Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Eye Research 78 (2004) 187–196
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

# Comparative anatomy of nitrergic intrinsic choroidal neurons (ICN) in various avian species

Falk Schroedl[a,*], M. Egle De Stefano[b], Sven Reese[c], Axel Brehmer[a], Winfried L. Neuhuber[a]

[a]*Anatomisches Institut I, FAU Erlangen-Nürnberg, Krankenhausstr. 9, 91054 Erlangen, Germany*
[b]*Dip. di Biologia Cellulare e dello Sviluppo, Università 'La Sapienza', P.le Aldo Moro 5, 00185 Rome, Italy*
[c]*Institut für Tieranatomie I, LMU München, Veterinärstraße 13, 80539 München, Germany*

Received 18 June 2003; accepted 7 November 2003

**Abstract**

Intrinsic choroidal neurons (ICN) represent a peculiar feature of eyes in higher primates and birds. They account for up to 2000 in human and duck eyes but are virtually absent or rare in all other mammalian species investigated so far. It has been suggested that ICN are involved in regulation of ocular blood supply, hence influencing intraocular pressure, and changes in choroidal thickness, thus influencing accommodation. The present study was undertaken in order to compare differences in various avian species with respect to ICN as well as to provide data on some avian species relevant for experimental ophthalmic research, i.e. chicken and quail.

Choroids from 12 avian species were processed for NADPH-diaphorase histochemistry or, in some cases, neuronal nitric oxide synthase immunocytochemistry. ICN were quantified and normalized to mean choroidal area. Three choroids of each galliformes (i.e. chicken, quail, turkey) and anseriformes (i.e. Muscovy duck, Mallard duck, goose) were rastered in squares of 1 $mm^2$ and *x*/*y* coordinates were transferred into a 3D-diagram with the amount of ICN represented in the *z*-axis.

ICN were detected in all species investigated. They were predominantly small cells with soma diameters of 20–30 μm. In turkey, and to a lesser amount in chicken, a subpopulation of ICN with somal diameters of up to 70 μm was observed. Highest mean cell counts were found in goose (6195·4; turkey 3558·4; chicken 1681·4; Muscovy duck 785·4; Mallard duck 640·8; quail 440·2). Normalized to choroidal area, highest mean cell counts were (per $mm^2$): 12·62 in goose, 4·42 in both chicken and turkey, 2·86 in quail, 2·66 in Mallard duck and 1·89 in Muscovy duck. In galliformes, ICN were found to be accumulated temporo-cranial, while in anseriformes they were arranged in a more belt-like fashion, passing from cranio-nasal to temporo-caudal.

Our results show that besides Muscovy duck, other avian species appear as suitable models for further functional experiments on ICN. The temporo-cranial accumulation of ICN in galliformes and the belt-like arrangement in anseriformes may reflect special functional requirements in regions of high visual acuity.


*Keywords:* eye; vascular innervation; VIP; neuronal nitric oxide synthase; non-vascular smooth muscle; accommodation

## 1. Introduction

During recent years, birds have become a more and more important model for studies of visual function and ocular vascular and refractory homeostasis (Lauber, 1991; Reiner et al., 1995; Wallman et al., 1995; Iuvone et al., 1997). One major difference between the eyes of mammals and birds is the absence of blood vessels in the avian retina. Although a specialized intraocular structure, the pecten, is a major source for retinal oxygen and nutrient supply (Kajikawa, 1923; Wingstrand and Munk, 1965; Meyer, 1986), the main source may be the choroid. Thus, the avian choroid is very prominent (Wittich, 1854; Franz, 1934; Duke-Elder, 1958) and also richly innervated. Beyond this, the choroid in birds represents a sponge-like framework of non-vascular smooth muscle cells surrounding lymphatic lacunae (Junghans et al., 1996; De Stefano and Mugnaini, 1997a,b). This cavernous body, by changing its thickness, is able to compensate for refractory error which was experimentally induced by spectacle lenses in chicks (Wildsoet and Wallman, 1995; Schmid et al., 1999). On the other hand it may act as

* Corresponding author. Falk Schroedl, Anatomisches Institut I, FAU Erlangen-Nürnberg, Krankenhausstr. 9, 91054 Erlangen, Germany.
*E-mail address:* falk.schroedl@anatomie1.med.uni-erlangen.de (F. Schroedl).

0014-4835/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
DOI:10.1016/j.exer.2003.11.007

a cooling system for the retina (Parver et al., 1980; Bill, 1985). The autonomic innervation of this specialized choroidal tissue in birds is provided by the superior cervical (Kirby et al., 1978; Guglielmone and Cantino, 1982; Bill, 1991), ciliary (Meriney and Pilar, 1987; Reiner et al., 1991; Cuthbertson et al., 1996) and pterygopalatine (Cuthbertson et al., 1997) ganglia. Primary afferents from the trigeminal ganglion may be included into this autonomic innervation because of their local effector function by releasing vasodilator peptides (Reiner, 1987; Holzer, 1988; Shih et al., 1999). Poorly recognized within this innervation concept is the observation of neuronal cell bodies within the choroid, the so called intrinsic choroidal neurons (ICN; Schrödl et al., 2000). Up to now, this neuronal population has been described in comparable amounts only in higher primates and birds while lacking or rare in rodents, cats and rabbits (Flügel et al., 1994; Bergua et al., 1996). Although their presence has been known since the 19th century (Müller, 1859), no major attention has. been paid to them until recently (Miller et al., 1983; Bergua et al., 1993; Flügel et al., 1994; Schrödl et al., 2000). Their targets are choroidal arteries and non-vascular smooth muscle cells of the choroidal stroma (Flügel et al., 1994; Bergua et al., 1996; Schrödl et al., 2000, 2003). Besides that, there is morphological evidence that ICN integrate sympathetic and primary afferent signals (Schrödl et al., 2001a,b), thus indicating a pivotal role in ocular autonomic innervation. Further, there is morphological evidence for intrinsic ICN-ICN contacts (Schrödl et al., 2000, 2003).

Among birds, there is great diversity of behavior supposed to require different visual abilities. For example, the refractory range is particularly wide (up to 50 diopters) in some aquatic birds (Duke-Elder, 1958), while others are used to dive and their eyes have to adapt to rapid and large pressure variations, as it has been reported for penguins with diving depths up to 200 m (Kooyman et al., 1982). It can be surmised that these different visual needs are also reflected in various choroidal functions that have to be regulated accordingly and this might be reflected by differences in ICN. Thus, the present study was undertaken to provide basic data on ICN in different avian species. It is divided into two parts: part one relates to anseriformes and galliformes which are easily accessible for experiments and include current models in ophthalmic research, i.e. chicken and quail. Part two was designed as a first screening of birds with different environmental needs (terrestrial, aquatic, predatory) which may require special regulation of their ocular homeostasis. Part of the manuscript has been presented in abstract form (Schrödl et al., 2002).

## 2. Materials and methods

In this study, 12 domestic, terrestrial, aquatic and predatory avian species were investigated as listed in Table 1. All wild animals were obtained in accordance with the German huntinglaw and were provided by the local hunting association. Animals in this study covered by the German nature conservation law, i.e. *Bubo bubo*, or protected all the year round, i.e. *Buteo buteo*, have been sacrificed because of incurable non-ocular diseases and were obtained from the clinic for avian diseases, Munich, Germany. Heads of all domesticated birds were provided from local poultry farms.

Immediately after sacrifice, eyes of birds were fixed by intraocular injection of phosphate buffered saline (PBS) containing 4% paraformaldehyde, and heads were kept on ice for about 20 min during transport to the laboratory. Eyes were enucleated and opened along the ora serrata and the vitreous body. Retina and retinal pigment epithelium (RPE) were carefully removed using blunt tweezers. Remaining RPE was detatched using tweezers or cotton swabs. Eye cups with choroid attached to the sclera were immersion-fixed for another 2 hr at room temperature followed by a rinse in PBS (pH 7·3). Choroidal wholemounts were prepared by dissecting the choroid from the scleral cup and from its insertion at the pecten oculi and rinsed in PBS.

Table 1
List of species and numbers of choroids investigated (of different animals each)

| Common name | Scientific name | Order | Environmental need | N choroids |
|---|---|---|---|---|
| Chicken | *Gallus domesticus* | Galliformes | Terrestrial | 7 |
| Quail | *Coturnix coturnix japonica* | Galliformes | Terrestrial | 5 |
| Turkey | *Meleagris gallopavo* | Galliformes | Terrestrial | 5 |
| Muscovy duck | *Cairina moschata* | Anseriformes | Terrestrial | 6 |
| Mallard duck | *Anas platyrhynchos* | Anseriformes | Aquatic | 5 |
| Goose | *Anser anser* | Anseriformes | Aquatic | 5 |
| Canada goose | *Branta canadensis* | Anseriformes | Aquatic | 2 |
| Mute swan | *Cygnus olor* | Anseriformes | Aquatic | 2 |
| Cormorant | *Phalacrocorax carbo* | Pelicaniformes | Predatory aquatic | 3 |
| Buzzard | *Buteo buteo* | Accipitriformes | Predatory day | 1 |
| Eagle owl | *Bubo bubo* | Strigiformes | Predatory night | 1/3 |
| Ostrich | *Struthio camelus* | Struthioniformes | Terrestrial | 1 |

### 2.1. NADPH-diaphorase (NADPH-d) histochemistry

Wholemounts were incubated for 1 hr at 37°C in the following solution: 1 mg NADPH (Biomol, Hamburg, Germany) and 0·25 mg nitroblue-tetrazolium chloride (Biomol, Hamburg, Germany) per ml phosphate buffer, containing 0·5% Triton X-100 (Merck, Darmstadt, Germany). Incubation was stopped by several rinses in PBS and wholemounts were coverslipped in Kaiser's Glycerol Gelatine (Merck).

In a previous study, virtually complete colocalization of NADPH-d activity and neuronal nitric oxide synthase (nNOS) has been established in duck ICN (Bergua et al., 1996). Thus, for quantitative analysis of nitrergic neurons, nNOS-immunohistochemistry was applied in one swan and one quail, using a protocol as described earlier (Schrödl et al., 2000).

### 2.2. Depigmentation

After NADPH-d reaction, in species with pigmented choroids a depigmentation was performed using the following protocol: 0·3% $H_2O_2$ and 0·5% KOH in distilled water for 1–4 hr depending on the depigmentation process as controlled under the microscope. Depigmentation was carried out at room temperature while tissues were kept in the dark. After neutralization in 1% of acetic acid, wholemounts were rinsed several times in PBS and coverslipped in Kaiser's glycerol gelatine.

### 2.3. Documentation

Results of NADPH-d histochemistry and nNOS immunohistochemistry were documented on a Leica Aristoplan microscope using Kodak Tmax 100 ASA black and white film and Ektachrome 400 ASA color film for immunohistochemistry, respectively.

For quantitative analysis in all species ICN were counted ( × 10 or × 25 objective lens) using a manual counting device. Results are indicated as mean ± standard deviation.

For topographic analysis, choroids of three right eyes each from galliformes (chicken, quail, turkey) and anseriformes (Muscovy duck, Mallard duck, goose) were treated as follows: wholemounts were rastered in grids of 1 × 1 mm and ICN per grid were counted ( × 10 or × 25 objective lens). To avoid double counting of ICN overlapping a grid, right and down margins were counted to the same, whereas the other sides where counted to the neighbouring grids. *XY*-coordinates of squares were transferred into a 3D-plot where the amount of ICN per square represented in the *z*-axis (Microsoft Excel, CorelDraw 10). Additionally, two eyes of Canadian Goose were processed in the same way.

Means of all ICN out of one species were calculated and levelled out with the mean choroidal area. Choroids were treated as ideal circles. For the excised insertion of the pecten oculi no special correction was used.

## 3. Results

### 3.1. Galliformes and anseriformes

ICN were detectable in all three Galliformes. Mean cell counts were 1681·4 ± 978·6 in chicken ($n = 5$), 440·2 ± 198·6 in quail ($n = 5$), and 3558·4 ± 1111·4 in turkey ($n = 5$) as indicated in Tables 2 and 3. Normalized to choroidal area, also the apparently lower amount in the quail was nevertheless in a comparable range as in the two other species (chicken 4·4 $mm^{-2}$; quail 2·86 $mm^{-2}$; turkey 4·42 $mm^{-2}$; see Table 3). ICN were round to ovoid or multidendritic (Fig. 1), and the longest cell diameter ranged from 20 to 30 μm. In turkey and chicken a subpopulation of ICN with large cell diameters (up to 70 μm; Fig. 1) was prominent (turkey: 638 out of 3092 in one choroid, 20·6%; chicken: 58 out of 1812 in one choroid, 3·2%), whereas in quail these large neurons were found only occasionally. A gradient with high amounts of ICN from suprachoroidal layers to only a few against the choriocapillaris side was clearly seen. A dense NADPH-d positive perivascular plexus around initial segments of blood vessels entering the choroid was observed in all of the tissues investigated. The topography of ICN was consistent: ICN were always found to be clustered in the temporo-cranial quadrant, termed here the peak region. ICN diminished nasally and vanished caudally. This distribution was slightly different in the turkey: ICN spread in high amounts all over the choroid, nevertheless showing a peak-region temporo-cranial (see Fig. 2). Highest amounts of ICN in the peak-region were

Table 2
Number of ICN in galliformes and anseriformes investigated (out of five eyes each)

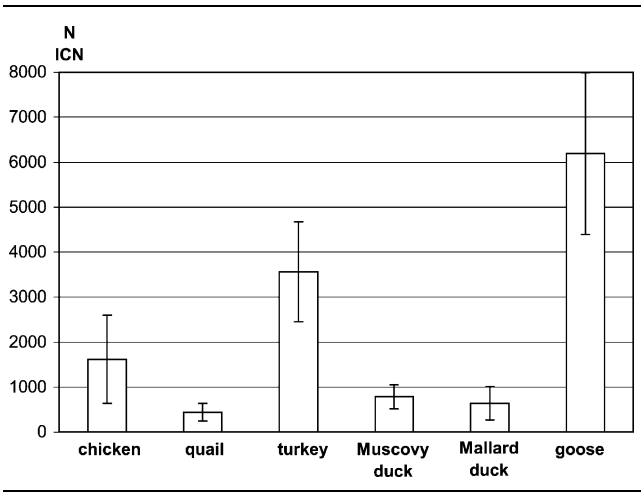

Table 3
Mean number of ICN in galliformes and anseriformes, normalized to mean amount of ICN per mean choroial area

| Order | Species | Mean (± S.D.) of ICN (out of five eyes) | Mean choroidal diameter (mm) | Choroidal area ($mm^2$) | Mean ICN/choroidal area ($mm^{-2}$) |
|---|---|---|---|---|---|
| Galliformes | Chicken | 1681·4 (±978·6) | 22 | 380·1 | 4·42 |
| | Quail | 440·2 (±198·6) | 14 | 153·9 | 2·86 |
| | Turkey | 3558·4 (±1111·4) | 32 | 804·2 | 4·42 |
| Anseriformes | Muscovy duck | 785·4 (±267·2) | 23 | 415·4 | 1·89 |
| | Mallard duck | 640·8 (±366·1) | 17·5 | 240·5 | 2·66 |
| | goose | 6195·4 (±1800·0) | 25 | 490·8 | 12·62 |

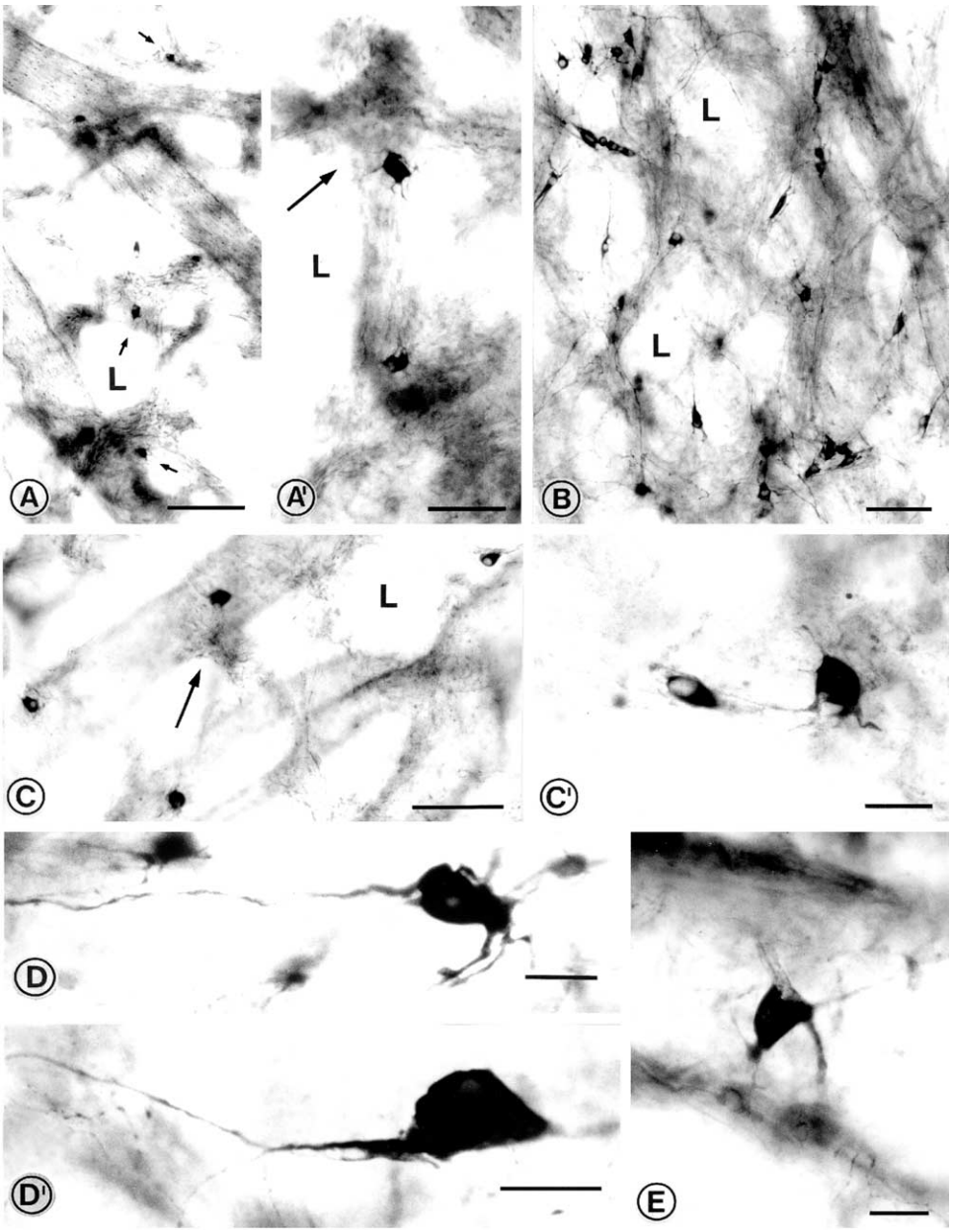


Fig. 1. NADPH-d-positive ICN in choroidal wholemounts from Muscovy duck (A,A′), goose (B), chicken (C,C′), turkey (D,D′) and quail (E). Most neurons are situated at connection points (arrows) of trabeculae lining lacunae (L). The plexus of NADPH-d-positive axons is best seen in the specimen from goose (B). Choroidal stromal smooth muscle cells display faint staining. The average maximum soma diameter was 20–40 μm. In turkey, a subpopulation of ICN (20,6%) showed a soma diameter of 40–70 μm (D′). (Scale bars: A, C, = 100 μm; A′, B, D′, E = 50 μm; C, D′ = 25 μm).

123 ICN $mm^{-2}$ in the chicken, 60 $mm^{-2}$ in quail and 58 $mm^{-2}$ turkey.

In the three Anseriformes, mean cell counts were 785·4 ± 267·2 in Muscovy duck ($n = 6$), 640·8 ± 366·1 in Mallard duck ($n = 5$), and 6195·4 ± 1800·0 in goose ($n = 5$; see Table 2). Normalized to choroidal area the lowest value was found in the Muscovy duck (1·89 $mm^{-2}$), followed by the Mallard duck (2·66 $mm^{-2}$) and the goose with 12·62 $mm^{-2}$, the highest value observed (see Table 3). The size and morphology of ICN resembled those in

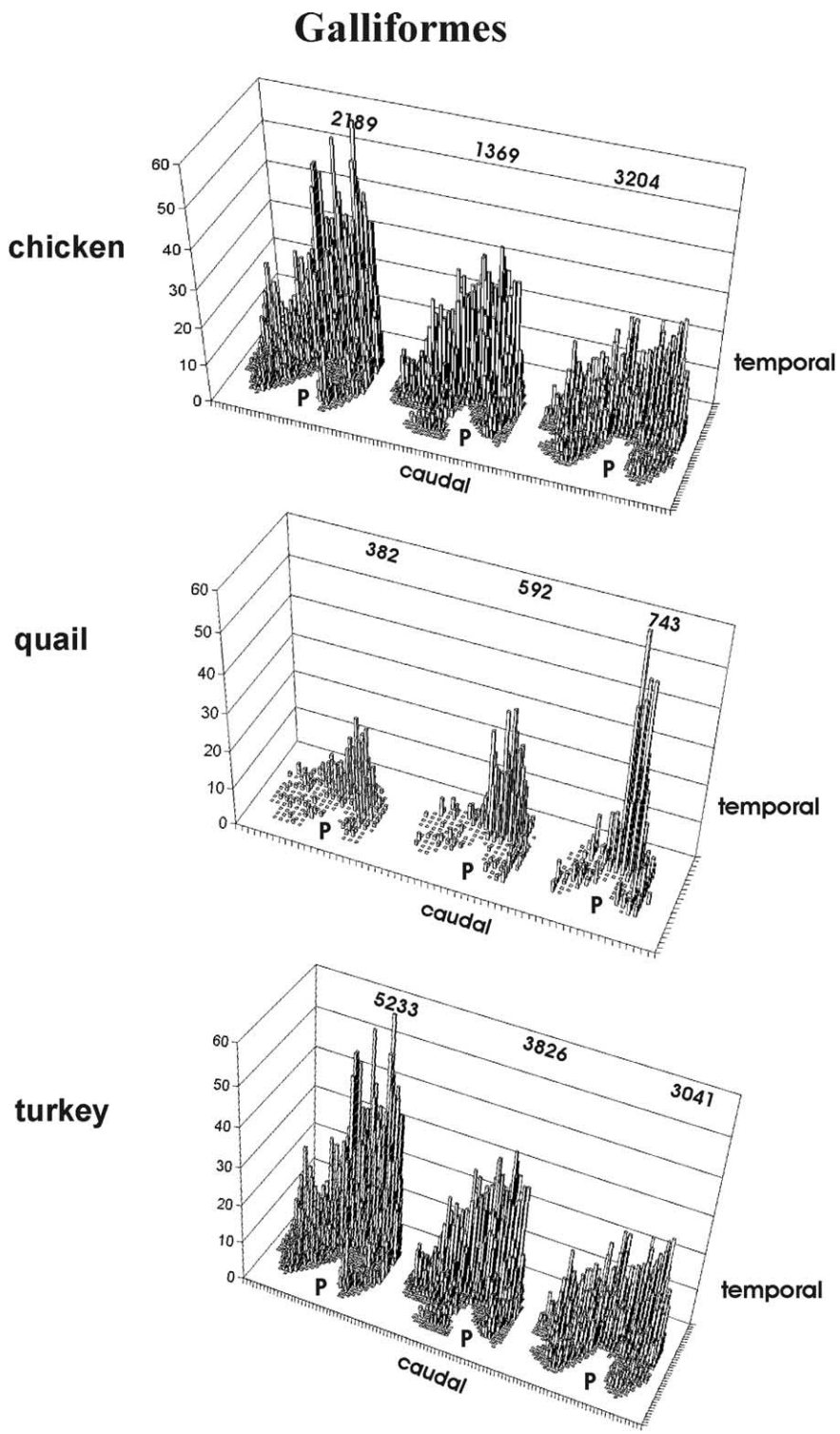


Fig. 2. 3D-plots of the topography of ICN of three right eyes in galliformes. Lamina suprachoroidea facing upwards. $z$-axis = amount of ICN $mm^{-2}$; P: excision site of the pecten oculi. Absolute numbers of ICN per choroid are indicated. Note the temporo-cranial accumulation of ICN in all galliformes.

the galliformes (Fig. 1), and met the description of Bergua et al. (1996). However, large neurons were observed only occasionally in ducks, while slightly more were present in the goose. The inward gradient of ICN was equivalent to galliformes. The topography differed significantly from that in galliformes. In all anseriformes, a belt-like distribution of ICN was found, passing from naso-cranial to temporo-caudal (see Figs. 3 and 4). This belt-like distribution was most prominent in Muscovy duck, confirming earlier descriptions (Bergua et al., 1996). Nevertheless, peak regions were found within this belt-like arrangement of ICN. In contrast to galliformes, a peak-region was prominent in the naso-cranial quadrant Muscovy duck which met our earlier descriptions (Schrödl et al., 2001b).

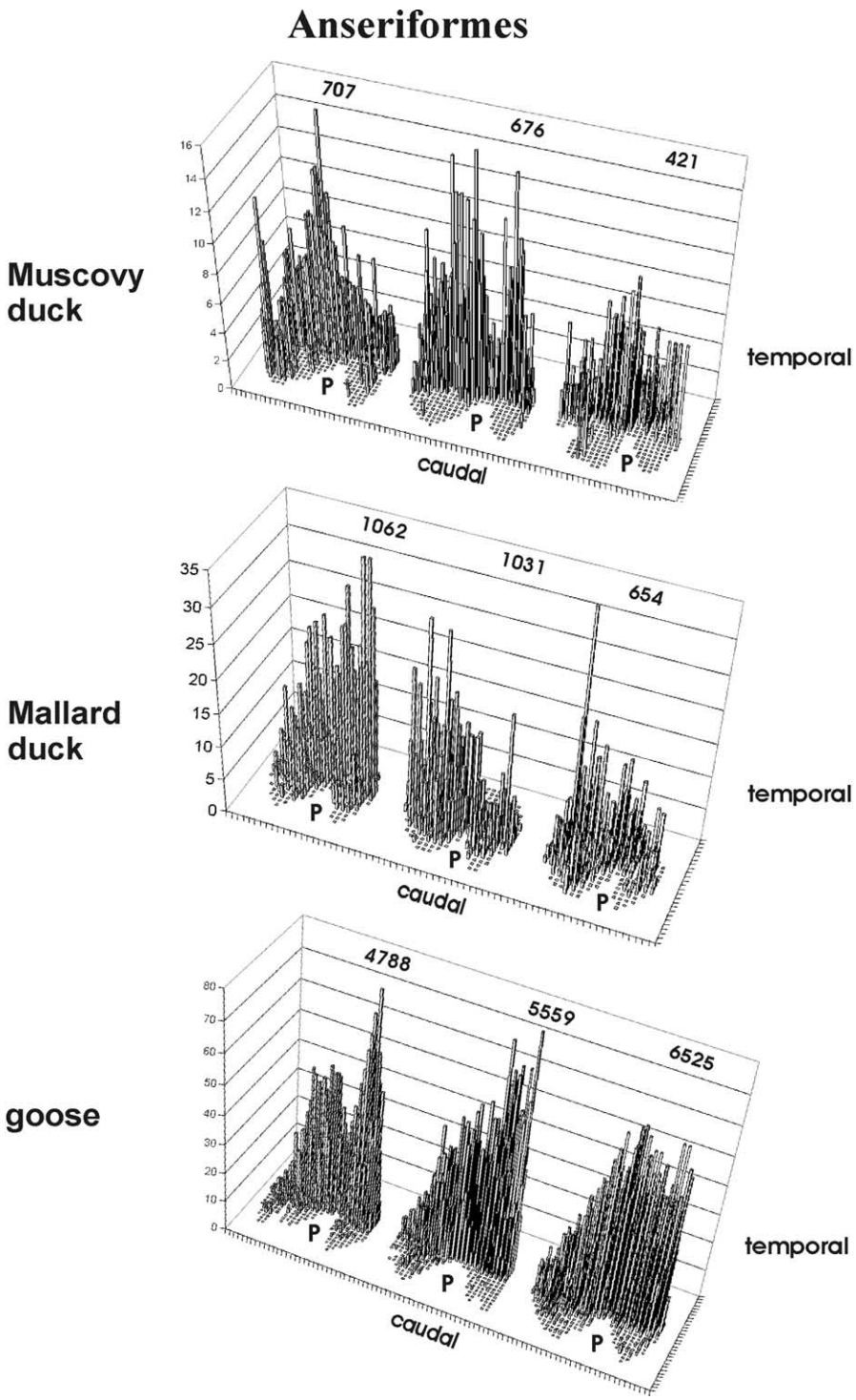


Fig. 3. 3D-plots of the topography of ICN of three right eyes in anseriformes. Lamina suprachoroidea facing upwards. $z$-axis = amount of ICN $mm^{-2}$; P: excision site of the pecten oculi. Absolute numbers of ICN per choroid are indicated.

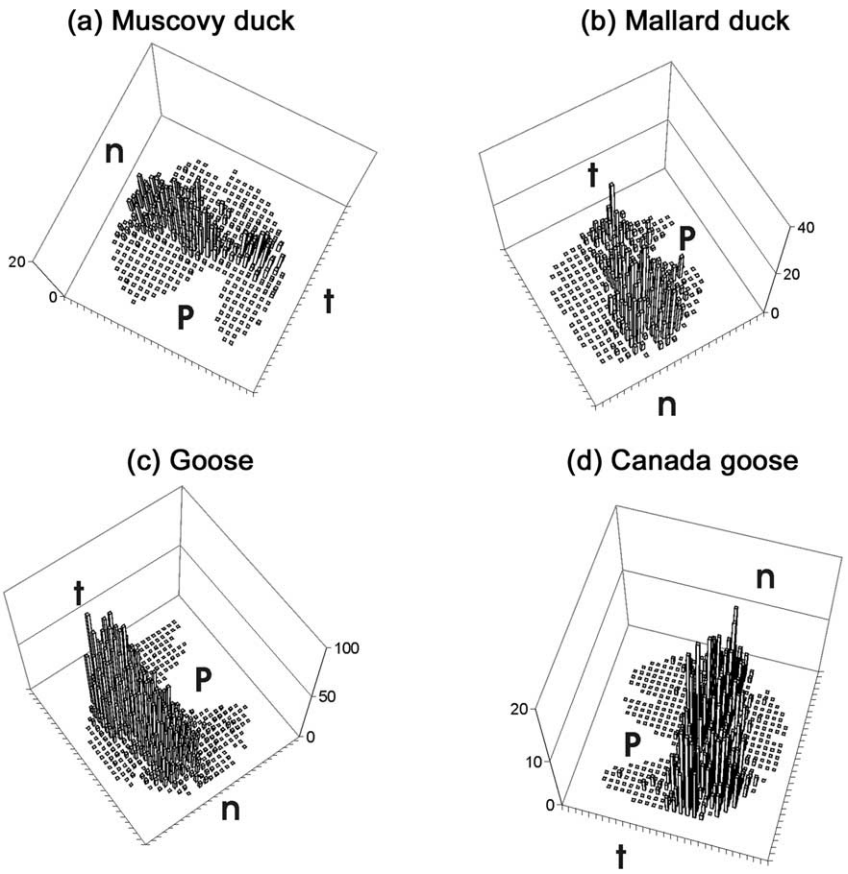


Fig. 4. 3D-plots of ICN in anseriformes: Different viewing angles are used in order to gain better visualization of the belt-like arrangement (P: excision site of the pecten oculi = caudal, t: temporal, n: nasal). Fig. a–c corresponds to Fig. 3, middle choroid of each spezies d: right eye of Canada gose.

In one Muscovy duck a second temporo-cranial peak-region could be observed, from the former one separated by a sharp inclination at the base of the pecten oculi (see Fig. 3). In the Mallard duck, two animals showed naso-cranial peak-regions, whereas one animal showed a temporo-cranial peakregion. In goose, a temporo-cranial peakregion within the ICN belt was prominent in all three animals. One animal showed a inclination at the pecten-base, ascending to a naso-cranial peakregion. Highest amounts of ICN in the peak-regions were: Muscovy duck 16 $mm^{-2}$ (temporal and nasal); Mallard duck 33 $mm^{-2}$ temporal, 28 $mm^{-2}$ nasal; goose 75 $mm^{-2}$ (temporal). In one goose, 9526 ICN were counted, the highest amount of ICN hitherto observed.

### 3.2. Other avian species

In general, the morphology of ICN in the following species were in accordance with the descriptions above, therefore only differences will be reported here.

*Canada goose* ($n = 2$) : Both animals show a belt-like organization of ICN, as described for the Muscovy duck (see Fig. 4). ICN counts reached 1178 and 2056, respectively. Peakregions were present in both cranial hemispheres (peak-maximum temporal 32 ICN $mm^{-2}$, nasal 21 ICN $mm^{-2}$). The choroidal diameter was 26 mm (3·04 ICN $mm^{-2}$).

*Mute swan* ($n = 2$) : although a dense perivascular plexus in initial ciliary artery segments could be observed, only 15 nNOS-immunoreactive ICN were counted. In the NADPH-diaphorase staining, 28 nitrergic neurons were present and some positive nerve fibres within the choroidal stroma could be observed. The choroidal diameter was 22 mm (0·05 ICN $mm^{-2}$).

*Cormorant* ($n = 3$) : in this species, no perivascular NADPHd-positive plexus could be observed, although ICN were present (502/236/438). They were mainly clustered in ganglia of up to 12 cells in anterior segments of the choroid. Only very few were counted in the central parts of the choroid (choroidal diameter 24·5 mm; 0·83 ICN $mm^{-2}$).

*Buzzard* ($n = 1$) : a typical NADPH-diaphorase positive perivascular plexus was not detectable, and only 52 ICN were counted. Nevertheless, endothelial NADPH-d activity was observed and choroidal stromal cells displayed medium NADPH-d activity. The choroidal diameter was 34 mm (0·05 ICN $mm^{-2}$).

*Eagle owl* ($n = 1$) : One third of a complete choroid could be prepared for a NADPH-diaphorase staining. In this sector, 221 ICN were counted, more concentrated in the cranial segment. The majority of ICN had an ovoid shape

and no processes were visible. Because initial segments of choroidal arteries were missing, we were unable to judge the presence of a perivascular plexus. The choroidal diameter was 42 mm.

*Ostrich* ($n = 1$) : 1784 ICN positive for NADPH-d were counted, most of which had large diameters. They were found to be concentrated in anterior segments of the choroid. A dense endothelial reaction product was observed, but a perivascular plexus was missing. The choroidal diameter was 61 mm (0·61 ICN $mm^{-2}$).

## 4. Discussion

Using NADPH-d cytochemistry and nNOS immunohistochemistry, we showed that intrinsic choroidal neurons (ICN) can be found in various avian species, including those already established as animal models in experimental ophthalmic research, i.e. chicken and quails. ICN showed a distinct topography with a temporo-cranial accumulation in galliformes, whilst in anseriformes they were arranged belt-like, passing from naso-cranial to temporo-caudal. Morphologically, ICN represent a consistent cell population in the species investigated here, being small, mostly multipolar cells with mean soma diameters ranging from 20 to 40 μm. However, in some species, e.g. turkey and chicken, a cell population with soma sizes of up to 70 μm was prominent, indicating subpopulations of ICN with probably specialized functions. In birds, the presence of ICN has been only occasionally described in the past (chicken: Kirby et al., 1978; Guglielmone and Cantino, 1982; pigeon: Cuthbertson et al., 1997). Only recently more systematic studies have been performed (Bergua et al., 1996; Schrödl et al., 2000; 2001a,b). To our knowledge, no data were available for quail and turkey up to now and, besides the Muscovy duck, for all the anseriformes as well. Since ICN are thought to represent a relevant component of ocular autonomic innervation in birds (Bergua et al., 1996; Schrödl et al., 2000; 2001a,b) the need for a thorough search of ICN in avian species relevant for experimental ophthalmic research, i.e. chicken and quail, was apparent.

From the data presented here, possible functional roles played by ICN can be narrowed down. First, comparing the size of eyes in different species, from the largest eye of any land vertebrate, i.e. ostrich (Walls, 1967), to the medium-sized eye of turkey and goose to the small eye of quail, no obvious correlation with the amount of ICN could be established. Therefore, we conclude that whatever the functional roles of ICN might be, they are not related to possible regulatory mechanisms depending on the size of the eye. Second, ICN were found to be present in some aquatic birds like ducks and goose and one could propose that these neurons might be involved in adaption to pressure differences caused by diving. However, in the cormorant, a diving predatory bird with, besides the penguin, one of the maximal diving depths ever investigated (up to 40 m; Keller and Vordermeier, 1994), a rather low amount of ICN was detected. Therefore, ICN seem not to be involved in balancing pressure differences during diving.

Describing differences in the amount of ICN, we cannot exclude effects of aging that might lead to a drop of ICN, since for obvious reasons the age of most wild species presented here could not be established thus making it difficult to compare wild and domesticated species (Hodos, 1993). However, the choroids of the Mute swan investigated here stemmed from 1-year-old animals, i.e. before sexual maturity, and nevertheless show low amounts of ICN. Therefore we desisted from further elaborating this issue.

ICN in both mammals and birds abut both the muscular wall of choroidal arteries (Flügel et al., 1994; Bergua et al., 1996; Schrödl et al., 2003) and non-vascular smooth muscle (Poukens et al., 1998; Schrödl et al., 2000, 2003), which in birds constitute a contractile trabecular meshwork around lymphatic launae (Junghans et al., 1996; De Stefano and Mugnaini, 1997a). By virtue of these targets, ICN are strategically placed in order to control important functions of the choroid. By influencing the vascular tone, ICN might play an important role in the autonomic regulation of intraocular pressure (Bill and Sperber, 1990; Bill, 1991), and be therefore instrumental in extending our knowledge of some pathogenic aspects of glaucoma (May et al., 1997). Another possible functional role of ICN would be the neuronal control of choroidal thickness. Regulation of the tone of non-vascular smooth muscle fibers leads to different filling of lymphatic lacunae. Subsequently, this will change choroidal thickness, with consequent shift of the adjacent retina. Thus, the optical properties of the eye will be altered.

This process has been extensively studied in the avian eye (Wildsoet and Wallman, 1995; Wallman et al., 1995) but the underlying mechanisms are still not understood. In this context, differences in ICN topography between galliformes and anseriformes are intriguing. In ground feeding birds, like galliformes (and pigeon, see below), the temporo-cranial retina processes naso-caudal visual information which reflects the bird's pecking region (Erichsen et al., 1989; Hodos and Erichsen, 1990). This region seems to be most important also for socialising effects amongst chicken (Stamp Dawkins, 1995). In this respect, a choroidal accommodation mechanism (Walls, 1967) would be of great importance for the bird's visual field, giving a full optical performance without lenticular or corneal (Walls, 1967; Meyer, 1986) accommodation. This functional retinal topography correlates strikingly with the temporo-cranial accumulation of ICN in galliformes. Assuming innervation of non-vascular smooth muscle cells by embedded ICN, this temporo-cranial accumulation may suggest that ICN are closely involved in shifting the overlaying retina in a sense of local choroidal accommodation. Interestingly, ICN in anseriformes display a belt-like distribution, matching the area linearis of the anseriform retina, which in turn reflects the linear arrangement of the visual field in this order of

birds (Slonaker, 1897; Wood, 1917; Franz, 1934; Duijm, 1958). In line with the above arguing, this again supports a functional involvement of ICN in control of choroidal thickness. The mechanisms or chemical signals triggering a choroidal shift may derive from the retina itself (Fischer et al., 1999; Crewther, 2000, 2003), or will be released in the choroid upon visual stimulation, as it has been shown for retinoic acid (Mertz and Wallman, 2000), directly influencing non-vascular smooth muscle cells. However, they may act via ICN as a neuronal relay. Interesting in this respect is the fact that both mechanisms in which ICN might play a role, i.e. choroidal blood flow and choroidal accommodation, are coupled mechanisms (Reiner et al., 1995; Papastergiou et al., 1998).

Since the choroid of all avian species is structurally similar and supposedly meets the same functional requirements, the question arises which neuronal populations may substitute for the role of ICN in avian species with few ICN (pigeon: Cuthbertson et al., 1997; swan, buzzard: this study). Nevertheless, the few ICN reported in pigeon, were found to be concentrated in the temporo-cranial region of the choroid (Cuthbertson et al., 1997), the field of high visual acuity in this bird (see Cuthbertson et al., 1996), and therefore compare favourally with our results.

Taken together, ICN represent a peculiar cell population in the eye of various avian species, including those already established as animal models in experimental ophthalmic research. Therefore, future studies can be extended now to these bird species to better understand the role of ICN in ocular homeostasis. The distinct topographical differences of ICN in the two orders of birds investigated, i.e. belt-like in anseriformes and peaking temporo-cranial in galliformes, leads us to propose the hypothesis, that ICN may be involved in neuronal control of choroidal thickness in regions of high visual acuity in the avian eye.

## Acknowledgements

We would like to thank the horticulture Schötzau, Burgstall, and the poultry farm Drechsel, Münchaurach, as well as C and S Gumbmann, Niederndorf, for their generous collaboration. The perfect technical assistance by Anita Hecht, Andrea Hilpert, Karin Löschner and Inge Zimmermann is gratefully appreciated. This work was supported by Deutsche Forschungsgemeinschaft (DFG), SFB 539 BII.3. This article is dedicated to Professor G.O.H. Naumann.

## References

Bergua, A., Jünemann, A., Naumann, G.O., 1993. NADPH-D reactive choroid ganglion cells in the human. Klin. Monatsbl Augenheilkd 203, 77–82.

Bergua, A., Mayer, B., Neuhuber, W.L., 1996. Nitrergic and VIPergic neurons in the choroid and ciliary ganglion of the duck *Anis carina*. Anat. Embryol. (Berl) 193, 239–248.

Bill, A., 1985. Some aspects of the ocular circulation. Invest. Ophthalmol. Vis. Sci. 26, 410–424.

Bill, A., 1991. The 1990 Endre Balazs Lecture. Effects of some neuropeptides on the uvea. Exp. Eye Res. 53, 3–11.

Bill, A., Sperber, G.O., 1990. Control of retinal and choroidal blood flow. Eye 4, 319–325.

Crewther, D.P., 2000. The role of photoreceptors in the control of refractive state. Prog. Ret. Eye Res. 19, 421–457.

Crewther, D.P., Crewther, S.G., Liang, H., Junghans, B.M., 2003. Evidence for Ionic control of refractive compensation. Invest. Ophthalmol. Vis. Sci. 44 ARVO E-abstract 2809.

Cuthbertson, S., Jackson, B., Toledo, C., Fitzgerald, M.E., Shih, Y.F., Zagvazdin, Y., Reiner, A., 1997. Innervation of orbital and choroidal blood vessels by the pterygopalatine ganglion in pigeons. J. Comp. Neurol. 386, 422–442.

Cuthbertson, S., White, J., Fitzgerald, M.E., Shih, Y.F., Reiner, A., 1996. Distribution within the choroid of cholinergic nerve fibers from the ciliary ganglion in pigeons. Vision Res. 36, 775–786.

De Stefano, M.E., Mugnaini, E., 1997a. Fine structure of the choroidal coat of the avian eye. Lymphatic vessels. Invest. Ophthalmol. Vis. Sci. 38, 1241–1260.

De Stefano, M.E., Mugnaini, E., 1997b. Fine structure of the choroidal coat of the avian eye. Vascularization, supporting tissue and innervation. Anat. Embryol. (Berl) 195, 393–418.

Duijm, M., 1958. On the position of a ribbon like central area in the eyes of some birds. Arch. Néerl Zool. 13, 127–145.

Duke-Elder, S., 1958. The eyes of birds. In: Duke-Elder, S., (Ed.), System of Ophthalmology, Kimpton, London, pp. 397–427.

Erichsen, J.T., Hodos, W., Evinger, C., Bessette, B.B., Phillips, S.J., 1989. Head orientation in pigeons: postural, locomotor and visual determinants. Brain Behav. Evol. 33, 268–278.

Fischer, A.J., McGuire, J.J., Schaeffel, F., Stell, W.K., 1999. Light- and focus-dependent expression of the transcription factor ZENK in the chick retina. Nat. Neurosci. 2, 706–712.

Flügel, C., Tamm, E.R., Mayer, B., Lütjen-Drecoll, E., 1994. Species differences in choroidal vasodilative innervation: evidence for specific intrinsic nitrergic and VIP-positive neurons in the human eye. Invest. Ophthalmol. Vis. Sci. 35, 592–599.

Franz, V., 1934. Höhere Sinnesorgane. In: Bolk, L., Göppert, E., Kallius, E., Lubosch, W. (Eds.), Handbuch der vergleichenden Anatomie der Wirbeltiere, Urban und Schwarzenberg, München, pp. 989–1292.

Guglielmone, R., Cantino, D., 1982. Autonomic innervation of the ocular choroid membrane in the chicken: a fluorescence-histochemical and electron-microscopic study. Cell Tissue Res. 222, 417–431.

Hodos, W., 1993. The visual capabilities of birds. In: Zeigler, H.P., Bischoff, H.J. (Eds.), Vision, Brain and Behaviour in Birds, MIT, Cambridge, p. 70.

Hodos, W., Erichsen, J.T., 1990. Lower-field myopia in birds: an adaptation that keeps the ground in focus. Vision Res. 30, 653–657.

Holzer, P., 1988. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 24, 739–768.

Iuvone, P., Bernard, M., Alonso-Gomez, A., Greve, P., Cassone, V., Klein, D., 1997. Cellular and molecular regulation of serotonin N-acetyltransferase activity in chicken retinal photoreceptors. Biol. Signals 6, 217–224.

Junghans, B.M., Crewther, S.G., Liang, H., Crewther, D.P., Wareing, L., Pirie, B., 1996. Lymphatics in the chick choroid? Aust. N Z J. Ophthalmol. 24, 47–49.

Kajikawa, J., 1923. Beiträge zur Anatomie und Physiologie des Vogelauges. Graefes Arch. Ophthalmol. 112, 260–346.

Keller, T., Vordermeier, T., 1994. In: Fischerei, B.L.f., Fischerei, Stranberg (Ed.), Abschlussbericht zum Forschungsvorhaben 'Einfluss des Kormorans (Phalacrocorax carbo sinensis) auf die Fischereibestände ausgewählter bayerischer Gewässer unter Berücksichtigung fischökologischer und fischereiökonomischer Aspekte', p. 9.

Kirby, M.L., Diab, I.M., Mattio, T.G., 1978. Development of adrenergic innervation of the iris and fluorescent ganglion cells in the choroid of the chick eye. Anat. Rec. 191, 311–319.

Kooyman, G.L., Davis, R.W., Croxall, J.P., Costa, D.P., 1982. Diving dephts and energy requirements of King Penguins. Science 217, 726–727.

Lauber, J.K., 1991. Review: avian models for experimental myopia. J. Ocul. Pharmacol. 7, 259–276.

May, C.A., Hayreh, S.S., Furuyoshi, N., Ossoinig, K., Kaufman, P.L., Lutjen-Drecoll, E., 1997. Choroidal ganglion cell plexus and retinal vasculature in monkeys with laser-induced glaucoma. Ophthalmologica 211, 161–171.

Meriney, S.D., Pilar, G., 1987. Cholinergic innervation of the smooth muscle cells in the choroid coat of the chick eye and its development. J. Neurosci. 7, 3827–3839.

Mertz, J.R., Wallman, J., 2000. Choroidal retinoic acid synthesis: a possible mediator between refractive error and compensatory eye growth. Exp. Eye Res. 70, 519–527.

Meyer, D., 1986. The avian eye. In: Sturkie, P., (Ed.), Avian Physiology, Springer, Tokyo, pp. 37–48.

Miller, A.S., Coster, D.J., Costa, M., Furness, J.B., 1983. Vasoactive intestinal polypeptide immunoreactive nerve fibres in the human eye. Aust. J. Ophthalmol. 11, 185–193.

Müller, H., 1859. Ueber Ganglienzellen im Ciliarmuskel des Menschen. Verh physik-med Ges Würzburg 10, 107–110.

Papastergiou, G.I., Schmid, G.F., Riva, C.E., Mendel, M.J., Stone, R.A., Laties, A.M., 1998. Ocular axial length and choroidal thickness in newly hatched chicks and one-year-old chickens fluctuate in a diurnal pattern that is influenced by visual experience and intraocular pressure changes. Exp. Eye Res. 66, 195–205.

Parver, L.M., Auker, C., Carpenter, D., 1980. Choroidal blood flow as a heat dissipating mechanism in the macula. Am. J. Ophthalmol. 89, 641–646.

Poukens, V., Glasgow, B.J., Demer, J.L., 1998. Nonvascular contractile cells in sclera and choroid of humans and monkeys. Invest. Ophthalmol. Vis. Sci. 39, 1765–1774.

Reiner, A., 1987. The presence of substance P/CGRPcontaining nerve fibres, VIP-containing fibres and numerous cholinergic fibres on blood vessels of the avian choroid. Invest. Ophthalmol. Vis. Sci. Suppl. 28, S81.

Reiner, A., Erichsen, J.T., Cabot, J.B., Evinger, C., Fitzgerald, M.E., Karten, H.J., 1991. Neurotransmitter organization of the nucleus of Edinger-Westphal and its projection to the avian ciliary ganglion. Vis. Neurosci. 6, 451–472.

Reiner, A., Shih, Y.F., Fitzgerald, M.E., 1995. The relationship of choroidal blood flow and accommodation to the control of ocular growth. Vision Res. 35, 1227–1245.

Schmid, G.F., Papastergiou, G.I., Lin, T., Riva, C.E., Laties, A.M., Stone, R.A., 1999. Autonomic denervations influence ocular dimensions and intraocular pressure in chicks. Exp. Eye Res. 68, 573–581.

Schrödl, F., Brehmer, A., Neuhuber, W.L., 2000. Intrinsic choroidal neurons in the duck eye express galanin. J. Comp. Neurol. 425, 24–33.

Schrödl, F., de Laet, A., Tassignon, m., Van Bogaert, P.-P., Brehmer, A., Neuhuber, W.L., Timmermans, J.-P., 2003. Intrinsic choroidal neurons (ICN) in the human eye: projections, targets and basic electrophysiological data. Invest. Ophthalmol. Vis. Sci. 44, 3705–3712.

Schrödl, F., De Stefano, M.E., Brehmer, A., Neuhuber, W.L., 2002. Topography of intrinsic choroidal neurons (ICN) in various avian species. Exp. Eye Res. 72, 21.

Schrödl, F., Schweigert, M., Brehmer, A., Neuhuber, W.L., 2001a. Intrinsic neurons in the duck choroid are contacted by CGRP-immunoreactive nerve fibres: evidence for a local pre-central reflex arc in the eye. Exp. Eye Res. 72, 137–146.

Schrödl, F., Tines, R., Brehmer, A., Neuhuber, W.L., 2001b. Intrinsic choroidal neurons in the duck eye receive sympathetic input: anatomical evidence for adrenergic modulation of nitrergic functions in the choroid. Cell Tissue Res. 304, 175–184.

Shih, Y.F., Fitzgerald, M.E., Cuthbertson, S.L., Reiner, A., 1999. Influence of ophthalmic nerve fibers on choroidal blood flow and myopic eye growth in chicks. Exp. Eye Res. 69, 9–20.

Slonaker, J., 1897. A comparative study of the area of acute vision in vertebrates. J. Morphol. 13, 445–503.

Stamp Dawkins, M., 1995. How do hens view other hens? The use of the lateral and binocular visual fields in social recognition. Behaviour 132, 591–606.

Wallman, J., Wildsoet, C., Xu, A., Gottlieb, M.D., Nickla, D.L., Marran, L., Krebs, W., Christensen, A.M., 1995. Moving the retina: choroidal modulation of refractive state. Vision Res. 35, 37–50.

Walls, G.L., 1967. The Vertebrate Eye and its Adaptive Radiation. Hafner, London.

Wildsoet, C., Wallman, J., 1995. Choroidal and scleral mechanisms of compensation for spectacle lenses in chicks. Vision Res. 35, 1175–1194.

Wingstrand, K., Munk, O., 1965. The pecten oculi of the pigeon with particular regard to its function. Biol. Skr Dan Vid Selsk 14, 1–89.

Wittich, V., 1854. Vergleichend histologische Mittheilungen. I. Über den Bau der Choroidea des Säugethier- und Vogelauges. Arch. Ophthalmol. Bd. II, 124–132.

Wood, C., 1917. The Fundus Oculi in Birds Especially as Viewed by the Ophthalmoscope: A Study in Comparative Anatomy and Physiology. Lakeside, Chicago, IL.