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Journal Title: INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE
Volume: Volume 54, Number 13
Publisher: ASSOC RESEARCH VISION OPHTHALMOLOGY INC |
2013-12-01, Pages 8275-8284
Type of Work: Article
Publisher DOI: 10.1167/iovs.13-12544
Permanent URL: https://pid.emory.edu/ark:/25593/vk1jb

Final published version: http://dx.doi.org/10.1167/iovs.13-12544

Accessed June 11, 2021 1:34 AM EDT
The Rat With Oxygen-Induced Retinopathy Is Myopic With Low Retinal Dopamine

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Submitted: June 5, 2013
Accepted: October 14, 2015
DOI:10.1167/iovs.13-12544

PURPOSE. Dopamine (DA) is a neurotransmitter implicated both in modulating neural retinal signals and in eye growth. Therefore, it may participate in the pathogenesis of the most common clinical sequelae of retinopathy of prematurity (ROP), visual dysfunction and myopia. Paradoxically, in ROP myopia the eye is usually small. The eye of the rat with oxygen-induced retinopathy (OIR) is characterized by retinal dysfunction and short axial length. There have been several investigations of the early maturation of DA in rat retina, but little at older ages, and not in the OIR rat. Therefore, DA, retinal function, and refractive state were investigated in the OIR rat.

METHODS. In one set of rats, the development of dopaminergic (DAergic) networks was evaluated in retinal cross-sections from rats aged 14 to 120 days using antibodies against tyrosine hydroxylase (TH, the rate-limiting enzyme in the biosynthesis of DA). In another set of rats, retinopathy was used to evaluate spherical equivalent (SE), electoretinography (ERG) was used to evaluate retinal function, and high-pressure liquid chromatography (HPLC) was used to evaluate retinal contents of DA, its precursor levodopamine (DOPA), and its primary metabolite 3,4-dihydroxyphenylacetic acid (DOPAC).

RESULTS. The normally rapid postnatal ramiﬁcation of DAergic neurons was disrupted in OIR rats. Retinography revealed that OIR rats were relatively myopic. In the same eyes, ERG confirmed retinal dysfunction in OIR. HPLC of those eyes’ retinae conﬁrmed low DA. Regression analysis indicated that DA metabolism (evaluated by the ratio of DOPAC to DA) was an important additional predictor of myopia beyond OIR.

CONCLUSIONS. The OIR rat is the ﬁrst known animal model of myopia in which the eye is smaller than normal. Dopamine may modulate, or fail to modulate, neural activity in the OIR eye, and thus contribute to this peculiar myopia.

Keywords: retinopathy of prematurity, myopia, dopamine, rat, development, ocular

In a recent magnetic resonance imaging (MRI) study, Chui et al.1 found the eyes of rats with oxygen induced retinopathy (OIR) to be characterized by short axial length, increased corneal power and lens power, and proportionally shallow anterior segment. Furthermore, using an untested approach, they calculated, but did not measure, the mature rat eye with a history of OIR to be relatively myopic. Dopamine (DA) has been implicated as a mediator of ocular growth, mostly as a stop signal. In form-deprivation myopia models, eye enlargement is accompanied by decreased retinal DA and DA agonists prevent the myopia.2–7 Thus, DA might participate in attenuating the growth of the OIR eye, and possibly (via downstream paracrine messengers) in the development of the anterior segment.

In the retina, DA is produced by a single class of amacrine cell8–13 that constitutes less than 0.1% of the population of retinal neurons.8,14,15 Stimulation of the retinal ON pathway by light induces DA release from these cells.6 Defects in retinal ON pathway signaling are associated with anomalous eye growth,16 and OIR in the rat produces persistent dysfunction of the neurosensory retina.17 OIR-induced changes in DA release might participate in this retinal dysfunction since DA regulates many aspects of retinal signaling. For example, DA alters retinal circuitry to favor cone-driven, contrast-sensitive pathways in bright light18 and rod-driven, light-sensitive pathways in dim light19 in part by inducing nitric oxide (NO) release.20,21 Dopamine also modulates connexin-36 gap junctions,22–24 glutamatergic synapses,25,26 voltage-gated potassium channels in ON-cone bipolar cells,22,27 Na⁺,K⁺-ATPase channels in photoreceptors,28 and cGMP-gated channels in cones.29 It is, therefore, plausible that DA participates both in the retinal dysfunction and the prevalent ametropia recently documented in OIR rats’ eyes.

In the present study of the OIR rat, the development of retinal dopaminergic (DAergic) neurons and the association of refractive state, retinal function, and DA metabolism are investigated. Herein is reported that (1) despite having recently been found to be characterized by a small eye,1 the OIR rat is myopic, (2) the OIR rat is characterized by delayed and attenuated development of retinal DAergic networks, and (3) diminished DA metabolism is associated with the magnitude of the myopia. Provocatively, human eyes with a history of retinopathy of prematurity (ROP) are, on average, also
FIGURE 1. Dopamine (DA) synthesis and metabolism. Inhibition of aromatic L-amino acid decarboxylase (AAAD) by m-hydroxybenzylhydrazine (NSD-1015) prevents the decarboxylation of L-dihydroxyphenylalanine (DOPA) into DA. Shadowed metabolites were assessed in isolated retinae by high-pressure liquid chromatography (HPLC). Other abbreviations: TH, tyrosine hydroxylase; MAO, monoamine oxidase; COMT, catechol-O-methyltransferase; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; DBH, dopamine β-hydroxylase; DHPG, 3,4-dihydroxyphenylethylamine glycol; MHPG, 3-methoxy-4-hydroxyphenyl glycol; PNMT, phenylethanolamine N-methyltransferase.

characterized by steep corneas, thick lenses, and shallow anterior segments that render them myopic in spite of short axial length. Thus, the rat with OIR may provide insight into the development of some of the ocular features of human ROP eyes.

METHODS

Experimental Design

All experiments were performed in Sprague-Dawley rats (Charles River Laboratories, Worcester, MA). In the first set of experiments, DAergic development in the retina was studied immunohistochemically (IHC) in retinal cross sections. Prior to these IHC studies, the antibodies used were validated by Western blot and by comparison of labeled cell structures to published literature. In the second set of experiments, individual rats were refracted, tested by electroretinography (ERG), and then their retinal DA content was evaluated by high-pressure liquid chromatography (HPLC). These data were analyzed for significant relations using regression analyses. All experiments were conducted with the approval of the Institutional Animal Care and Use Committee at Boston Children’s Hospital.

Animals and Induction of Retinopathy

Retinopathy was induced in rats following the “50/10” method of Penn et al. Neovascularization and an avascular peripheral retina characterize this retinopathy. Although the rat is not born prematurely, the rat pup’s eyes are in a state of maturity that corresponds to approximately human midgestation. On the day of birth until postnatal day (P) 14, newborn pups and dam were placed in an oxygen-controlled environment (OxyCycler; Biospherix Ltd., Redfield, NY) where the ambient oxygen concentration alternated every 24 hours between 50% and 10% ± 1%. This oxygen regimen targeted the rats’ retinae during a period of rhodopsin and sensitivity development similar to the timing of the supplemental oxygen used to manage very prematurely born human infants. In the OIR rat, and the human infant with active ROP, retinal sensitivity and vessel tortuosity are about the same. Room-air-reared animals served as controls (‘RAR rats’). A total of 87 animals were studied. Numbers of OIR and RAR rats used in each experiment are given in the respective section of the Results. The light cycle was 12 hours dark, 12 hours of 75 lux (or less) light. All samples and measurements were obtained during the light period, at least 2 hours after lights on and 2 hours before lights off, because synthesis and turnover of DA are known to follow a circadian rhythm. All procedures were carried out in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research.

Immunohistochemistry

Validation of Antibodies by Western Blot. A mouse monoclonal antibody (MAB318; Millipore, Billerica, MA) that targets tyrosine hydroxylase (TH, the rate-limiting enzyme in the biosynthesis of DA) was tested for quality by Western blot on one rat’s retinae. Homogenization was performed at 4°C with a lysis buffer containing phosphatase inhibitor cocktail and protease inhibitor according to the instruction of the vendor. Lysates were centrifuged at 14,000g at 4°C for 10 minutes. Protein concentration was assayed by the Bradford method. The samples were boiled for 5 minutes in 2× Laemmli sample buffer (Bio-Rad, Hercules, CA). Protein (15–50 μg) was subjected to electrophoresis on 10% Tris-HCl ready gel (Bio-Rad), followed by transfer to a nitrocellulose membrane that was blocked for 1 hour in a 5% suspension of dried milk in washing buffer and incubated overnight at 4°C with monoclonal anti-TH (1:1000). Visualization was performed using goat anti-mouse secondary antibody conjugated to horseradish peroxidase (GzM-HRP; Millipore). The band was developed with a chemiluminescence detection system (X-OMAT 2000A; Eastman Kodak, Rochester, NY).

Validation of Antibodies by Target Features. An enucleated eyeball was punctured through the cornea and fixed in 4% paraformaldehyde for 1 hour. The retina was flatmounted and the mouse monoclonal primary antibody (MAB318, 1:500; Millipore) was applied. Then, the retina was incubated with 1:1000 secondary antibody (Alexa Fluor 954-conjugated goat anti-mouse), washed, and photographed (40×, DM5500 microscope; Leica, Wetzlar, Germany). The labeled cells were compared with descriptions of DAergic cells in the literature.

Development of Dopaminergic Amacrine Cell Networks. Eyecups for cryosectioning were fixed as described above; then placed in 30% sucrose at 4°C overnight, embedded.
in optimal cutting temperature (OCT) compound (Sakura Finetek USA, Inc., Torrance, CA) and frozen. Retinal cross-sections (16 \mu m) were cut with a cryostat and kept frozen until immunostaining. The monoclonal (MAB318, 1:500; Millipore) and a rabbit polyclonal (AB512, 1:500; Millipore) antibodies against TH were used to detect DAergic cells. The retinal sections were then incubated with 1:1000 respective secondary antibodies (Alexa Fluor 954-conjugated goat anti-mouse and 488-conjugated goat anti-rabbit; Millipore). After washing, each section was counterstained with 4',6-diamidino-2-phenylindole (DAPI) and treated with Antifade (Life Technologies, Grand Island, NY) to prolong immunofluorescence.

Digital images (40%) of each section were obtained using respective filters and overlaid to generate a tricolor (pseudo-RGB) image. To quantify TH+ labeling, a region of interest (ROI) along the border between the inner nuclear layer (INL) and the inner plexiform layer (IPL) was identified and circumscribed by an operator (NZ). Automated segmentation was then performed on the ROI in ImageJ, and the pixels above threshold were counted. The operator then traced the length of the INL-IPL boundary and divided total the number of counted pixels by this length.

**Retinoscopy**

Two experienced retinoscopists (AMB, ABF) performed streak retinoscopy two times in both eyes, at least 15 minutes after instillation of 0.2% cyclopentolate hydrochloride and 1% phenylephrine hydrochloride (Cyclomydral; Alcon, Fort Worth, TX). Each retinoscopist was masked as to the other’s results. Because the “small eye artifact” results in relatively hyperopic estimates of spherical equivalent (SE), the SE in OIR rats was expressed relative to that in RAR rats.

**Electroretinography**

ERG were recorded as previously described. In brief, rats were anesthetized with an intraperitoneal injection of 75 mg kg\(^{-1}\) ketamine and 10 mg kg\(^{-1}\) xylazine. Mydriasis was
induced using Cyclomydrl (Alcon). Body temperature was maintained with a warming pad. Gold loop electrodes were placed on both eyes, the reference was placed in the mouth, and the ground was affixed to the tail or hind foot. ERG stimuli were delivered using an Espion e2 with Colordome Ganzfeld stimulator (Diagnosys LLC, Lowell, MA). Responses were elicited using a series of flashes of doubling intensity, ranging from one that elicited a small b-wave to one that saturated the a-wave (green light emitting diode [LED] 0.000125–2.05 cd s m\(^{-2}\) then white xenon arc 8.20–524 cd s m\(^{-2}\)). The saturating amplitude (\(R_m\)) and sensitivity (\(S\)) of the rod photoresponse were estimated by fit of a model (\(P_3\)) of the biochemical processes involved in the activation of phototransduction to the ERG a-waves. The saturating amplitude (\(R_m\)) and sensitivity (\(1/K\)) of the dark-adapted postreceptor retina were derived from the Naka-Rushton equation fit to the response versus intensity relationship of \(P_2\). The oscillatory potentials (OPs), which characterize activity in retinal cells distinct from those that generate \(P_3\) and \(P_2\), such as inner-retinal amacrine and ganglion cells, were also studied. Their sensitivity (\(1/K_{OPs}\)) and saturating amplitude, estimated by the square root of saturating OP energy (\(E_{m}^{1/2}\)), were also measured. Thus, \(P_3\), \(P_2\), and the OPs can be loosely thought of as including predominating contributions from cells with respectively deeper retinal positions: photoreceptors, bipolars, and amacrine and ganglion cells. All ERG data were recorded as the log change from normal (\(D\log\text{Normal}\)). By expressing the data in log values, changes in observations of fixed proportion become linear, consistent with a constant fraction for physiologically meaningful changes in parameter values.

**Detection of DAergic Activity**

The utilization of DA can be estimated by monitoring the decline in levels of DA and its metabolites when its synthesis is interrupted. As shown in Figure 1, DA is synthesized from L-tyrosine by a two-step process: (1) hydroxylation of L-tyrosine by TH to produce levodopamine, the carboxylated form of dihydroxyphenylalanine (DOPA), and (2) decarboxylation of DOPA by aromatic L-amino acid decarboxylase (AAAD) to produce DA. The primary metabolite of DA is 3,4-dihydroxyphenylacetic acid (DOPAC); DA can also be converted to norepinephrine by dopamine \(\beta\)-hydroxylase, though not significantly in retina. \(m\)-Hydroxybenzylhydrazine (NSD-1015) inhibits AAAD, resulting in accumulation of DOPA, which can be used to estimate in situ TH activity/DA synthesis; the decline in DA and its metabolites in the presence of NSD-1015 is a measure of DA utilization. In order to analyze DA turnover in OIR rat retinae as compared with RAR rat retinae, NSD-1015 (150 mg kg\(^{-1}\)) was administered intraperitoneally. Approximately 30 minutes later, animals were killed with CO\(_2\) and their retinae were removed and flash frozen in liquid nitrogen; elapsed time was carefully noted. Uninjected rats served as controls. The retinal samples were later homogenized with 0.1 N HClO\(_4\) containing 0.1% sodium metabisulfite to prevent the oxidation of catecholamine, and retinal levels of DA, DOPA, and DOPAC were assayed using HPLC with coulometric detection. Regression on elapsed time versus metabolite concentration provided an estimate of the rate of change in each metabolite following injection of NSD-1015. The coefficients of this regression enabled us to estimate the steady-state levels of each metabolite.

**Data Analysis**

Parametric statistical analyses were performed in SPSS Statistics 21 (SPSS, Inc., An IBM Company, Chicago, IL). The TH+ labeling in retinal sections was studied as a function of age by
**Table.** Mean (SD) Key Parameters of Refracted Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>RAR</th>
<th>OIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight at P55–59</td>
<td>g</td>
<td>356 (107)</td>
<td>287 (62)</td>
</tr>
<tr>
<td>Mean refraction</td>
<td>D</td>
<td>1.82 (2.59)</td>
<td>0.19 (3.00)</td>
</tr>
<tr>
<td>$Rm_{P2}$</td>
<td>$\mu V$</td>
<td>946 (295)</td>
<td>321 (79)</td>
</tr>
<tr>
<td>$S$</td>
<td>$m^2/cd^3$</td>
<td>3420 (1260)</td>
<td>2490 (770)</td>
</tr>
<tr>
<td>$1/K_{C_{1P2}}$</td>
<td>$m^2/cd^s$</td>
<td>954 (439)</td>
<td>367 (187)</td>
</tr>
<tr>
<td>$Em^{1/3}$</td>
<td>$\mu V$</td>
<td>201 (49)</td>
<td>40 (26)</td>
</tr>
<tr>
<td>$1/K_{C_{1P2}}$</td>
<td>$m^2/cd^s$</td>
<td>6.10 (7.85)</td>
<td>3.27 (1.11)</td>
</tr>
<tr>
<td>DOPA</td>
<td>pg/mL</td>
<td>159 (60)</td>
<td>148 (39)</td>
</tr>
<tr>
<td>DA</td>
<td>pg/mL</td>
<td>2130 (160)</td>
<td>1330 (300)</td>
</tr>
<tr>
<td>DOPAC</td>
<td>pg/mL</td>
<td>1140 (370)</td>
<td>640 (310)</td>
</tr>
</tbody>
</table>

ANOVA. Refractive state in OIR and RAR rats was compared by a second ANOVA. Within-subjects comparisons of ERG parameters and HPLC data obtained from the refracted rats were performed by additional, respective ANOVA, and the relation of ERG and HPLC parameters to refractive state (SE) was studied by multiple regression.

**RESULTS**

Normal Postnatal Development of Retinal DAergic Networks Is Disrupted in OIR

One RAR rat's retinae were lysed for Western blot analysis of the monoclonal anti-TH antibody, another's retinae were flat mounted for inspection of the structure of the cells labeled by this antibody, and 32 RAR and 32 OIR rats' retinae were sectioned for evaluation of the development of the DAergic retinal cell networks. First, the specificity of the monoclonal anti-TH antibody in the lysate was confirmed: the results of the Western blot showed a single band at approximately 59 kDa (Fig. 2A), consistent with the description provided by the vendor. Second, the monoclonal anti-TH antibody was used to label the flat-mounted retina. As shown in Figure 2B, the telltale features of DAergic amacrine cells were revealed: a dense plexus of fibers was seen when the plane of focus was at

![Figure 6](image-url). Response amplitude and sensitivity in rod photoreceptors ($Rm_{P2}, S$), postreceptor bipolar cells ($1/K_{C_{1P2}}$), and inner retinal amacrine and ganglion cells ($Em^{1/3}, 1/K_{C_{1P2}}$) derived from the dark-adapted ERG are plotted on the abscissa; the ordinate value 0 represents normal. At every retinal depth, response amplitude and sensitivity was significantly subnormal in ROP rats.
The OIR Retina Is Dysfunctional

Following retinoscopy, retinal function was tested in the same rats by ERG. The respective amplitude and sensitivity ERG parameters for the photoreceptors (RmP2, S), postreceptor cells (RmP2, 1/KP2), and inner retina (EmV, 1/Kop) were analyzed by repeated-measures ANOVA with factors group, category (amplitude, sensitivity), depth (photoreceptor, postreceptor inner retina), and eye (left, right). As shown in Figure 6 and the Table, ERG response amplitude and sensitivity were both significantly reduced in OIR rats at every retinal depth (Fgroup = 7.56; df = 1.19; P = 0.015). Differences in other factors (depth, category, and eye) and their interactions were all insignificant (all P ≥ 0.39).

Steady-State Levels of Retinal DA Are Reduced in OIR Without a Defect in DA Turnover

In the same rats refracted and tested by ERG, DA level was measured using HPLC. Five of the RAR rats and six of the OIR rats were injected with NSD-1015 and killed 19 to 48 minutes later, along with the remaining rats who did not receive an injection. The results of HPLC of DOPA, DA, and DOPAC in excised retinae are shown in Figure 7 and given for (rats not treated with NSD-1015) in the Table. DOPA, DA, and DOPAC were all measurable in both RAR and OIR retinae and were analyzed by repeated-measures ANOVA with factors group, metabolite (DOPA, DA, DOPAC), and injection (none, NSD-1015). In rats not treated with NSD-1015, compared with control retinae, OIR retinae had similar levels of DOPA, but significantly decreased levels of DA and DOPAC (Fgroup × metabolite = 5.63; df = 2.34; P = 0.057). NSD-1015 had very similar effects on both OIR and control retinae: marked increase in DOPA, marked decrease in DA, and no significant change in DOPAC (Fmetabolite × injection = 84.9; df = 2.34; P < 0.001). These results are evidence that enzymatic conversion of L-tyrosine to DA is normal in OIR retina, but that instead there are fewer DAergic cells or processes.

The coefficients for regression on elapsed time since NSD-1015 injection versus metabolite were 1.01 for DOPA, −0.20 for DA, and −0.028 for DOPAC, suggesting that, following NSD-1015 injection, DOPA levels rise rapidly, DA levels fall moderately, and DOPAC levels fall very slowly.

DA Cell Networks May Contribute to OIR Myopia

In order to evaluate what factors could contribute to the OIR myopia, forward-stepwise multiple regression using information criterion for parameter entry and removal with parameters group (RAR = 0, OIR = 1), photoreceptor amplitude (RmP2) and sensitivity (S), postreceptor amplitude (RmP2) and sensitivity (1/KP2), inner-retinal amplitude (EmV) and sensitivity (1/Kop), and loglevels of DOPAC/DA were run; utilization of DA is commonly estimated by the ratio of DOPAC to DA.59,73 As shown in Figure 8A, the resulting model (P = 0.031) included only group (RAR or OIR) and ratio (DOPAC/DA). As shown in Figure 8B, these two parameters alone were sufficient to produce a model with excellent agreement between predicted and observed SE (r² = 0.71). To ascertain whether the predictions regarding the steady-state levels of DA and DOPAC in the NSD-1015—injected animals were reasonable, these animals’ predicted refractions, as calculated from the regression model, were added to the plot. The agreement remained excellent.
**DISCUSSION**

Dopamine released by DAergic amacrine cells reaches target neurons either locally at the synaptic terminals or via paracrine diffusion. Thus, DA is poised to regulate a wide range of retinal functions, and loss of DA will have sundry ocular effects. In the present study, despite the fact that the OIR rats’ axial lengths were presumably low, development of DAergic processes was delayed and, when the retina was mature, remained less prominent than in RAR retinae (Figs. 3, 4). In addition, as summarized in the Table, the mature eye of the OIR rat is characterized by neurosensory retinal dysfunction and myopia. Some of these features may have been, in part, consequences of altered retinal DA.

**Maturation of Retinal DAergic Cells**

Development of TH+ processes in the normal retina proceeded steadily from P14 through P20; TH+ immunoreactivity was higher still at P57 and then decreased by P120 (Fig. 4). In the rat retina, most progenitor cells destined to become DAergic neurons reach their cell fate within a prenatal period of amacrine cell differentiation between embryonic days 16 and 20.74 Although cell number is approximately fixed, DAergic perikarya increase in size at least through P21 and their processes become greater in density at even older ages.75 In these sections, dendritic and axonal ramifications were not distinguished. The elaboration of DAergic dendritic processes seems to be complete by approximately P15, but axonogenesis is only just beginning; there is little evidence of axon terminals in the form of rings until approximately P17 and, while the rings are mostly in place at P21, maturation of DAergic axons continues into adulthood.75 These results are consistent with postnatal maturation of DAergic networks, but also indicate a regression in the mature eye. Since soma were not counted, cell death, decreased ramification per cell, or simply loss of TH immunoreactivity could not be distinguished.76 Interestingly, there is a decrease in many ERG parameters at ages older than 30 to 60 days in rats77 and mice.78 Notably, the induction of OIR occurs from P0 to P14, a timeframe during which DAergic cells are mainly undergoing development instead of differentiation, meaning there may well be a full complement of DAergic cells, but with altered morphology in OIR. In particular, it is likely that the development of dendritic processes is impacted and, thus, the persistently low TH+ might be mostly associated with loss of DAergic dendrites.

**DA and OIR**

The postnatal development and maturation of DAergic amacrine cell networks was found to be delayed and diminished. Dopamine released by these cells presumably acts on the RPE to regulate eye growth.79,80 Dopamine production is decreased in form-deprivation myopia in both monkeys and chicks.81,82 Thus, DA is a stop signal in myopia. However, complete depletion of DA causes an overall reduction of eye size in fish.83 Refractive development in OIR rat eyes, being myopic but small,1 would seem to depend on mechanisms beyond DA.

Regression analysis suggested a relationship between refractive state and DA metabolism (Fig. 8). While DA released in the retina could reach the anterior segment via vitreal or uveal routes, more likely (if it indeed plays a role at all), it would regulate anterior segment development via alternative pathways. For example, NO has been reported to inhibit DA release from DAergic neurons,84,85 whereas DA has been reported to induce release of NO in retina.20,21 Other data suggest that there is altered nitric oxide synthase (NOS) activity in OIR.86 These data point to the possibility that altered NO production is an important step during OIR pathogenesis, perhaps leading to the alterations in postnatal development and maturation of DAergic amacrine cells in OIR retina.

**Retinal Function**

The neural retina was dysfunctional in the OIR rats (Table; Fig. 6). Significant attenuation was observed in responses originating in photoreceptors, bipolar cells, and inner retinal neurons, with the most marked dysfunction in the inner retinal response amplitudes (OPs). The OIR rats were approximately four-fifths the size of the RAR rats, and it is possible that the size of the rat (or its eye) could cause the observed change in response parameters, rather than residual retinal pathology. However,
within each group (RAR, OIR), there was no straightforward association between weight and ERG amplitude. Furthermore, evidence suggests that the association between eye size and ERG amplitude is quite small and in the opposite direction: smaller eyes tend to have larger amplitudes. Thus, any effect of size would likely have led to an underestimation of the impact OIR on retinal function.

**Relationship to ROP Myopia**

The axial length of the eye of the OIR rat has been previously shown to be short. These data confirm that the OIR rat is also myopic (Table; Fig. 5). The OIR rat's eye has some provocative similarities to the eye with a history of ROP; it is, therefore, possible that similar processes are at work in OIR and ROP eyes, although the relevance to human ROP myopia is equivocal. On the one hand, in both ROP myopia and OIR eyes, the dioptric power of the anterior segment is high, more than offsetting the short axial length. On the other hand, the OIR rat is not born prematurely and does not suffer from cicatricose consequences when left untreated, these are the features most feared in severe ROP (i.e., ROP that would not be treated). In mild ROP, the incidence of myopia is not much increased, but myopia does seem to be a feature of the OIR rat. Furthermore, the rats in this study were born full term, and thus, did not suffer any effects from premature externalization. Nevertheless, further study of the OIR rat may provide insights into ocular development difficult or impossible to obtain using conventional (axial) myopia models.

**Acknowledgments**

Supported by National Institutes of Health (NIH) Grants R31EY020508 (JDA), R01EY004864 and P30EY006360 (PMD), Research to Prevent Blindness, Inc. (PMD), and the Massachusetts Lions Eye Research Fund (RMD).

Disclosure: N. Zhang, None; T.L. Favazza, None; A.M. Baglieri, None; I.V. Bendor, None; E.R. Noonan, None; A.B. Fulton, None; R.M. Hansen, None; P.M. Iuvone, None; J.D. Akula, None

**References**


28. Shulman LM, Fox DA. Dopamine inhibits mammalian photoreceptor Na+, K+-ATPase activity via a selective effect on the...


60. Hoed DC, Birch DG. A computational model of the amplitude and implicit time of the b-wave of the human ERG. Vis Neurosci. 1992;8:107–126.


