Altered Retinal Function and Structure after Chronic Placental Insufficiency

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PURPOSE. To consider whether growth restriction secondary to chronic placental insufficiency results in postnatal deficits in retinal structure and function.

METHODS. Chronic placental insufficiency was induced just before midgestation in guinea pigs through unilateral ligation of the uterine artery. Eight weeks after birth, electroretinograms were recorded from prenatally compromised (PC, n = 6) and control (n = 15) animals. Data were collected for b-wave amplitude and implicit time, also the modeled receptoral (P3) response and oscillatory potentials were extracted. After electroretinography, retinas were prepared for structural analysis (PC, n = 6; control, n = 7). A separate cohort of PC (n = 8) and control (n = 9) animals underwent tyrosine hydroxylase immunoreactivity (TH-IR, dopaminergic neurons) and nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry (neuronal nitric oxide synthase, nNOS)—these being markers of amacrine cell subpopulations.

RESULTS. Electroretinography revealed two PC guinea pigs with marked changes to saturated receptor amplitude (RmP30), sensitivity (log S) and postreceptoral waveforms. Grouped PC data revealed significantly reduced RmP30, whereas log S was not affected. The b-wave amplitudes were normal, but b-wave implicit times were delayed (P < 0.05) in PC animals. Amplitudes and peak times of oscillatory potentials were also significantly reduced and delayed (P < 0.05). Morphologic analysis revealed significant reductions in all cellular and plexiform (synaptic) layers in both the central (P < 0.05) and peripheral (P < 0.05) retina in PC animals. The outer retina, which contains the photoreceptors and the outer plexiform layer was particularly affected. The reduced growth of plexiform layers suggests a reduction in the growth of the neuropile in PC animals compared with control animals. The total number (P < 0.03) and density (P < 0.05) of TH-IR neurons was reduced, whereas the total number and density of nNOS-positive amacrine cells was not significantly different between PC and control animals.

CONCLUSIONS. Chronic placental insufficiency results in morphologic and functional alterations to the retina. Electroretinogram deficits in PC animals indicated both inner and outer retinal anomalies. Such affects could contribute to the visual impairments reported in very-low-birth-weight children, some of whom are growth restricted. (Invest Ophthalmol Vis Sci. 2002;43:805–812)

Infants of very-low-birth-weight (VLBW), approximately 15% of whom are growth restricted (i.e., small for gestational age), are at increased risk for visual impairment, including deficits in acuity, color vision, and contrast sensitivity. Poor visual function early in life may result in motor and cognitive dysfunction. Indeed, poor motor skills and performance IQ in VLBW children are best predicted by reduced contrast sensitivity at higher spatial frequencies, which is often associated with retinal disease. However, the cause of visual impairments in VLBW infants is unknown. It is important to determine whether the deficits relate to adverse pre-, peri-, or postnatal events, particularly in light of the increasing survival rate of VLBW infants.

A compromised prenatal environment, after dietary manipulation, has been shown to alter retinal function as measured using the electroretinogram (ERG). Chronic placental hypoxemia and malnutrition result in abnormal retinal neuronal growth and optic nerve myelination in guinea pig fetuses near term. The purpose of the present study was to determine whether prenatal compromise (PC) could result in altered retinal structure and function later in life.

The long gestation period of the guinea pig (~67 days) offers better temporal resolution of retinal development than is possible in species with a shorter gestation time, such as the rat. Additionally, at midgestation discrete classes of neurons and synapses develop in the guinea pig retina, more closely mimicking human intrauterine growth. In the present study, chronic placental insufficiency was induced by unilateral ligation of the uterine artery just before midgestation. At this stage of guinea pig retinal development, neurogenesis is largely complete, but cellular differentiation and synaptogenesis are ongoing. Offspring were examined 8 weeks after birth, which is equivalent to adolescence in humans.

Of particular interest was the effect of PC on the number and distribution of tyrosine hydroxylase-immunoreactive (TH-IR) amacrine cells, which are known to be dopaminergic. These cells may have a role in contrast processing and were reduced in number at term and 8 weeks after hypoxemia in an ovine model of chronic placental insufficiency. We also assessed a chemically distinct subset of amacrine cells which contain neuronal nitric oxide synthase (nNOS) and which are known to be resistant to hypoxic injury in the central nervous system. Indeed, the nitric oxide (NO)-producing cells may be involved in mediating neurotoxicity. We have previously shown that nNOS-immunoreactive amacrine cells also stain with nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry, and we used this technique in the present study.

METHODS

Chronic Placental Insufficiency

All investigations conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Pregnant guinea pig dams,
were anesthetized under dim red light. Exposure was calculated as previously described. These measurements yielded an unligation remained in place until delivery. At birth, animals were classified as PC (n = 14) if their birth weight was 2 SDs below that of age-matched control animals. Animals from mothers that underwent a sham procedure served as control subjects (n = 16). PC and control animals were randomly assigned to two cohorts: The first underwent ERG and measurement of the thickness of the retinal layer (n = 7 control and n = 6 PC), and the second cohort underwent neurochemical assessment (THIR and NADPH-d histochemistry: n = 9 control and n = 8 PC). An additional eight animals from sham-treated mothers served as ERG control subjects only.

Electroretinography

ERGs were recorded from the left eye of control (n = 15) and PC (n = 6) animals at postnatal week 8. Darkadapted (>12 hours) animals were anesthetized under dim red light (λmax = 650 nm). Mydriasis (≥4 mm) was achieved with tropicamide (Mydriacyl 0.5%; Alcon Laboratories, Newbury Park, CA) was delivered into a Ganzfeld sphere. Flash exposure was digitized at 2 kHz. 3000 Hz, model P55; Grass Instruments, Inc., West Warwick, RI) and a single photomultiplier electrode (Hansen Ophthalmic Development Laboratory, Solon, IA) was used to detect changes in the ERG signal. ERGs from right eyes were collected and the raw data was averaged at each exposure with an interstimulus interval of 40 to 180 seconds. Responses were amplified (gain ×1000, −5 dB at 0.1 and 3000 Hz, model P55; Grass Instruments, Inc., West Warwick, RI) and digitized at 2 kHz.

A commercial photographic flash unit (285V; Vivitar Photographics, Newbury Park, CA) was delivered into a Ganzfeld sphere. Flash exposure was calculated as previously described. These measurements yielded an unfiltered photopic exposure of 3.5 log cd s/m², which was attenuated using calibrated neutral-density filters (Wratton; Eastman Kodak Co., Rochester, NY).

ERG Analysis. Conventional ERG b-wave (peak-to-peak) amplitudes and implicit times were determined from the raw data. In addition, we modeled the leading edge of the a-wave using a computational model of phototransduction arrived at by,

\[ P5(t, t_{0}) = Rm_{pca} \cdot \left[ 1 - e^{-(t-t_{0})/\tau} \right] \]  

for t > t₀, where P5 is the summed rod photocurrent as a function of stimulus exposure, t (in candelas per second per square meter) and time t (in seconds) and Rm_pca (in microvolts) is its saturated response. Sensitivity (S, in cubic second square meters per candela) is scaled by t, whereas t₀ (in seconds) is a delay that includes biochemical and other recording latencies. This model was fitted to the raw data as an ensemble (0.7–2.5 log cd s/m²) up to the first minimum of each a-wave or a maximum of 20 ms. Optimization was achieved by minimizing the root-mean-square error term with the solver module of a spreadsheet program (Excel; Microsoft Corp., Redmond, WA).

Oscillatory potential (OP) visualization is hampered by intrusion from the a-wave and b-wave. As a consequence, we isolated the OP by digitally subtracting the a-wave and b-wave from the raw data and band-pass filtering the resultant waveform13 (55–250 Hz, 512-tap, finite impulse response filter, Blackman window). After OP extraction, we modeled the data in the time domain using a Gabor function (equation 2c), which represents the multiplication of a Gaussian envelope (equation 2a) with a sine wave carrier (equation 2b).

\[ f(x) = \sin 2 \cdot \pi \cdot p \]  

As a function of time (x), the Gaussian envelope (equation 2a) is described by its maximum amplitude (a, OP amplitude in microvolts), peak envelope position (m, OP implicit time, in milliseconds), and spread (seconds, milliseconds). The sine wave carrier (equation 2b) is described by its frequency (b, in hertz) and phase relative to the start of the waveform (p, in degrees). Fitting was achieved by floating all parameters and minimizing the mean-square-error term, using a customized Levenberg-Marquardt optimization routine. Such modeling presumes no physiological basis but allows OPs to be easily compared between control and treatment groups. The model provides an excellent fit to the extracted waveforms, as shown in Figure 4A. Importantly, an excellent correlation is found between the amplitude of the Gaussian envelope (a in equation 2a) and the normal parameters used to describe these oscillations, such as the amplitude of the largest OP (r² = 0.90) and the root-mean-square amplitude for all OPs (r² = 0.91).

**Morphologic Analysis**

At the conclusion of the ERG recording, deeply anesthetized animals (pentobarbital sodium, 200 mg/mL intraperitoneally) were perfused through the left ventricle with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB, pH 7.4). Retinas from right eyes were dissected 1 hour after perfusion and prepared for structural analysis (PC, n = 6; control, n = 7). A second cohort of animals (PC, n = 8; control n = 9) were perfused at 8 weeks after birth and their retinas processed for THIR immunohistochemistry and NADPH-d histochemistry.

**Araldite Embedding.** Right eyes from control and PC animals were enucleated, the cornea removed, and the eyecup placed directly into 1% glutaraldehyde in 4% PFA (pH 7.4). Small blocks of retina were sampled at both central (immediately adjacent to the optic disc in nasal retina) and peripheral (inferior temporal quadrant, 5 mm from the optic disc) locations. Sections were postfixed in 1% osmium tetroxide for 30 minutes, stained with 1% uranyl acetate, and embedded in Epon Araldite. Seminith (1 μm) transverse sections were cut from two blocks of central and peripheral retina from each animal.

**Analysis of Retinal Layers.** Measurements were made of the mean thickness of total retina, ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and photoreceptor layer (PRL), including both inner and outer segments. Sections were projected at ×600 magnification, and individual layers were measured using a computerized digitizing pad (Sigma Scan Pro ver. 4.0; SPSS Science, Chicago, IL). The mean thickness of each layer was calculated in each animal.

**Immunohistochemistry**

**THIR Immunohistochemistry.** The right retina from each animal was prepared as a wholemount and reacted for THIR using the avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA), as previously described. The primary antiserum, mouse monoclonal anti-TH (Chemicon International, Temecula, CA) was diluted at 1:1000 and incubated for 72 hours. Retinas were then incubated for 45 minutes in the secondary antibody (1:200, biotinylated anti-mouse IgG; Vector Laboratories, Burlingame, CA) followed by incubation in the avidin-biotin complex (1:200; Vector Laboratories, Burlingame, CA). Retinas were reacted with 0.5% 3,3′-diaminobenzidine (DAB) solution in 0.01% hydrogen peroxide to produce a brown reaction product. Control experiments, performed by omitting the primary antibodies, failed to show staining.

**NADPH-d Histochemistry.** The left retina from each animal was washed in 0.1 M Tris buffer (pH 7.6) and reacted for 45 minutes in the NADPH-d reaction solution, which contained 0.25 mg/mL nitroblue tetrazolium (Sigma Chemical Co., St. Louis, MO), 1 mg/mL β-NADPH (Roche Molecular Biochemicals, Mannheim, Germany) and 0.90).

\[ \text{d} = \text{PC} \]
0.5% Triton X-100 (Sigma) in 0.1 M Tris buffer (pH 7.6) at 37°C. Tissue was then washed in Tris buffer, mounted, and coverslipped with aqueous mounting medium (Glycergel; Dako, Carpinteria, CA). Control experiments were performed by omitting β-NADPH, whereupon staining failed to occur.

**Analysis of Immunohistochemistry and Histochesistry.** Density, total number, and somal area for each class of TH-R (type I and II–) and NADPH-d-positive (ND1 and ND3) amacrine cell were determined. Mean cell density was measured, using a computer-assisted stereological tool (Castgrid, ver. 1.10; Olympus, Birkeroed, Denmark) set to randomly sample 100 fields (0.04 mm² per retina). The total area of the retina was determined from a projected image of retinal wholemounts using a computerized digitizing pad (Sigma Scan Pro ver. 4.0; SPSS Science). Total numbers were calculated from the mean density and retinal area measurements. To analyze somal area, 50 to 100 randomly selected somata were sampled throughout each retina for each cell class using the computer-assisted stereological system at ×1000 magnification (oil immersion), and mean somal area was calculated.

**Shrinkage**

The retinal areas of one control and one PC animal were measured before and after the tissue was reacted for TH-R. Shrinkage was determined to be less than 0.5% for both control and PC tissue. Consequently, shrinkage was not taken into account when assessing neuronal density or total number.

**Statistical Analysis**

Anatomic measurements were made using a single-blind paradigm with coded slides, and these data are shown as the mean (± SEM). Our ERG data were found to be non-Gaussian. As a consequence, nonparametric indices are used to describe group distributions (median and interquartile range). The ERG figures show 5th and 95th percentiles and outliers for control animals, whereas PC animals are shown by individual data points. Statistical comparisons of group parameters was achieved using nonparametric tests (α = 0.05). In the case of the ERG the Kruskal-Wallis test was used, whereas anatomic comparisons were performed with the Mann-Whitney U test.

**RESULTS**

At birth, body weights (control 108.3 ± 3.6 g vs. PC 61.6 ± 2.5 g; P < 0.0001) were reduced in PC animals. Body weight (control 566.3 ± 22.9 g vs. PC 450.1 ± 13.2 g; P < 0.0005), brain weight (control 4.04 ± 0.08 g vs. PC 3.60 ± 0.05 g; P < 0.0001), and crown-to-rump length (control 24.9 ± 0.5 cm vs. PC 22.9 ± 0.4 cm; P < 0.005) remained lower at 8 weeks in PC than in control animals. However, liver weight (control 19.7 ± 1.2 g vs. PC 18.5 ± 0.9 g; P < 0.3) and brain-to-body weight ratio (control 7.0 ± 0.4 × 10⁻⁷ vs. PC 8.0 ± 0.3 × 10⁻⁷; P < 0.08) were not significantly different between control and PC animals.

**Electroretinography.** Representative waveforms for a control animal (Fig. 1, N), show characteristic changes with intensity, where a- and b-waves increase in amplitude and have faster implicit times. Note the double-peaked b-waves in the guinea pig, in which a faster cone-dominated process (~50 ms) becomes apparent at 1.0 log cd · s/m². PC animals showed varying degrees of functional deficit at postnatal week 8 (Fig. 1, PC1–PC6), ranging from subtle (Fig. 1, PC1, reduced OPs) to marked ERG loss (Fig. 1, PC4 and PC5, reduced a-wave, b-wave, and OPs). In the most significantly growth-restricted animal (40 g at birth; Fig. 1, PC6), the b-wave was absent at all intensities.

Figures 2A and 2B consider the photoreceptor (P3) contribution to the ERG and show that four PC animals (filled circles) had saturated responses (RmP₃) that lay below the 5th percentile of control responses (box plot area). In contrast, only two of the most severely affected animals had transduction sensitivities (log S) below the 5th percentile. As a group, PC animals had RmP₃ significantly removed from the control group (control: −98.7 μV, [5th, 95th percentiles: −106.2, −82.30]; PC: −46.0 μV, [−77.4, −17.9]). However, log S (control: 2.77 m²/cd · s², [2.69, 2.95]; PC: 2.90 m²/cd · s² [2.18, 3.11]) was not significantly different between PC and control animals. As a consequence of altered receptor function we can expect altered postreceptorial waveforms.

The peak-to-peak intensity–response function (Fig. 3A) shows a large notch at intermediate intensities characteristic in this species. This notch results from an interaction between a negative-going photoreceptoral potential and a positive postreceptoral response. Although significant b-wave losses were observed in three PC animals (Fig. 3A) at high intensities, the PC group (filled symbols, n = 6) was not statistically different from control animals (box plots, n = 15) across all intensities. The lack of significance may reflect the limited power available from our small sample. However, b-wave implicit times were significantly slower in PC animals than in control animals at −1.7 log cd · s/m² and above 0.1 log cd · s/m² (Fig. 3B, P < 0.05). We will argue later that this finding is consistent with an abnormal RmP₃.

Representative extracted (symbols) and modeled OPs (lines) in Figure 4A show that PC guinea pigs (filled circles, thick lines, PC2) had slower and smaller OPs than did control animals (unfilled squares, thin line), particularly at high light levels (>1.0 cd · s/m²). Nonparametric statistical comparison showed that OP envelope amplitude and peak time were significantly smaller (P < 0.05, >1.0 log cd · s/m²) and slower (P < 0.05, at all stimulus exposures except 2.5 log cd · s/m²), respectively in PC animals (filled symbols) compared with control animals (box plots; Figs. 4B and 4C, respectively).

**Retinal Layers.** Measurements of the six individual layers of the retina and the total retinal thickness were made for both central and peripheral retina in control and PC animals at 8 weeks after birth. The basic laminar morphology was fundamentally the same in both groups. In PC retinas there was no evidence of displaced cell bodies to indicate delayed or aberrant migration of cells from the germinal layer to their appro-
priate neuronal layers. However, total mean thickness of central and peripheral retina was reduced \((P < 0.05)\) in PC compared with control animals, because of a significant reduction in all retinal layers (Table 1, Figs. 5A, 5B). In the most severely growth-restricted animal (Fig. 1, PC6), which showed no postreceptoral response, the OPL was 1.5 \( \mu \text{m} \) in thickness and in some places appeared to be absent—this, compared with an average thickness of 8.7 ± 0.6 \( \mu \text{m} \) in control animals.

**TH-IR Amacrine Cells.** In contrast to previous findings, two morphologically distinct classes of dopaminergic amacrine cells (types I and II) were observed in the guinea pig retina. Type I neurons are large, are intensely immunoreactive for TH, and exhibit two to three primary dendrites extending from the soma (Figs. 5C, 5E), whereas type II neurons are smaller, are less immunoreactive, and have no stained dendritic processes (Figs. 5D, 5F). Both classes of TH-IR cells (types I and II) were distributed across the entire retina in control and PC animals.

At 8 weeks after birth, there was no significant difference between the total retinal areas of PC and control animals. However, a significant reduction in the mean density (32%, \( P < 0.05 \)) and number (33%, \( P < 0.05 \)) of TH-IR amacrine cells was found in PC (Fig. 5D) compared with control animals (Fig. 5C; Table 1). This reflects a nonselective reduction in density and total number of both type I (34% and 36%, \( P < 0.02 \)) and type II (34% and 34%, \( P < 0.02 \)) amacrine cells. Despite these changes, no significant difference was observed in the somal area of type I or II amacrine cells between the two groups (Table 2).

**NADPH-d-Positive Amacrine Cells.** Two types of NADPH-d-positive cells were present in the guinea pig retina, ND1 and ND3, according to the classification of Cobcroft et al. ND1 cells had large oval somata and several thick, intensely stained dendrites arising from each of the cell bodies. ND3 cells were much smaller and rounder in appearance than the ND1 cells, with no evidence of dendritic processes. Both classes of NADPH-d-positive amacrine cells (ND1 or ND3) were distributed across the entire retina. There was no significant difference between groups in the total number (control, 8,956 ± 1,200 vs. PC, 10,175 ± 1,458) or density (62 ± 5 vs. 74 ± 8 cells/mm\(^2\)) of NADPH-d-positive cells. The total number (ND1: 2802 ± 287 vs. 3191 ± 242; ND3: 6154 ± 938 vs. 6984 ± 1324) and density (ND1: 19.5 ± 1.0 vs. 23.5 ± 1.0 cells/mm\(^2\); ND3: 42.4 ± 4.6 vs. 50.7 ± 8.2 cells/mm\(^2\)) of ND1 and ND3 cells were not different between the groups. Additionally, the mean somal areas of ND1 (136 ± 10 vs. 124 ± 6 mm\(^2\)) and ND3 cells (94 ± 7 vs. 84 ± 2 mm\(^2\)) were not significantly different between control and PC animals.

**Process Outgrowth.** The morphology of TH-IR and NADPH-d-positive amacrine cell processes could not be as-

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**FIGURE 3.** Peak-to-peak (b-wave) amplitude and implicit time versus stimulus exposure. (A) B-wave amplitude for control animals (box plots) showing the 5th and 95th percentile confidence limits and outliers (○). (●) PC animals. Every second stimulus exposure has been omitted for clarity. †Statistical significance for group comparison, \( P < 0.05 \). (B) B-wave implicit time for control (box plot) and PC (●) animals. Other details as in (A).

**FIGURE 2.** Phototransduction parameters for control (N, box plot) and PC animals (filled circles). (A) Saturated amplitude \((R_{mP3})\) for control animals with the 5th and 95th percentile confidence limits and outliers denoted by the dashed area and unfilled circles, respectively. †Statistical significance for group comparison, \( P < 0.05 \). (B) Transduction sensitivity \((\log S)\) for control animals and PC animals. Other details as in (A).
sessed quantitatively because of the density of the process network. However, qualitative examination suggests that TH-IR processes may be reduced in PC (Fig. 5F) compared with control animals (Fig. 5E).

**DISCUSSION**

In this study, chronic placental insufficiency caused deficits in retinal structure and function later in life. The insult was initiated during neurogenesis, but before the onset of critical developmental steps such as synaptogenesis and photoreceptor formation. Guine pig shows a similar pattern of retinal histogenesis to humans, and thus model intrauterine compromises with relevance to the human situation. Chronic placental insufficiency in the guinea pig results in pups with significantly lighter birth weight. These animals remain significantly lighter than age-matched control animals and show sustained morphologic and functional changes at 8 weeks after birth.

Consistent with the low birth weights, our PC animals showed significant reductions in the thicknesses of all retinal layers. Total retinal thickness was reduced by 20% centrally (P < 0.002) and 22% peripherally (P < 0.007). This effect was pronounced in the outer retina, specifically the outer segment (~27%) and OPL (~36%), consistent with the observed reduction in Rm_p. Although any firm conclusion regarding structural and functional relationships in terms of photoreceptor function (Rm_p) must be guarded because of our sample size, we note that four of six of our PC animals had both reduced Rm_p and shorter outer segment lengths than any of our control animals. In addition, all PC animals had smaller OP amplitudes and thinner INLs than control animals.

Inner retinal deficits may arise from outer retinal changes. Consistent with this proposal, Figure 6 shows excellent correlation between changes in Rm_p and inner retinal amplitudes (Fig. 6A, b-wave r^2 = 0.74; Fig. 6B, OP r^2 = 0.78). Although a reduction in b-wave amplitude was expected in our PC group, we failed to find any statistically significant change. However, b-wave timing was significantly delayed in PC animals. These findings appear incongruous but are supported by the proposal of Hood and Birch who showed that reduced Rm_p with normal log S levels can produce delayed b-wave timing without affecting b-wave amplitude. Most of our PC animals conformed to this expectation; thus, most of the functional and

<table>
<thead>
<tr>
<th>Table 1. Retinal Layers in Central and Peripheral Regions</th>
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<tr>
<td><strong>Central Retina</strong></td>
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<tr>
<td>Total retinal thickness</td>
</tr>
<tr>
<td>GCL</td>
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<tr>
<td>Inner plexiform layer</td>
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<tr>
<td>Inner nuclear layer</td>
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<td>Outer plexiform layer</td>
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<td>Outer nuclear layer</td>
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<tr>
<td>Inner segments</td>
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<tr>
<td>Outer segments</td>
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<td>Total photoreceptor layer</td>
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</tbody>
</table>

Data are mean micrometers ± SEM for seven control and six PC animals.
anatomic deficits in the inner retina appear to be secondary to a receptoral dysfunction.

As previously mentioned, the maturation of photoreceptor outer segments and the formation of their synapses are critical steps in the establishment of normal visual function. Photoreceptor maturation in the guinea pig begins at day 45 of gestation and is complete by day 62 (lamellae formation), some 5 to 6 days before birth.11 The intrauterine growth restriction in the present study was induced during this critical period of photoreceptor development and as a consequence, significant reductions were found in outer segment length and ONL thickness in the present study. It is important to note that similar changes in outer segment length have been shown to predict

Table 2. TH-IR Amacrine Neurons in Retina

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Prenatally Compromised</th>
</tr>
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<tbody>
<tr>
<td>All cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total retinal area (mm²)</td>
<td>144 ± 7</td>
<td>143 ± 4</td>
</tr>
<tr>
<td>Mean density cells/mm²</td>
<td>63 ± 6</td>
<td>42 ± 14*</td>
</tr>
<tr>
<td>Total number cells/retina</td>
<td>9122 ± 921</td>
<td>5982 ± 640†</td>
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<tr>
<td>Type I cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean density cells/mm²</td>
<td>18 ± 2</td>
<td>12 ± 1†</td>
</tr>
<tr>
<td>Total number cells/retina</td>
<td>2603 ± 272</td>
<td>1782 ± 207†</td>
</tr>
<tr>
<td>Somal area (μm²)</td>
<td>133 ± 5</td>
<td>129 ± 5</td>
</tr>
<tr>
<td>Type II cells</td>
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<td></td>
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<tr>
<td>Mean density cells/mm²</td>
<td>45 ± 5</td>
<td>29 ± 3*</td>
</tr>
<tr>
<td>Total number cells/retina</td>
<td>6519 ± 723</td>
<td>4199 ± 495‡</td>
</tr>
<tr>
<td>Somal area (μm²)</td>
<td>77 ± 2</td>
<td>77 ± 3</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM for nine control and eight PC animals. * P < 0.03, † P < 0.05, ‡ P < 0.02, § P < 0.01.
reductions in \( R_{\text{mP}} \) in other species\(^{28-30} \) and may explain the \( R_{\text{mP}} \) deficits found in our PC animals.

However, we also found selective postreceptor anomalies greater than would be predicted from an isolated receptoral loss (Fig. 1. PC5 and PC6). The reduced phototransduction sensitivity (Fig. 2B, log \( S \)) in PC5 and PC6 confirms the presence of more extensive functional defects.\(^{20,31} \) Previous ultrastructural assays have identified poor formation of outer segments in the retina of prenatal PC guinea pigs,\(^{10} \) which may account for the change in log \( S \) in our severely affected animals (Fig. 2, PC5 and PC6). Similarly, the gross reduction in OPL thickness (83%) may underlie the complete absence of inner retinal function found in PC6. These functional and morphometric changes argue for a more generalized lesion in some PC retinal function found in PC6.\(^{32} \) These functional and morphometric changes may underlie the complete absence of inner retinal function found in PC6. These functional and morphometric changes argue for a more generalized lesion in some PC retinal function found in PC6.

Our morphologic and functional findings are consistent with a graded effect of chronic placental insufficiency. Mild placental insufficiency may result in a primary receptoral lesion with consequent deficits across the entire retina (animals PC1–PC4). The cause of this lesion is not clear, but may involve metabolic and/or hypoxic mechanisms. More severely affected animals (i.e., PC5 and PC6) appeared to sustain substantial inner retinal deficits in addition to receptoral lesions. Consistent with this suggestion, functional\(^{33,34} \) and anatomic\(^{35} \) studies have found that severe oxygen deprivation and vascular insufficiency have a graded effect across the retina, with the most severe cases having greater losses in the inner retina (ganglion and bipolar cells and b-wave) than in the outer retina.

Although this proposal remains speculative, previous findings of smaller and fewer mitochondria in photoreceptor inner segments of prenatal PC guinea pigs\(^{36} \) suggests the presence of altered metabolism. Similarly, the significant reduction in dopaminergic amacrine cells found in our animals is consistent with oxidative stress.\(^{30} \) In particular, that NADPH-d–NOS-IR-positive amacrine cells were not reduced further supports this proposal, because these cells are known to show relative resistance to hypoxic injury.\(^{14,16,17} \) The significance of this differential susceptibility of inner retinal cell classes to hypoxic injury in the developing retina remains to be elucidated.

An interesting finding was the preferential loss of OPs in a few PC animals (Fig. 6B). OPs have been shown to be dependent on retinal circulation\(^{37} \) and are sensitive indicators of retinal ischemia,\(^{38} \) such as in retinopathy of prematurity,\(^{26} \) diabetic retinopathy,\(^{39} \) central retinal vein occlusion,\(^{40} \) and systemic hypertension.\(^{41} \) The origin of the OPs remains equivocal; however, amacrine cells\(^{12} \) and dopamine\(^{43} \) may be involved in initiating neuronal events that underlie the OPs. Hence, the increased susceptibility to oxidative stress\(^{30} \) of both dopaminergic amacrine cells and OPs may result in the present study may reflect a common hypoxemic mechanism.

We believe that retinal damage resulting from PC is likely to arise from several factors, because this form of compromise is known to induce both hypoxemia\(^{44} \) and malnutrition,\(^{45} \) as well as an altered endocrine status.\(^{46,47} \) Currently, we are unable to distinguish between the individual contributions of these factors to the morphologic and functional outcomes observed in this study. Nonetheless, our data argue that the sequelae to hypoxic insult can account for most of these outcomes.

**Conclusions**

Chronic placental insufficiency can result in long-term structural and neurochemical alterations in the retina that are associated with disruption of normal visual function, as detected by the ERG. Postnatal functional deficits vary in severity from subtle deficits in \( R_{\text{mP}} \) and OPs to a complete absence of the b-wave. In compromised human fetuses a similar susceptibility of retinal neurons may contribute to the cause of visual deficits.

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**References**


812  Bui et al.


