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COMPARATIVE ANALYSIS OF RETINA STRUCTURE AND PHOTOPIC ELECTRORETINOGRAMS IN DEVELOPING ALTRICIAL PIGEONS (*COLUMBA LIVIA*) AND PRECOCIAL JAPANESE QUAILS (*COTURNIX COTURNIX JAPONICA*)

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Resumen. – Análisis comparativo de la estructura retiniana y electroretinogramas en la paloma, artricial, y la codorniz, precocial, en desarrollo. – Electroretinogramas (ERG) fueron obtenidos en condición fotópica de pichones y adultos de la paloma (*Columba livia*), altricial-nidícola, y de la codorniz (*Coturnix japonica*), precocial-nidífuga; luego, las retinas fueron procesadas para análisis histológicos (microscopía electrónica), con el objetivo de analizar la madurez retiniana en función de la edad, utilizando palomas y codornices de 0-, 7-, 15-, 21- y adultos de 75 días de nacidos. Las respuestas de los ERG fueron comparadas basadas en la amplitud de las ondas a y b obtenidas estimulando los ojos con flashes de luminancia decreciente (0,0; -0,2; -0,4; -0,6 y -0,8 unidades logarítmicas; intensidad máxima: 3,31 cd-s/m^2). Las palomas de 0 y 7 días de edad no mostraron una respuesta medible al ERG. Las respuestas comenzaron en palomas de 15 días e incrementaron su amplitud con la edad. En la codorniz, estas respuestas se observaron en pichones de 0 día. Sin embargo, los ERGs de las codornices adultas (75 días) decrecieron en amplitud al compararlos con los de las codornices de 21 días. Las palomas no presentaron fotorreceptores al nacer; no obstante, las palomas de 15 días mostraron fotorreceptores plenamente desarrollados. En la codorniz recién nacida (0 día de edad), los fotorreceptores se encontraban totalmente desarrollados, distinguiéndose fácilmente entre conos y bastones. En la paloma, las capas nuclear y plexiforme interna fueron más delgadas que en la codorniz y el número de células ganglionares menor. La falta de respuesta al ERG y la falta de fotorreceptores en las palomas de 0 día de edad muestran que, al contrario de las codornices, las palomas son ciegas al nacer. La alta densidad de células en la capa nuclear interna, el mayor espesor de la capa plexiforme interna, y el gran número de células ganglionares son indicaciones de que la codorniz tiene una mejor agudeza visual que la paloma.

Abstract. – Photopic electroretinograms (ERGs) were obtained of nestlings and adults of altricial-nidicolous Common Pigeons (*Columba livia*), and chicks and adults of precocial-nidifugous Japanese Quails (*Coturnix japonica*); thereafter the retinas were processed for histological analysis (electron microscopy) in order to test retinal maturity as a function of age, using 0-, 7-, 15-, 21- and adult 75-day old (after hatching) pigeons and quails. ERG responses were compared based on the a- and b-wave amplitudes following flashes of decreasing luminance (0.0, -0.2, -0.4, -0-6 and -0.8 log units; maximal intensity: 3.31 cd-s/m^2). Hatching and 7-day pigeons presented no measurable ERG response. The responses started at 15 days and increased in amplitude with age. Quail chicks started to show measurable responses at the 0-day stage. However, the ERGs of adult (75-day) quails decreased compared to those of 21-day old chicks. Pigeon hatchlings (0-day stage) had no photoreceptor; however, fully developed photoreceptors were present in

15-day old birds. On the other hand, in quail chicks, fully developed and easily distinguished rods and cones were present at hatching. The inner nuclear and inner plexiform layers averaged thinner and the number of ganglion cells lower in pigeons than in the quails. The lack of ERG response and of photoreceptors in pigeon hatchlings shows that, contrary to precocial-nidifugous quail chicks, they are blind. The high density of cells in the inner nuclear layer, the high number of ganglion, and the higher thickness of the inner plexiform layer are indications of better visual acuity for the quails, compared to the pigeons. *Accepted 5 July 2007.*

Key words: Electroretinogram, retina, Common Pigeon, Japanese Quail, *Columba livia, Coturnix japonica.*

INTRODUCTION

One striking feature of postnatal growth in birds is the dichotomy between precocial and altricial development (Ricklefs 1983). Some hatchlings like those of songbirds, woodpeckers, hummingbirds, pigeons, parrots, are helpless and depend entirely on their parents; others like those of ducks, shorebirds, quails, grouses, are mobile and able to find their food by themselves (Gill 1994). The terms "altricial" and "precocial" refer to the extremes of the spectrum of increasing maturity at hatching and decreasing dependence on parental care (Gill 1994). Altricial birds are naked, blind, and immobile when they hatch and thus are completely dependent on their parents; they appear to have hatched prematurely. Precocial birds, on the contrary, are well-developed chicks, usually covered with fuzzy down; they can feed themselves, run about, and regulate their body temperature soon after they hatch (Gill 1994). Their brains are quite large compared with those of altricial nestlings (Gill 1994). Precocial birds lay larger eggs than do altricial ones of the same size, with 30–40% yolk, compared to 15–27% (Gill 1994). Incubation period is longer in precocial birds than in altricial ones. Altricial birds grow about three to four times more rapidly than precocial birds of the same body size (Ricklefs 1979a, Starck 1993).

Birds, except the kiwis (*Apteryx* sp.) are the more highly visually dependent animals of all vertebrates (Martin 1990, Martin *et al.* 2007). Many aspects of their adaptation to their environment and their survival depend on precise and subtle visual discrimination (Hodos 1993). Behaviors such as foraging, territory and nest defense, mate selection, orientation, homing and navigation depend on a well developed and highly sensitive visual system (Hodos 1993). As shown by Ricklefs (1983), the central nervous system, in particular the motor and visual systems, are more highly developed at the time of hatching in precocial than in altricial birds.

The retinas of most mammals are incompletely developed at birth and, during the first week of life, maturation proceeds rapidly; on the contrary, in some non-mammalian vertebrates, retinal maturation occurs during the incubation period and, at birth, retinal properties are fully developed (Bagnoli *et al. 19*85). In Common Pigeon (*Columba livia*) hatchlings, eyes are usually closed. Sometimes the lids may open but vision seems non-functional. Generally, eyelid opening occurs at about 2–5 days after hatching (Heaton & Harth *et al.* 1974) and photoreceptors are lacking. Bagnoli *et al.* (1985) have shown that photosensitive lamellae in the outer photoreceptors segments and a few synapses in the outer plexiform layer of the retina can be seen at the time the first electroretinograms (ERGs) can be recorded, i.e., at 4–6 days post hatching; in contrast, numerous synapses are already present in the inner plexiform layer when photoreceptor lamellae have yet to appear. The chicks of the Japanese Quail (*Coturnix*

japonica) have their lids open and their retinas already have all their layers at birth. In the chicken (*Gallus gallus*), the segregation of the outer and inner plexiform layers begins on day-6, and is completed on day-14 of embryonic development (Meller & Tatzlaff 1976, Spence & Robson 1989). The first synapses appear in the inner plexiform layer on day-13 of the embryonic development, but appear in the outer plexiform layer only by day-17 of the incubation period (Hering & Kröger 1996). Yamada *et al.* (1998) consider that the visual system of the Japanese Quail is fully established at 30 days of age.

The ontogeny of visual function in birds can be reflected by the papillary light reflex. The onset of the papillary reflex was reported as taking place before hatching, i.e., around 67–70%, 84%, and 87% of total incubation period in precocial (ducks and quails), semi-precocial (chicken) and altricial species (Common Pigeon), respectively, and around the 7th day after hatching in the altricial Common Grackle (*Quiscalus quiscula*) (Heaton 1971, 1973; Heaton & Harth 1974). ERG in response to flash stimuli is often used for viewing the ontogeny of the retinal function in vertebrates (see Bagnoli *et al. 19*85).

In spite of many studies dealing with the visual system of birds, as far as we know, there is no comparative study of the structure and function of the retina of developing post-hatch altricial and precocial birds, from the time of birth to the adult age. The present study was conducted to compare the ontogeny of the retinal structure and function of altricial-nidicolous Common Pigeons and precocial-nidifugous Japanese Quails, and to correlate their electrophysiological responses with a morphological analysis of their retina, using post-hatch individuals of both sexes ranging in age from newly hatched to 75-day old adults. The study of retinal function was limited to photopic conditions taken the fact that both species are strictly diurnal birds, and that recording ERG in scotopic, in addition to photopic, conditions would have lengthened the experimental protocol and demanded too much from so small animals such as newly born nestlings and chicks.

METHODS

In order to test retinal maturity as a function of age, we used 0-, 7-, 15-, 21- and 75-day old (after hatching) pigeons and quails, hereafter referred to as P_0-Q_0, P_7–Q_7, etc. The birds were obtained from farms. They were maintained in laboratory until reaching the required age.

ERG recording. The electroretinogram (ERG) is the recording of electrical potentials produced by the retina in response to a light stimulus, and which can be recorded at a distance, i.e., at the cornea (Ikeda 1993). A typical ERG consists of two waves which arise in different layers of the retina, reflecting light-evoked potentials generated by different retinal cells. The first one (a-wave), negative, is generated mainly by the photoreceptors; the second one (b-wave), positive, takes origin in the inner nuclear layer (Armington 1974). The waveform of the ERG and its components exhibit changes depending on the intensity and wavelength of the stimulating flash, as well as the state of retinal adaptation (i.e., photopic, scotopic), and thus can be used to compare the retinal sensitivity of different animal species.

The number of nestlings or chicks used for ERG recording varied between 8 and 10 for each age class. ERGs were recorded in a dark room with the use of a LKC EPIC-2000 visual electrodiagnostic system (LKC Technologies Inc., Gaithersburg, MD), which includes a 41-cm diameter Ganzfeld full field stimulator (LKC Ganzfeld-2503B), using a method previously reported (Rojas *et al.* 1997, 1999a, 1999b). The birds were anesthetized

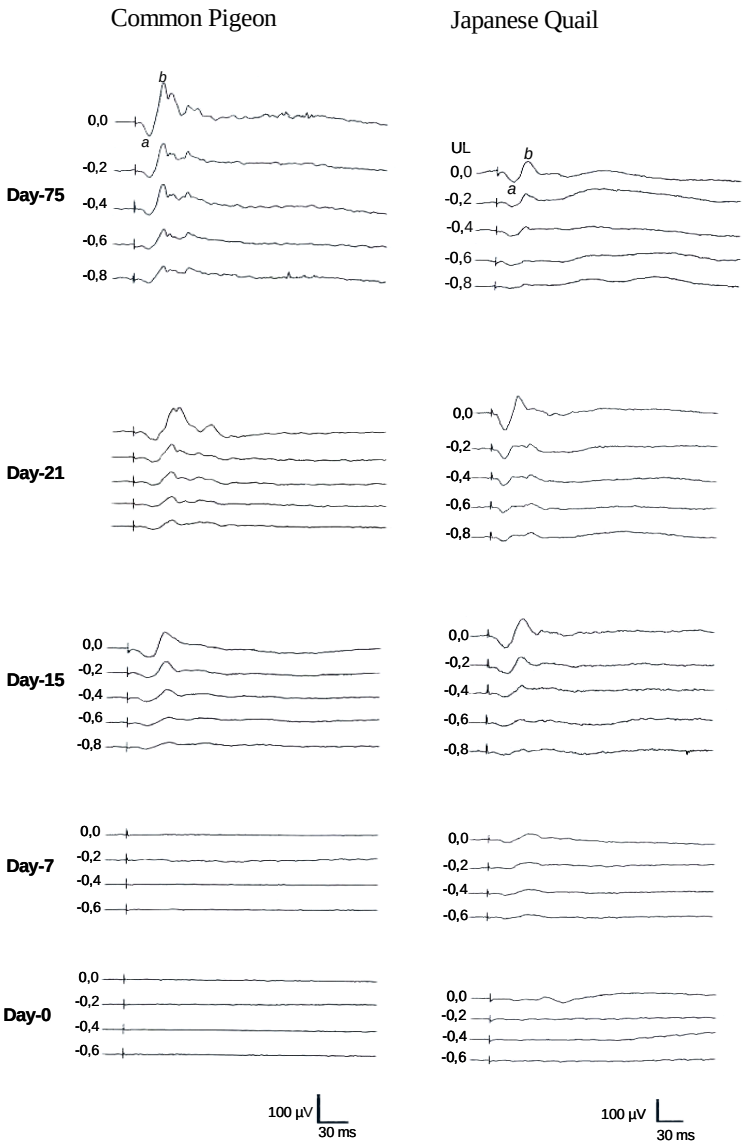

FIG. 1. Representative ERG responses of 0-, 7-, 15-, 21- and 75-day (after hatching) Common Pigeons and Japanese Quails obtained under photopic conditions. Nomenclature: a = peak of the a-wave; b = peak of the b-wave. The figures on the left represent light intensity values (Log units).

with a 1:1 mixture of ketamine-xylazine (0.0044 cc/kg injected in the pectoral muscle), and immobilized on a home-made recording holder with the head kept inside the Ganzfeld and the left eye maintained open upward. The left eyelids and nictitating membrane were kept retracted with a speculum, the cornea was anesthetized with 0.5% propa-

racaine hydrochloride, and the pupil was dilated with 1% tropicamide. The maximum pupil diameter (mm) was measured at the beginning and at the end of the experiment. Due to the fact that P_0 individuals have their eyes closed, a transversal cut was realized on the eyelid to expose the cornea. The active electrode consisted of a DTL® fiber (Sauquoit Industries, Scranton, PA) which was placed on the cornea (Hébert *et al.* 1996;, Lachapelle *et al.* 1993). Subdermal needles (Grass Instruments, Astro-med Inc., Warwick, RI), inserted under the skin of the crown and in the pectoral muscle, served as reference and ground electrodes, respectively.

The birds were then light-adapted for 10 min to a background luminance of 35.7 cd · m^2, following which the photopic ERGs (average of 4 at 4.1-sec intervals) were evoked to flashes of decreasing luminance (0.0, -0.2, -0.4, -0.6, and -0.8 log units; maximal intensity: 3.31 cd · m^{-2} · sec^{-1}). For P_0, P_7, Q_0 and Q_7, due to the fact that birds of these age classes tended to die before the end of a too long ERG protocol, experiments with flashes of -0.8 log units were not conducted for these age categories. Previous studies have indicated that the above parameters result in adequate and reproducible segregation of rod and cone functions in birds (Rojas *et al.* 1997, 1999a, 1999b).

Histological preparation. Once the ERG recordings were completed, four individuals of each species and age classes were kept for histological analysis. The former were euthanized under anesthesia. The left eye was removed and the axial length and equatorial diameter were measured (Martin 1986). The eye was then injected with 2.5% glutaraldehyde in 0.1M phosphate buffer (pH = 7.4 and 7.5 for pigeons and quails, respectively), punctured at the cornea, and placed in the same fixative for 45 min. Working with the eye in the fixative, the anterior part of the eye was removed and the retina, still attached to the choroid, was cut into 9 sectors, using the pecten as landmark. This division is the same as that used by Rojas de Azuaje (1993) and Rojas *et al.* (1997, 1999a, 1999b), and corresponds to that of Meyer & May (1973), although the sector numbering is different. For this study, only the sector 5 (central) of the retina was used. Still in the fixative, each sector was subdivided into 2-mm^2 portions, of which two were retained for analysis. After 45 min in the fixative, the retinal portions, were washed in 0.1M phosphate buffer for 15 min, postfixed in 1% OsO_4 in 0.1 M phosphate buffer for 1 h, rinsed in phosphate buffer followed by two baths in distilled water (10 min each), dehydrated in graded ethanol (from 50% to 100%, 5 min per step), and bathed in propylene oxide (10 min). The tissues were successively infiltrated with a 2:1 mixture of Epon and propylene oxide for 6 h, and pure Epon-812 medium for another 2 h. Finally, they were embedded in silicone rubber molds filled with Epon-812 and polymerized at 60°C for 48 h.

Semithin (0.6 μm) sections were obtained and mounted on glass slides and stained with toluidin blue for observation under a Zeiss photomicroscope. Cuts were made perpendicularly to the retina by reorienting the blocks until achieving sections longitudinal to the photoreceptors. Rods, cones and ganglion cells were counted in 280-μm wide fields, for a total of 5 counts. As in other avian retinas (Meyer & May 1973, Meyer 1977, Tansley & Erichsen 1985, Waldvogel 1990), double cones, in addition to single cones, were present in both species, and they were counted as two cones. In addition, the thickness of each retinal layer was measured. Ganglion cells were identified according to morphology and coloration criteria (Hayes & Brooke 1990, Inzunza *et al.* 1991): rounded or oval large cells with an oval pale nucleus and an easily distinguishable pale blue nucleolus. In most cases, ganglion cell bodies were

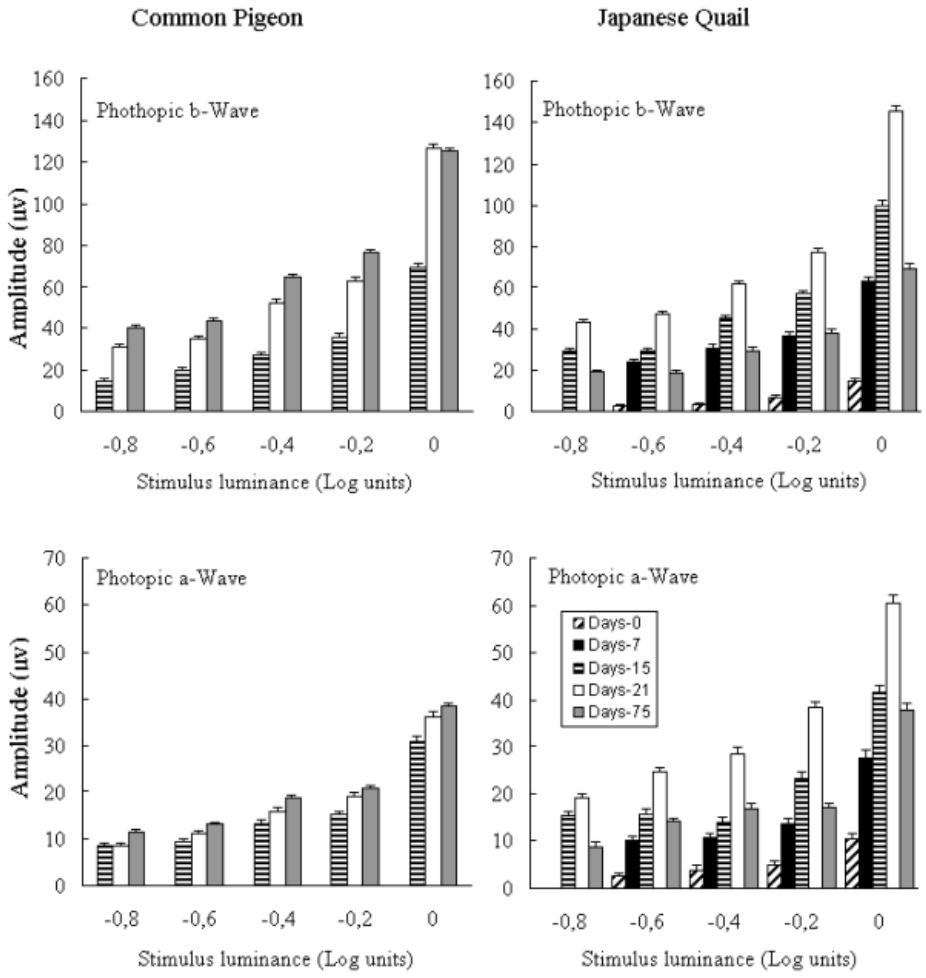

FIG. 2. Luminance-response function (Mean ± 95% confidence intervals) of the b- and a-waves of 0-, 7-, 15-, 21- and 75-day Common Pigeons and Japanese Quails obtained under photopic conditions.

arranged side by side in a 1-cell thick layer, but in the specialized thickened areas (e.g., central retina), they occurred in two or three layers. Displaced amacrine cells, on the other hand, appeared as small pale stained bodies lying next to the inner plexiform layer.

Additionally, for histological analysis of cellular components of the retina, thin (70 µm) sections were cut and stained with uranile acetate and lead citrate. Histological observations were made from microphotographs obtained with the use of a Hitachi H-600 transmission electron microscope.

Data analysis. Results were analyzed by using the means ± 95% confidence intervals, conventional two-way variance analysis

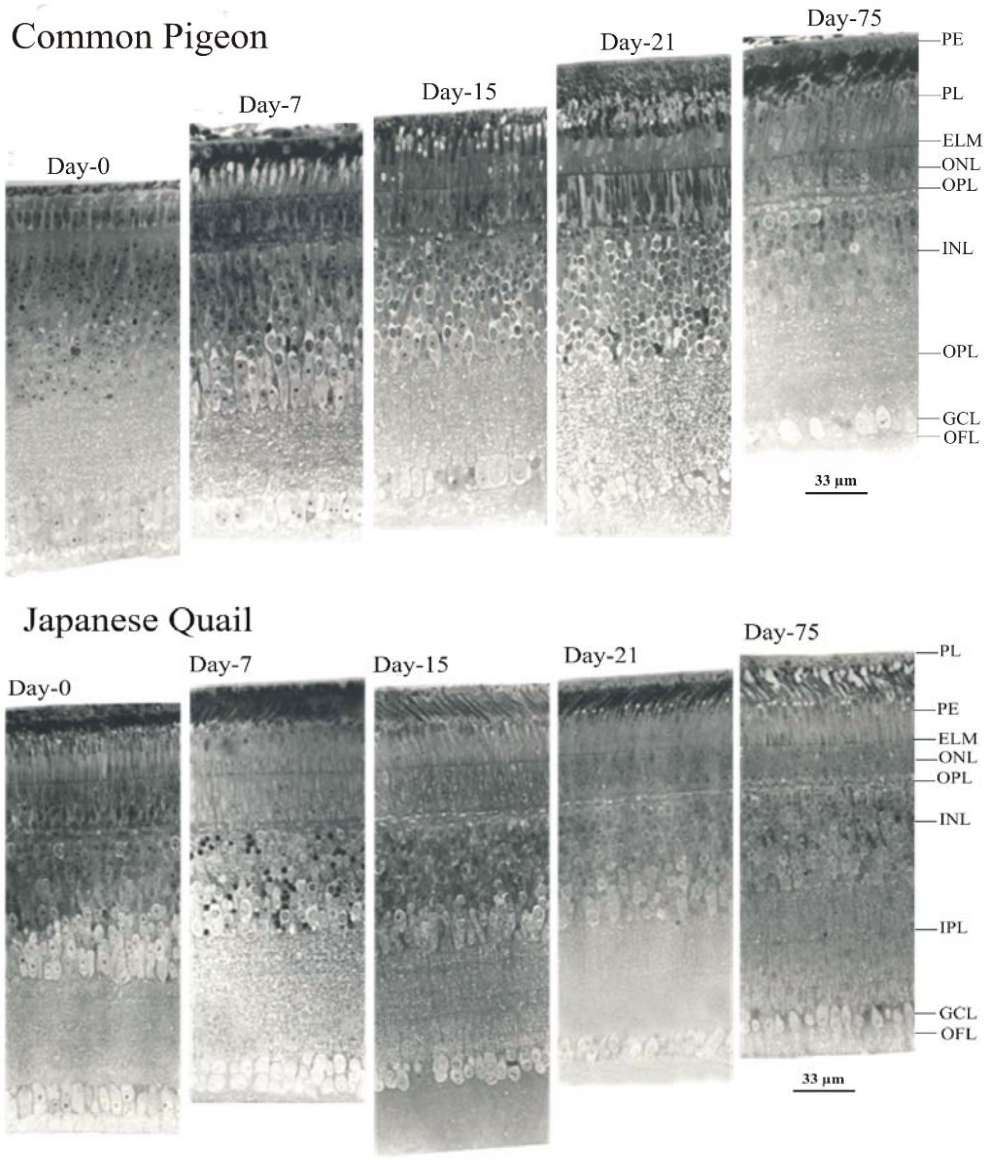

FIG. 3. Photomicrographs showing the layers of sector 5 of the retina of 0-, 7-, 15-, 21- and 75-day Common Pigeons and Japanese Quails. Nomenclature: ELM = external limiting membrane; GCL = ganglion cell layer; ILM = inner limiting membrane; INL = inner nuclear layer; IPL = inner plexiform layer; OFL = optic fiber layer; ONL = outer nuclear layer; OPL = outer plexiform layer; PL = photoreceptor layer.

(ANOVA), and Duncan *a posteriori* tests (Sokal & Rohlf 1979) for within and between group comparisons of the different variables for morphometric parameters and photopic ERG recordings (a- and b-wave amplitudes), evoked to flashes of 0.0 log units from which

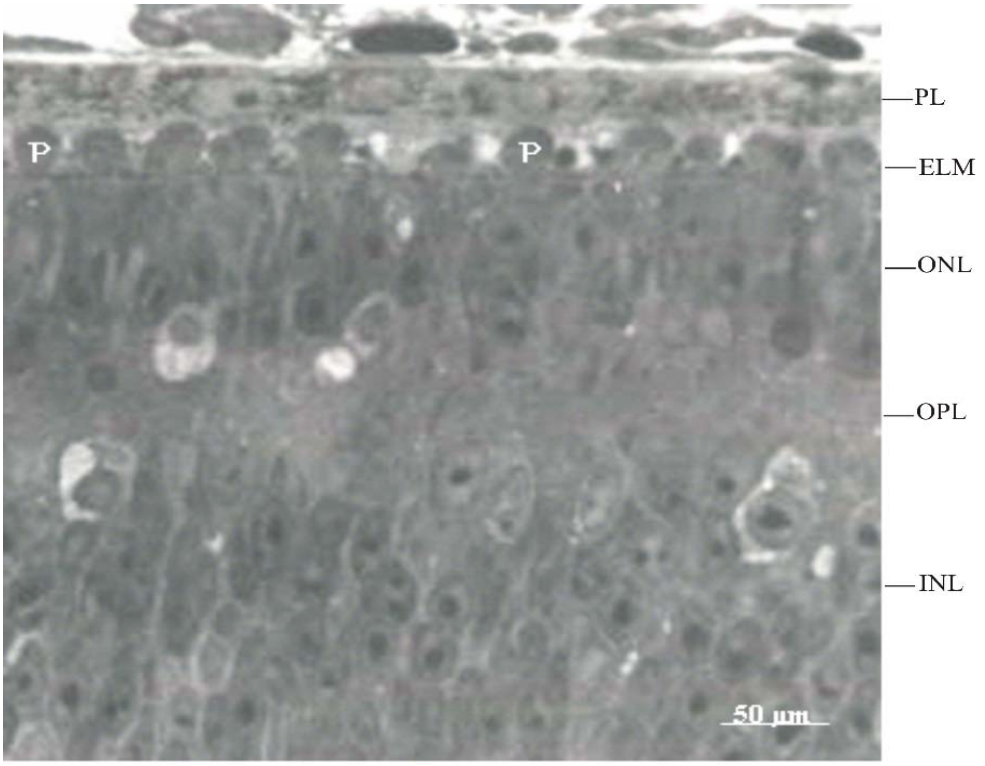

FIG. 4. Amplified view of the photoreceptor layer of 0-day pigeon hatchlings. Nomenclature: PL = photoreceptor layer; ELM = external limiting membrane; ONL = outer nuclear layer; OPL = outer plexiform layer; INL = inner nuclear layer; P = developing photoreceptor bud.

luminance-response function curves were generated.

RESULTS

Analysis of ERG recordings included the measurements of photopic a- and b-wave amplitudes for which luminance-response function histograms were generated (mean ± 95% confidence intervals). The analysis of morphological measurements also included calculation of the means (± 95% confidence intervals) of dilated pupil diameter, cell densities (rods, cones and ganglion cells), thickness of each retinal layer as well as the rod:cone ratios for each species.

Electroretinography. Representative ERGs obtained in photopic conditions for 0-, 7-, 15, 21- and 75-day old pigeons and quails are presented in Figure 1. They differ both in amplitude and shape between age classes of both species. The luminance-response function generated from amplitude measurements are graphically represented in Figure 2 for b- and a-waves.

As seen in Figure 2, 0- and 7-day old pigeon nestlings produced no ERG response at all. However, starting with 15-day old individuals, photopic b- and a-waves increased progressively in amplitude, both with the intensity of luminance stimulus and the age of individuals (Fig. 2), except for the b-wave of

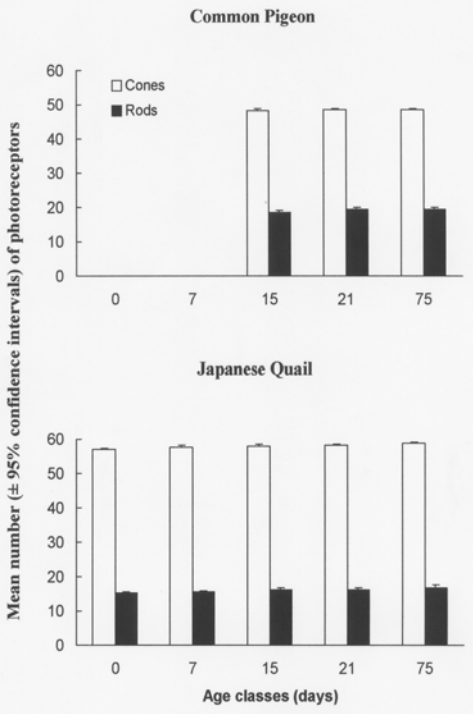

FIG. 5. Mean (± 95% confidence intervals) rod and cone numbers per 280 μm (± 95% CI) of 0-, 7-, 15, 21- and 75-day Common Pigeons and Japanese Quails.

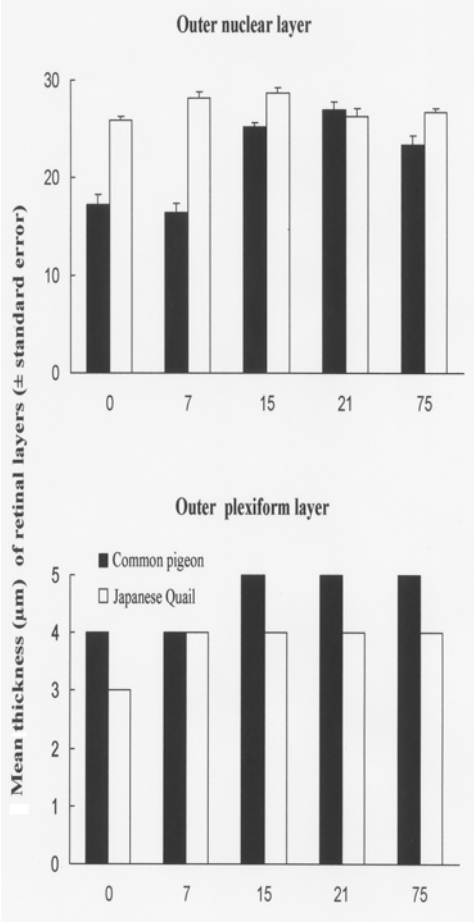

FIG. 6. Overall mean (± Standard error) thickness (μm) of outer nuclear (ONL) and plexiform (OPL) layers per 238 μm in 0-, 7-, 15, 21- and 75-day Common Pigeons and Japanese Quails.

21- and 75-day birds, at log unit 0, where response amplitudes were the same. Contrary to pigeons, 0-day hatchlings and 15-day chicks of the Japanese Quail produced ERG responses, but of very week amplitude (Fig. 1). Quail photopic b- and a-waves tended to increase in amplitude with the intensity of the luminance stimulus. However, contrary to pigeons, adult (75-day old) quails, at all stimulus intensities, suffered a decrease in the amplitude of their responses compared with 15- and 21-day individuals (Fig. 2).

At all tested stimulus intensities, 15- and 21-day quails presented higher photopic b-wave amplitudes than pigeons of the same ages (Fig. 2), while the opposite was observed for 75-day adults. The same was obtained for photopic a-wave amplitudes at all stimulus intensities of 15- and 21-day quails, but results tended to be opposite for 75-day adults (Fig. 2).

Morphology. Eye size and dilated pupil diameter increased as a function of age from 0-day to 75-day individuals in both species, and were greater in Common Pigeons than in Japanese Quails, as shown by the following figures: 6.8 ± 0.2 and 11.4 ± 0.3 mm, 9.4 ± 0.3

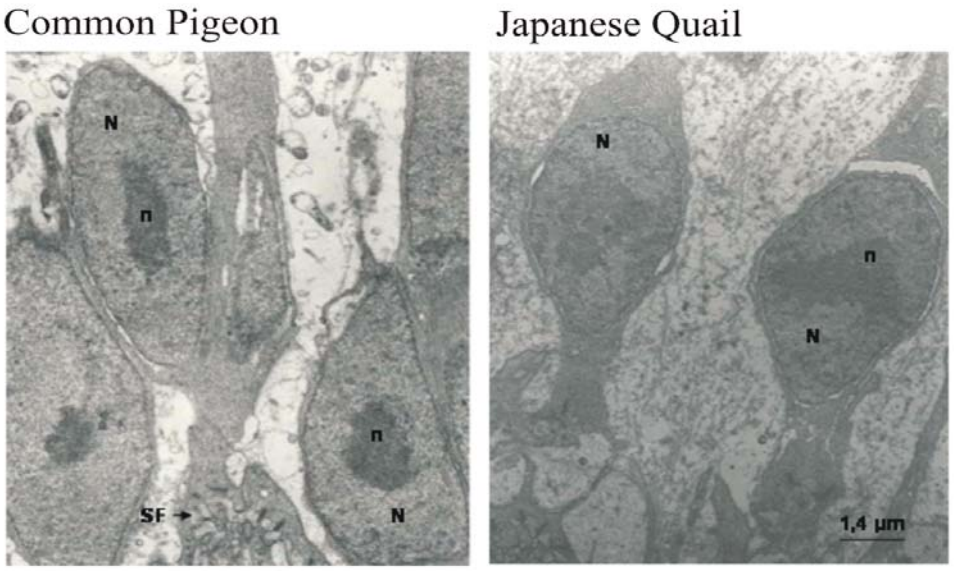

FIG. 7. Ultrastructure of the outer nuclear layers of 7-day Common Pigeons and Japanese Quails. Nomenclature: N = nucleus; n = nucleolus; SF = synaptic foot.

and 14.5 ± 0.2 mm, and 3.0 ± 0.0 and 4.8 ± 0.1 mm for axial length, equatorial diameter and dilated pupil diameter in 0-day hatchlings and 75-day Common Pigeons, respectively, compared to 5.2 ± 0.4 and 8.4 ± 0.5 mm, 7.1 ± 0.3 and 10.2 ± 0.4 mm, and 2.0 ± 0.0 and 2.9 ± 20.2 mm for the same parameters in 0-day chicks and 75-day Japanese Quails.

Photomicrographs showing the principal layers of sector 5 (central) of the retina of each species and age class are presented in Figure 3. At hatching, Japanese Quails chicks had fully developed retinal layers, including the photoreceptor layer, with typical rods and cones (Fig. 3). On the contrary, in 0-day pigeon hatchlings, photoreceptors were lacking (Fig. 3). Small buds were present but they corresponded to developing photoreceptors (Fig. 4). Progress in developing photoreceptors was observed in 7-day nestlings, but the rods and cones (with 2-µm oil droplets) can be considered as immature compared to those of 15-day pigeons. Excepting 0- and 7-day pigeons (lack of rods and cones), rod and cone numbers were very stable from one age class to another in both species (Fig. 5); rods and cones per 280µm in the central sector were *ca.* 19.0 and 48.5, and 16.0 and 58.0, resulting in rod:cone ratios of 0.4:1.0 and 0.3:1.0 for the pigeons and quails, respectively. Thus, compared to Common Pigeons, Japanese Quails had more cones and fewer rods. Fully grown oil droplets in the cones of both species varied in diameter between 3 and 4 µm.

The outer nuclear and plexiform layers in pigeons (measuring from 13 to 20 µm and 4 µm, respectively) were thinner in 0-day hatchlings than in older individuals; in the quails, the outer nuclear layer, varying in thickness from 24 to 32 µm, showed only little variation as a function of age classes, but the outer plexiform layer of 0-day chicks was thinner (3 µm) than that of older chicks (5 µm) (Fig. 6). In 7-day individuals of both species, the outer nuclear layer showed the pres-

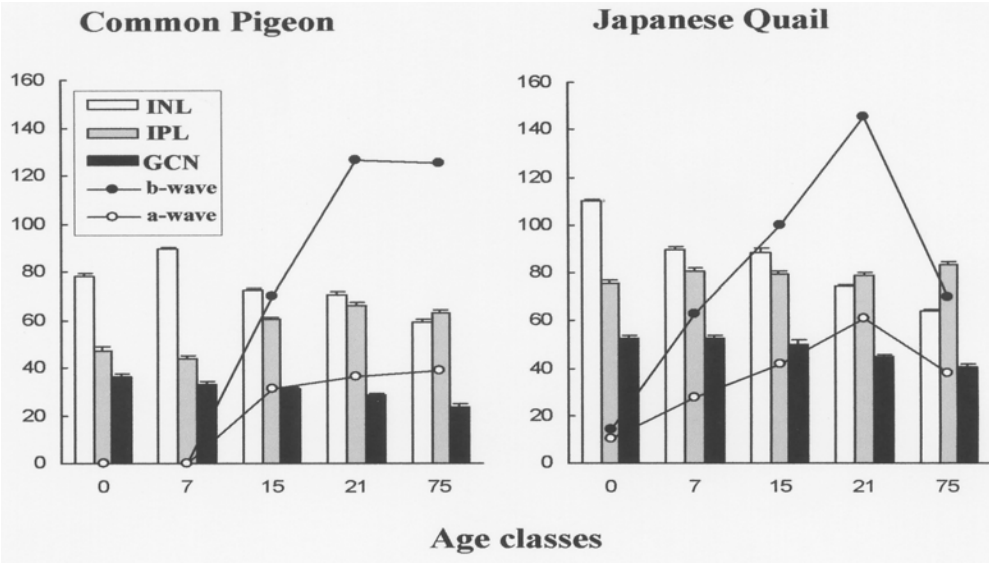

FIG. 8. Overall mean thickness (μm) of inner nuclear (INL) and plexiform (IPL) layers and overall mean ganglion cell number (GCN) per 238 μm in 0-, 7-, 15, 21- and 75-day Common Pigeons and Japanese Quails. The lines with black and open circles correspond to the luminance-response function of the b- and a-waves for each age class as transposed from Figure 2.

ence of large nuclei (the nuclei of photoreceptors) with prominent nucleoli, but the latter were less abundant in quails (Fig. 7). The thickness of the inner nuclear layer and the ganglion cell number tended to decrease with age in both species, but the thickness of the inner plexiform layer was lower in 0- and 7-day pigeons compared to older one, and showed no variation with age classes in Japanese Quails (Fig. 8). The inner nuclear layer of 7-day pigeons involved the presence of large nuclei with one or two nucleoli in bipolar and amacrine cells; quails of same age only had sparse nucleoli (Fig. 9). The nuclei of outer and inner nuclear layers of quails were characterized by the presence of less condensed chromatin. The ganglion cells of 0-day pigeon hatchlings were less organized compared to those of 21-day individuals which presented a more continuous pattern (Fig. 10); the nucleoli were lacking, or were very sparse, in 21-day individuals of both species.

DISCUSSION

Pigeons are blind at hatching with their eyes closed; 0-day hatchlings lack photoreceptors and their retina and that of 7-day individuals produce no ERG response at all. Progress in developing photoreceptors is observed in 7-day nestlings, but rods and cones (with oil droplets) still look immature compared to those of 15-day pigeons. The first ERG was observed only starting with the 15-day age class. The large nucleoli in the nuclei of the outer nuclear layer and the nuclei of bipolar and amacrine cells of 0- and 7-day pigeons are indicative of active RNA and protein synthesis. Indeed, the photoreceptor disks, which contain rod and cone photosensitive protein, first appear in 4–6 days nestlings (Bagnoli *et al.* 1985). According to Porciatti *et al.* (1985) and Bagnoli *et al.* (1985, 1987), the first photoreceptors and synapses appear in the central retina and follow a cen-

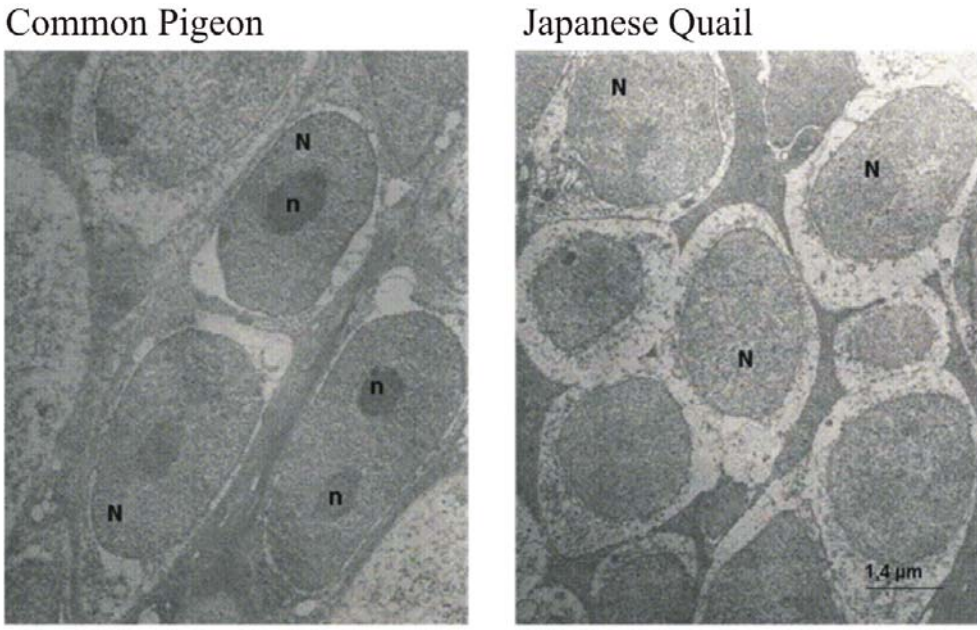

FIG. 9. Ultrastructure of the inner nuclear layers of 7-day Common Pigeons and Japanese Quails. Nomenclature: N = nucleus; n = nucleolus; SF = synaptic foot.

tro-peripheral progression. On the first day after hatching, pigeons have various synapses in the inner plexiform layer and only a few ones in the outer plexiform layer. The inner plexiform layer is made of the dendrites and neurites of bipolar and amacrine cells, while the outer plexiform layer is made of horizontal bipolar cells and photoreceptors. According to the same authors, the outer plexiform layer is formed at the same time as photoreceptor disks. The results of this study show that 0-day pigeon hatchlings already have the two plexiform layers, but synapses are probably inactive because of the lack of photoreceptors.

Hatching quails, on the contrary, have open lids and fully grown retinas with all their layers, and produce ERG responses, although of low amplitude. The presence of less condensed chromatin in the nuclei of their outer and inner nuclear layers indicates that protein synthesis largely took place before hatching. In the chicken, the segregation of the outer and inner plexiform layers begins on day-6, and ends around day-14 of embryonic life (Meller & Tetzlaff 1976, Spence & Robson 1989). The first synapses are found in the inner plexiform layer on day-13 of embryonic life, but appear between the photoreceptors and the bipolar cells of the outer plexiform layer beginning only on day-17 of prehatching life (Hering & Kröger 1996).

From the age of 15 days, the number of rods and cones and their ratios remained the same in the pigeons, but their photopic a- and b-waves continued increasing in amplitude until the age of 21 days, and remained relatively stable thereafter (Fig. 2). In the quails, on the contrary, the photopic a- and b-waves increased in amplitude up to the age of 21 days, but thereafter, between the 21

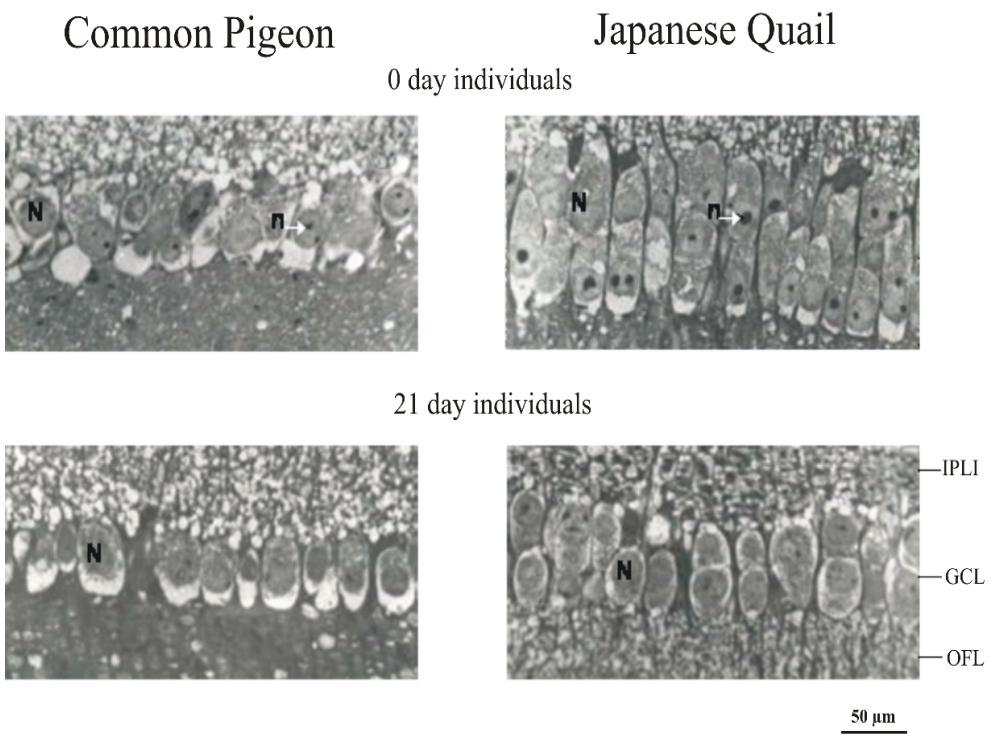

FIG. 10. Ultrastructure of the ganglion cell layer of 0- and 21-day Common Pigeons and Japanese Quails. Nomenclature: GCL = ganglion cell layer; IPL = inner plexiform layer; N = nucleus; n = nucleolus; OFL = optic fiber layer.

and 75 days of age, although rod and cone numbers did not decrease, they suffered a decrease in the order of 37% and 52%, respectively (Fig. 2).

The decrease in the amplitude of the photopic a-wave of quails could be due to a decrease in the production of photosensitive pigments as a result of cell maturation. The decrease of b-waves, on the other hand, could be due to the progressive decrease in the thickness of the inner nuclear layer from 0- to 75 days of age (Fig. 8), decrease which was observed also in pigeons, but was more pronounced in quails. Regressive processes in the subcellular levels of the retina may occur in quails as was experimentally demonstrated in the chicken, another precocial bird. Indeed, according to Mey & Thanos (2000), the neuronal populations produce longer dendrites, a greater number of dendritic branches with more synaptic spines, and larger axonal arborizations during development than are later present in the mature system; overproduction of cells and neurites in younger individuals can serve size-matching between connected systems that develop independently, and fine tuning of synaptic connections. Mey & Thanos (2000) assume that such a regressive process involves some kind of competition for synaptic sites or neurotrophic factors, and enumerate a series of selection mechanisms in the chick visual system: 1) the initial arborization of axon terminals in the optic tectum and retina covers larger territo-

ries because produce more cells and dendrites than necessary, implying that non-functional neurites are eliminated by a selection process; 2) cell death and removal of axon collaterals affect preferentially the neurons whose axons have grown to an inappropriate target; 3) excessive branches which degenerate later often have failed to reach appropriate sites in the tissue and have not become functional; 4) after a depletion of ganglion cells, only survive those neurons which have larger sizes and more dendritic branches, and that again provides circumstantial evidence for competition among these neurons for synaptic input. It thus appears that posthatching retinal development in quails, including the degeneration of many neurons of the inner plexiform layer, could be slow and may continue for some time after the 21th day of age, allowing the remaining neurons to produce more dendrites and neurites, thus increasing or maintaining the thickness of the inner plexiform layer. A decrease in the functionality of rods and cones between the 21th and the 75th days of age could also be a factor responsible for the decrease in b-wave amplitude. Indeed, Yamada *et al.* (1998) also reported a decrease in the quail retina thickness until the 30 days of posthatching age. During the same age interval, they observed a decrease in the ganglion and inner nuclear cell density. They observed a notable decrease in the inner nuclear and plexiform layers particularly between the 20th and the 30th days of age. However, in the present study, the inner plexiform layer did not suffer any decrease (see Fig. 8).

In the quails, the number of ganglion cells also suffered a progressive decrease from birth to the 75 days of age. In 12- to 16-day embryos, some 30-40% of ganglion cells degenerate (Hughes & McLoon 1979). Such a reduction in the ganglion cell number would not affect too much visual acuity in quails. Visual acuity is depending both on cones and ganglion cells, i.e., both structures are responsible for the detection of movement and fine details (Dowling 1987, Hodos *et al.* 1991, McIlwain 1996). Quails have more cones and ganglion cells than pigeons; this may allow them finer vision compared to pigeons. In quails, ganglion cells are more abundant in the central region of the retina and they gradually decrease in number from the center towards the periphery of the retina (Ikushima *et al.* 1986). In pigeons, ganglion cells are more abundant in the central and dorso-temporal areas (Binggeli & Paule 1969).

Posthatching growth of altricial birds is three or four times faster than that of precocial ones (Ricklefs 1979b). For example, the altricial European Starling (*Sturnus vulgaris*) grows four times faster than the semiprecocial Common Tern (*Sterna hirundo*), and two and half times faster that the precocial Japanese Quail (Ricklefs 1979b). Precocial birds have, at birth, a relatively larger brain giving them the capacity to forage, run about, and regulate their body temperature soon after they hatch, but their growth rate is very slow, resulting in a relatively smaller brain in adults; the opposite is observed in altricial birds (Starck 1993).

The main facts shown in this study are that ERG responses are lacking in 0-day hatchlings and 7-day nestlings of the Common Pigeon; beginning with 21-day nestlings, they reach maximal intensity and remain relatively stable or increase only slightly thereafter. In the Japanese Quail, on the other hand, measurable ERG responses take place in 0-day chicks and increase in amplitude until the age of 21 days, but decrease some time between 21 and 75 days of age. This is in agreement with the morphological features of the retinas, in particular with the lack of photoreceptors in 0- and 7-day pigeons, and with the presence of fully developed and easily distinguishable rods and cones in 0-day quail hatchlings.

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