

# AUTONOMIC NERVES TERMINATING ON MICROVESSELS IN THE PINEAL ORGANS OF VARIOUS SUBMAMMALIAN VERTEBRATES

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In earlier works we have found that in the mammalian pineal organ, a part of autonomic nerves – generally thought to mediate light information from the retina – form vasomotor endings on smooth muscle cells of vessels. We supposed that they serve the vascular support for circadian and circannual periodic changes in the metabolic activity of the pineal tissue. In the present work, we investigated whether peripheral nerves present in the photoreceptive pineal organs of submammals form similar terminals on microvessels.

In the cyclostome, fish, amphibian, reptile and bird species investigated, autonomic nerves accompany vessels entering the arachnoidal capsule and interfollicular meningeal septa of the pineal organ. The autonomic nerves do not enter the pineal tissue proper but remain in the perivascular meningeal septa isolated by basal lamina. They are composed of unmyelinated and myelinated fibers and form terminals around arterioles, veins and capillaries. The terminals contain synaptic and granular vesicles. Comparing various vertebrates, more perivascular terminals were found in reptiles and birds than in the cyclostome, fish and amphibian pineal organs.

Earlier, autonomic nerves of the pineal organs were predominantly investigated in connection with the innervation of pineal tissue. The perivascular terminals found in various submammals show that a part of the pineal autonomic fibers are vasomotoric in nature, but the vasosensory function of some fibers cannot be excluded. We suppose that the vasomotor regulation of the pineal microvessels in the photosensory submammalian pineal – like in mammals – may serve the vascular support for circadian and circannual periodic changes in the metabolic activity of the pineal tissue. The higher number of perivascular terminals in reptiles and birds may correspond to the higher metabolic activity of the tissues in more differentiated species.

*Keywords:* Pineal organ – vasomotor nerves – fine structure – cyclostome – fish – amphibia – reptiles – birds

## INTRODUCTION

Autonomic nerves in the submammalian pineal organs were described in birds, in the snake and lizard, further in some frog as well as fish species [5, 6, 11, 12, 13, 16, 17, 24]. The significance of pineal autonomic fibers were primarily investigated in mammals, focusing on the innervation of the pineal tissue and assuming a mediator func-

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tion in light information from the retina [6, 13, 17, 23]. A similar role for pineal autonomic nerves were supposed in frogs [12]. In earlier works we found that some of the autonomic nerves in the mammalian pineal terminate on smooth muscle cells of pineal arterioles and veins, therefore they obviously represent vasomotor nerves. Since sympathetic nerves, in general, serve for transmitting signals of the vegetative nervous system, further, light information from the optic system may reach the organ via its stalk [8, 9], we supposed that autonomic pineal nerves predominantly serve the vascular support for circadian and circannual periodic changes in the metabolic activity of the pineal tissue [3, 4, 18, 19].

We also found nerve terminals ending on vessels in the pineal organ of birds, an observation that suggests that pineal autonomic nerves form vasomotor terminals in the light-sensitive pineal organ of some submammals as well [3, 20, 21, 22]. As there are no detailed fine-structural data about the relation of autonomic nerve terminals and vessels in the submammalian pineal, in the present work we investigated the pineal autonomic nerves in various submammalian species from the cyclostome to birds.

## MATERIALS AND METHODS

The pineal organ in both sexes of the following species – 3 to 5 from each – were investigated: *Petromyson fluviatilis*, *Raja clavata*, *Chimera monstrosa*, *Carassius auratus*, *Ctenopharyngodon idella*, *Triturus cristatus*, *Rana esculenta*, *R. catesbeiana*, *Lacerta muralis*, *L. agilis*, *Natrix natrix*, *Gallus domesticus*, *Buteo buteo*.

The animals were kept under normal laboratory conditions and anaesthetised with phenobarbital prior to fixation. The fixation buffer containing 1% glutaraldehyde were perfused through the aorta, some pineals were dissected and immersed in the fixative. The pineal organs were further fixed in 1% OsO<sub>4</sub> for 1.5 h. The material was dehydrated and embedded in Poly Bed (Polysciences, St. Goar, FRG). Semithin serial-sections and ultrathin sections were cut on a Reichert Ultracut S ultramicrotome. The semithin sections were stained by toluidine blue-azur II and the ultrathin sections with uranyl acetate and lead citrate and were examined and photographed in a Hitachi H7600 electron microscope.

## RESULTS

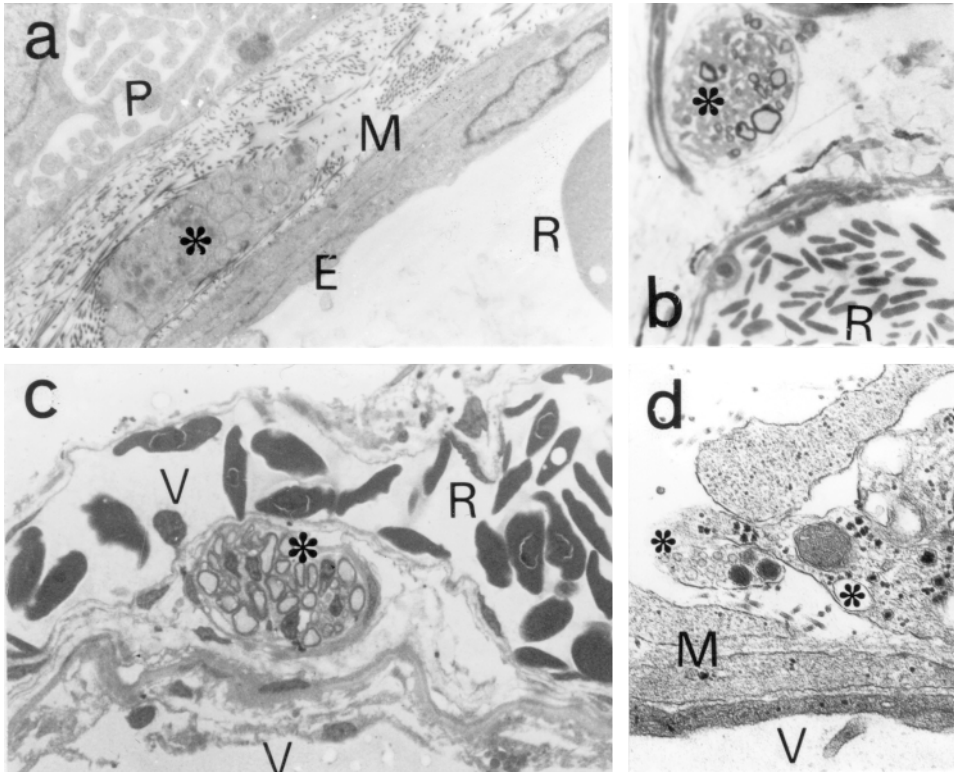
In all species investigated so far autonomic nerves reach the pineal organ alongside meningeal vessels. They contain unmyelinated and myelinated fibers encapsulated by Schwann-cells. Autonomic nerves accompany vessels entering the arachnoidal capsule and interfollicular meningeal septa. Being the continuation of the roof of the epithalamus, the pineal tissue proper is separated from the perivascular meningeal tissue by glial limiting membrane and basal lamina. The autonomic nerves form terminals containing synaptic and granular vesicles around arterioles, veins and capillaries.

Comparing various vertebrates, more perivascular terminals were found in reptiles and birds than in the cyclostome, fish and amphibian pineals.

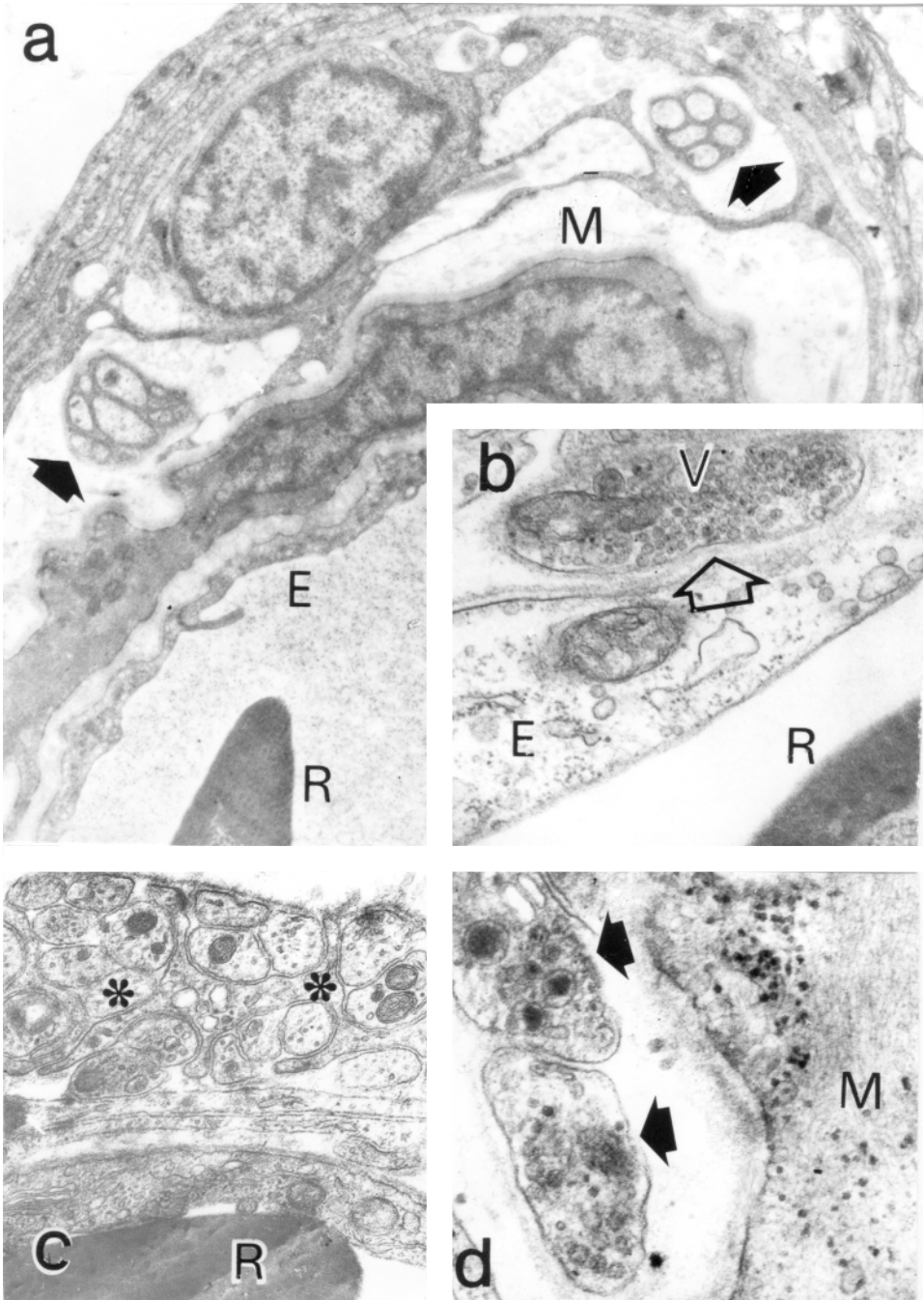
In the saccular pineal of the lamprey, perivascular autonomic nerves are rare (Fig. 1a). The wall of the vessels around the organ is thin and formed by endothelial cells and some pericytes and/or smooth muscle cells.

The pineal organ of fish and urodelan amphibia is a tube-like organ with a terminal enlargement. Small vessels lie around the pineal tube, among them peripheral nerves (Fig. 1b, c) containing 30–40 fibers and occasionally nerve terminals are found.

In anuran amphibians the pineal organ forms saccular evaginations and is better vascularised than the cyclostome, fish and newt pineals. Autonomic nerve terminals



*Fig. 1.* Autonomic nerves supplying of cyclostome, fish and amphibian pineal vessels. *a:* Unmyelinated fibers (asterisk) near an arteriole of the pineal organ of the lamprey *Lampetra fluviatilis*. E: endothelial cell, M: smooth muscle cells, P: pineal tissue, R: red blood cell,  $\times 11000$ . *b:* Autonomic nerve (asterisk) containing several unmyelinated and some myelinated fibers among pineal vessels of the fish *Ctenopharyngodon idella*. R: red blood cell,  $\times 300$ . *c:* Autonomic nerve (asterisk) among pineal vessels (V) of the newt (*Triturus cristatus*). R: red blood cell,  $\times 650$ . *d:* Nerve terminals (asterisks) containing granular and synaptic vesicles on smooth muscle cells (M) of a pineal arteriole (V) of the frog (*Rana esculenta*)



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Fig. 2. Perivascular autonomic nerves of the pineal organ of reptiles and birds. *a*: Autonomic nerves (arrows) in the wall of a pineal arteriole of the snake *Natrix natrix*. E: endothelial cells, M: smooth muscle cell, R: red blood cell,  $\times 1250$ . *b*: Nerve terminal (asterisk) on the wall of a pineal vein of the lizard (*Lacerta muralis*). E: endothelial cell, R: red blood cell, V: synaptic vesicles,  $\times 31000$ . *c*: Unmyelinated fibers (asterisks) and nerve terminals on a pineal vessel of *Gallus domesticus*. R: red blood cell,  $\times 11100$ . *d*: Nerve terminals (asterisks) on an arteriolar smooth muscle cell of a pineal arteriole of *Buteo buteo*, M: myofilaments of the smooth muscle cell,  $\times 5300$

close to the vascular smooth muscle cells contain synaptic and granular vesicles (Fig. 1d).

The pineal organ of reptiles is larger and more differentiated than that of frogs, its wall forms secondary evaginations. Pineal vessels are more numerous, they penetrate the meningeal septa separating the lobular evaginations of the organ. There are several autonomic fibers around larger arterioles running on both sides of the pineal (Fig. 2a). Nerve terminals were found not only on arterioles but also on veins and capillaries (Fig. 2b).

In birds investigated so far the pineal is relatively larger than in reptiles, it is a multilobulated organ. Numerous vessels run in the interlobular meningeal septa. The vessels are followed by large autonomic nerves (Fig. 2c), that form terminals containing granular and synaptic vesicles close to smooth muscle cells of vessels (Fig. 2d).

In all species studied similar perivascular nerves were found around meningeal vessels and arterioles of various brain areas. No essential fine-structural difference was found between nerve fibers innervating pineal vessels and those found around vessels supplying various brain areas or meninges.

## DISCUSSION

Several types of peripheral fibers were already identified in the pineal organ of submammals [11, 12, 13, 16, 17, 24]. Experimental works demonstrated the importance of sympathetic fibers in the melatonin secretion by mediating light information from the retina to the pineal organ in mammals that are thought not to be photosensitive [6, 13, 17, 23]. Also the vasomotoric nature of pineal autonomic fibers was supposed [5, 9, 10, 11, 14].

The unmyelinated autonomic fibers in the present work terminate on smooth muscle cells of pineal arterioles of various submammals. This finding supports the view that in vertebrates some of these fibers are vasomotoric in nature [4, 5, 14, 20, 21, 22]. The vasosensor nature of some of these fibers cannot be excluded. The peripheral nerves found to terminate around microvessels in the submammalian pineal organs do not differ from vasomotor fibers of vessels in the meninges and brain tissue [1, 2, 4, 7, 15].

Autonomic fibers were found to reach the mammalian pineal not only from the superior cervical ganglion but also from the sphenopalatine and trigeminal ganglia

[9, 10]. They can represent vasoconstrictor, vasodilator and vasosensor fibers, respectively. The submammalian pineal organs contain photoreceptor cells and there is no reason to attribute the autonomic fibers a role in mediating information about environmental light periodicity perceived by the retina [4]. We suppose that – like in mammals – the vasomotor regulation of the pineal microvessels of submammals may serve the vascular support for circadian and circannual periodic changes in the metabolic activity of the pineal tissue. The higher number of perivascular terminals found in reptiles and birds may correspond to the higher metabolic activity of the tissues in more differentiated species.

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