authors worked on different animal groups and different tissues (mammalian kidney and anuran lung, respectively). To test both recommendations fixatives Nos. 3 and 4 were composed.

The results were ambiguous. While the solution of high tonicity (No. 3) gave a perfect fixation of the parapineal organ and a very bad one of the pineal organ, the result with the low-tonicity fixative (No. 4) was exactly the opposite. Since both organs are limited by a thin connective tissue capsule and their walls do not exceed some 10—20 cells in thickness, it was concluded that there must exist a clear-cut difference in the reaction of different tissues towards glutaraldehyde fixatives. The question has to be further investigated.

Discussion

The teleostean parapineal organ, described in this study in detail for the first time, presents a series of enigmatical traits. Although two of its cell types are almost identical to those of the pineal organ (supporting cells and receptor cells), the scarcity of receptor cells, the peculiar principal cell type, the different innervation, and the absence of a connection with the third ventricle make it impossible to regard the parapineal organ as a miniature pineal organ.

Considering the close ontogenetic relationship of the two organs (HILL, 1894; FRIEDRICH-FREKSA, 1932) it can safely be concluded that their cells have a common origin as descendants from the ependymal cells of the roof of the third ventricle. The receptor cells, as well as the supporting cells, thus have the same

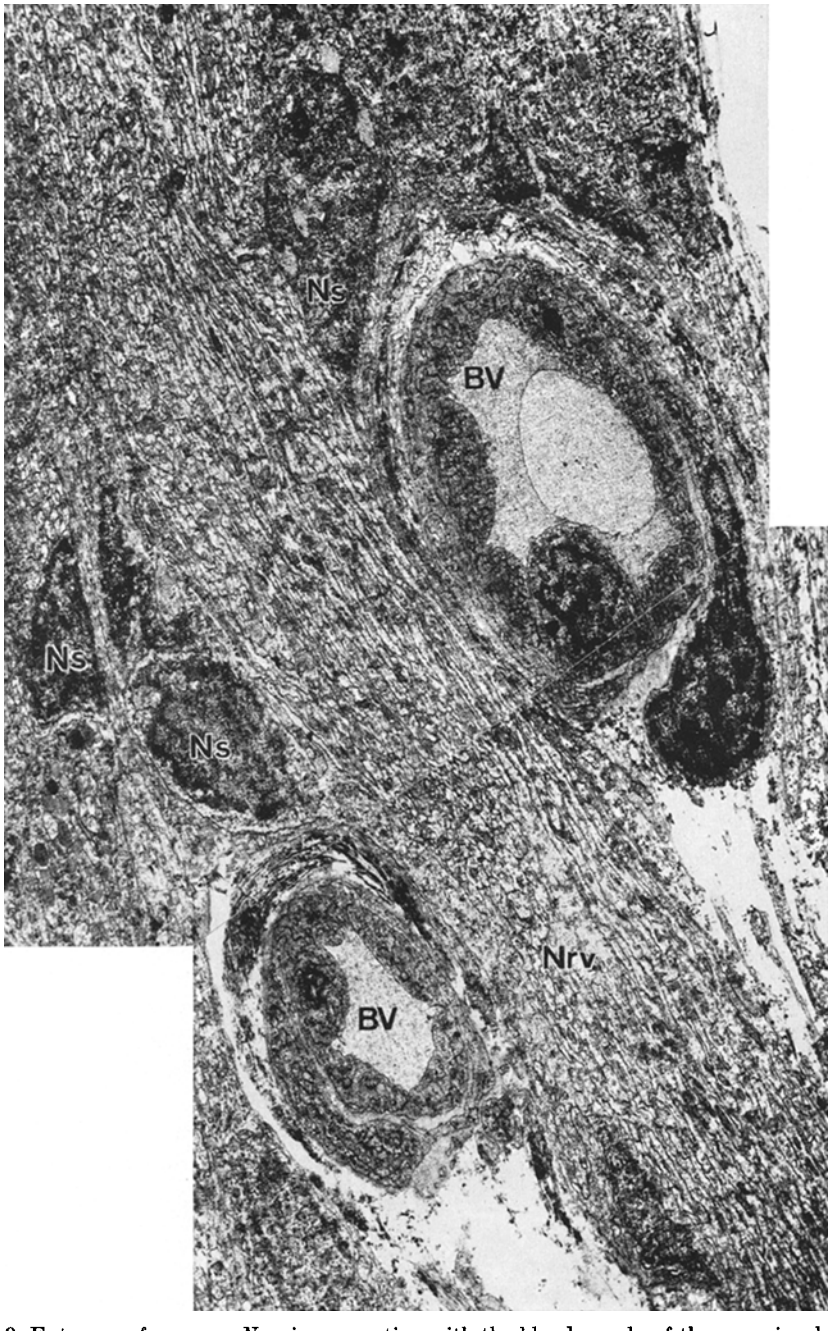

Fig. 9. Entrance of a nerve, *Nrv*, in connection with the blood supply of the parapineal organ of *Salmo gairdneri*. This relatively thick nerve, containing only unmyelinated fibres, merges into the fibrous zone (Fig. 2a) of the parapineal organ. *BV* blood vessel; *Ns* supporting cell nucleus. Fixative 1 (OsO_4). × 6,000

origin, although in a different position, as have rods, cones and pigment epithelial cells of the retina (*cf.* ARIËNS KAPPERS, 1965).

Can the ependymal cells become transformed into nerve cells? The step from some forms of pineal receptors, as described, *e.g.*, by OKSCHE and VAUPEL- VON HARNACK (1965) and RÜDEBERG (1968), to the neuron probably functioning as a chemoreceptor in the subfornical organ of the dog (ANDRES, 1965) or to the neuron with possible receptive character in the ependyma of the *regio hypothalamica* of the rabbit (LEONHARDT, 1968), is not very wide. The pineal receptors already display a pronounced polarity, transmit information to neurons (see. *e.g.* MORITA, 1966) via elongated axon-like projections, and contain tubules in their cytoplasm. If neurons can serve as receptors, the difference is really very subtle and the question posed above may be answered positively.

The numerous nerve cells of the parapineal organ can be explained in at least two ways. Either they represent the result of transformation of the majority of former receptor cells, or the concentration of the kind of nerve cells which is also to be found in the pineal organ and which originally may have migrated to their final position. In either way some transformation must be assumed. If the parapineal nerve cells represent former receptor cells, these must be thought to have undergone a complete reduction of inner as well as outer segments. Further, their formerly relatively short cell processes must have grown very much in length in order to form the parapineal tract, and their large filament bundles (own unpublished observations) must have disappeared. If, on the other hand, the parapineal nerve cells may be considered homologous with the nerve cells of the pineal organ, they must be supposed to have lost their dendrites. Incidentally, this seems to be the case also with the afore-mentioned type of neuron described by LEONHARDT.

Against the first theory speak the findings of COLLIN (1966, 1967), OKSCHE and VAUPEL-VON HARNACK (1966), and OKSCHE and KIRSCHSTEIN (1966, 1968), who found that in the former photoreceptors of the pineal organs of lizards and birds the inner segment is retained also when the outer segment has become completely reduced. In these animal groups, however, a transformation from a sensory to a secretory function supposedly takes place. If one can assume a transformation from sensory function to simple conduction, the theory may still be valid. However, it may be more likely that nerve cells have lost their dendrites when the near-absence of receptor cells no longer required the use of their former connections with the central nervous system. With the information available it is not yet possible to exclude either one or both of the proposed alternatives.

Taking into consideration the fact that the parapineal organ normally is degenerated in the adult fish (FRIEDRICH-FREKSA, 1932; U. HOLMGREN, 1965), one may ask if the organ has any function at all in cases where it is still retained. I believe that the organ functions at least during ontogeny and that the species now investigated represents a borderline case, as does also *Esox lucius*. The original function probably is maintained as long as the organ remains, but since the function may be of little importance for the adult animals, the organ has regressed in more specialized species. It is also possible that the function of the

parapineal organ is taken over during ontogeny by the cells of, *e.g.*, the habenular nuclei. Also in this way the prerequisite condition for the complete reduction of the parapineal would be created.

Assuming, consequently, that the parapineal organ performs some function of unknown character, the information necessary for the normal activity of the organ must be supposed to reach every cell. However, the lumen is not connected to the third ventricle, neither are most of the cells in connection with the small lumen. There is an indirect connection with the vascular system on one side of the organ. Only some small nerves reach the organ from other directions than the parapineal tract, and no fibres have been observed to penetrate the tissue and to establish synaptic connection with the nerve cell bodies. If the small nerves mentioned are efferent and, also, if the parapineal tract contains some efferent fibres, it must be supposed that the nervous interaction is taking place in the neuropil-like zone, where synaptic vesicles but not typical synapses have been observed.

The exact function of the parapineal organ of teleosts may never be made clear. The organ is so small that it can not be isolated under the dissecting microscope even in fixed tissue, let alone be extirpated or explored with a microelectrode in living animals. There is no morphological evidence linking the organ to a well-defined function, nor are there any known light or electron microscopically discernible products that may be experimentally influenced.

As can be concluded from the introduction, there has been disagreement regarding the original position of the parapineal organ. Whereas HILL (1894) believed in a symmetrical paired position of the pineal and parapineal organs, FRIEDRICH-FREKSA (1932) observed an original medial situation of both organs. TERRY (1910) and U. HOLMGREN (1965) merely reported that the position was asymmetrical; according to FRIEDRICH-FREKSA this position was the result of a secondary displacement. But while the three authors found the parapineal organ to the left of the midline, U. HOLMGREN alone observed it to the right. Since the only support for the statement of HOLMGREN are two aberrant individuals of *Coregonus* observed by FRIEDRICH-FREKSA, it can be supposed either that HOLMGREN referred to such aberrant individuals or that the teleost *Salvinellus fontinalis* L. differs from all other known species. However, U. HOLMGREN has also studied *Salmo salar* which, according to HILL (1894), normally has a left-sided parapineal organ. Therefore, the possibility that the statement of U. HOLMGREN is erroneous, can not be excluded.

I believe the observation of FRIEDRICH-FREKSA (1932) regarding the median origin of the parapineal organ, to be quite correct. Aberrant individuals can thus be explained simply as resulting from an erroneous migration of the parapineal organ when the rapid growth of the pineal organ forces it to move sideways. It is, further, probable that the parapineal organ develops from the same *Anlage* as the pineal organ, as was proposed by HILL (1894), and as may be concluded from the drawings of FRIEDRICH-FREKSA (1932). This opinion finds further support in the observations of EYCLESHYMER and DAVIS (1897) who found the parapineal organ of the holostean *Amia calva* to originate medially from the anterior wall of the pineal evagination. RUNNSTRÖM (1925) found the parapineal and pineal organs of *Lampetra* (Petromyzontidae) also to have a common medial origin.

The present discussion gives support to the theory of ARIËNS KAPPERS (1965, 1967), according to which the parapineal organ of the petromyzontids, the parapineal organ of the teleosts, the frontal organ of the anurans, and the parietal eye of the lizards are all to be considered homologous. The most important common characteristic of these structures is the fact that they all originate from the *anterior end* of the pineal *Anlage*.

The fact that these organs are generally connected to the left habenular ganglion (see ARIËNS KAPPERS, 1965, 1967, for very exhaustive accounts[2]) may also be of importance, but this would exclude the frontal organ of the anurans from this homologous series. However, the nervous connections are secondary established characteristics and have probably changed in the course of phylogeny. Therefore, the condition mentioned should not be regarded as a reason for not considering the frontal organ as a homologue of the parietal eye of lizards and the parapineal organ of both teleosts and petromyzontids.

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