Evidence for a Brief Period of Enhanced Oxygen Susceptibility in the Rat Model of Oxygen-Induced Retinopathy

Olga Dembinska,1 Luz Marina Rojas,2 Sylvain Chemtob,5 and Pierre Lachapelle1,4

PURPOSE. Findings in a previous study have shown that the retina of newborn rats exposed to hyperoxia during the first days of life sustain permanent functional (as determined with the rod ERG) and structural (as determined with histology) damage that appears to be determined by the level of retinal maturity reached at the time of oxygen exposure—the retinas of rat pups being more susceptible to hyperoxic shock during the second week of life than during the first week. Given that the cone ERG has been shown to mature later than the rod ERG, the purpose of the present study was to examine whether cone responses also demonstrate a similar maturational susceptibility to postnatal hyperoxia. Also examined was whether the oscillatory potentials (OPs) were affected by postnatal hyperoxia.

METHODS. Newborn rats were exposed to hyperoxia during selected postnatal day intervals either initiated at birth (early-onset exposure) or at a later postnatal age (late-onset exposure). Photopic and scotopic (mixed cone-rod) electroretinograms were recorded at 30 days.

RESULTS. Data analysis reveals that photopic and scotopic responses (b-wave and OPs) demonstrated a similar maturational susceptibility to postnatal hyperoxia, in which exposure regimens initiated during the second week of life were most detrimental to retinal function. The results also revealed a temporal window of enhanced oxygen susceptibility at approximately postnatal day 10. The duration of this window was longer when estimated with the scotopic responses, but the extent of the functional damage was more pronounced when estimated with the photopic signals. Finally, compared with the b-wave, the OPs, especially the short-latency OPs, were proportionally more affected.

CONCLUSIONS. The results suggest that cone function is significantly more susceptible to postnatal hyperoxia than rod function, and the OPs appear to be the most susceptible ERG components, thus suggesting a differential susceptibility to oxygen toxicity of the different retinal components. However, despite a clear demonstration of its existence, the exact nature of the temporal window of enhanced oxygen susceptibility as well as a possible equivalence in other animal models of oxygen-induced retinopathy, including the human form (retinopathy of prematurity), remains to be determined. (Invest Ophthalmol Vis Sci. 2002;43:2481–2490)

Retinopathy of prematurity (ROP), a major eye disease of the premature infant, is characterized by a wide range of vascular, functional, and, most probably, structural changes of the retina.1–3 Similar features were also reported for oxygen-induced retinopathy (OIR), which is the animal model of ROP, suggesting that the latter is a good working model of the former.4–12

In newborn rats, functional damage resulting from postnatal hyperoxia appears to be mostly situated at the postreceptorial level, as revealed by the large reduction in the amplitude of the b-wave of the electroretinogram (ERG) and the relative sparing of the a-wave.4,11,12 Apart from the typical vasculopathy known to characterize the rat model of ROP (e.g., vasoconstriction, vaso-obliteration, and neovascularization),4,6–8 previous studies reporting on the structural consequences of postnatal hyperoxia on the retina have also shown a marked decrease in the thickness of the outer plexiform layer (OPL) and in the count of the horizontal cells, whereas the other retinal layers appear to be spared.11,12 Furthermore, we have recently reported a dose-dependent correlation between the increase in duration of the oxygen exposure and the decrease in the amplitude of the b-wave of the rod-dominated ERG, as well as the decline in thickness of the OPL.12 This relationship was found to be dependent on the maturational stage of the retina—retinal damage being more severe if the hyperoxic shock occurred during the second week of life of the newborn rat than if it occurred during the first week.12 The latter window of oxygen sensitivity follows that previously demonstrated for the retinal vasculature, which has been shown to occur during the first week of life,1,3 and precedes that recently evidenced for the photoreceptors, which has been shown to take place between postnatal days 16 and 22.14

In our previous study, we examined the functional consequences of postnatal hyperoxia using the rod-mediated ERG.12 Because it has been documented that the cone ERG can also be affected by postnatal hyperoxia11 and that cone responses mature in rats at a later age than the rod ERG,15 the purpose of the present study was to investigate whether a similar temporal window of enhanced oxygen susceptibility could also be demonstrated with the cone ERG, and if so, to examine whether, compared with rods, it occurs at a later postnatal age. We also examined the maturational susceptibility of the OPs, because these retinal potentials were also shown to be severely reduced (in amplitude and number) by postnatal hyperoxia.11

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Supported by grants-in-aid from the McGill University-Montreal Children’s Hospital Research Institute, the Canadian Institutes of Health Research (Grant MT-12153 and MT-13383), the Quebec Research Foundation on Nature and Technologies—Research Group in Experimental Neuropsychology, and the Vision Network of the Quebec Health Research Foundation.

Submitted for publication November 9, 2001; revised February 19, 2002; accepted March 22, 2002.

Commercial relationships policy: N.

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METHODS

The experimental protocol was reviewed and approved by the McGill University Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care. Animal management was conducted in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Newborn Sprague-Dawley rats were exposed daily to 80% O₂ (mixture of medical grade 100% O₂ and room air) measured with an OxyChek model 2000; Critikon, Tampa, FL) for 22.5 hours interrupted by 3 periods (approximately 8 AM, 12 PM, and 4 PM) of 0.5 hour at 21% O₂.11,12 In the first group, identified as early-onset oxygen exposure, rats were exposed from birth to postnatal day 6 (0–6, n = 8), 9 (0–9, n = 7), 12 (0–12, n = 5), or 14 (0–14, n = 8), whereas in the second group, identified as late-onset oxygen exposure, they were exposed from postnatal day 6 (6–14, n = 7), 9 (9–14, n = 7), or 12 (12–14, n = 5) to postnatal day 14. After a preliminary analysis of the data, two new sets of rats were included. A first set, which included rats exposed from postnatal days 9 to 12 (9–12, n = 8) and a second one, which included rats exposed from birth until postnatal day 9. After a brief return to normoxia from postnatal days 9 to 12, the latter group were returned to the hyperoxic environment from postnatal days 12 to 14. This last group of rats was identified as (0–9)+(12–14) (n = 9).

Electroretinography

Photopic and scotopic ERGs were recorded by using a previously reported procedure.11,12 In short, after a 12-hour period of dark adaptation, the rats were anesthetized with an intramuscular injection of ketamine hydrochloride (80 mg/kg) and xylazine (6 mg/kg). The cornea was anesthetized with proparacaine hydrochloride 0.5%, and the pupil was dilated with drops of cyclopentolate hydrochloride 1%. The rats were exposed daily to 80% O₂ (mixture of medical grade 100% O₂ and room air measured with an OxyChek model 2000; Critikon, Tampa, FL) for 22.5 hours interrupted by 3 periods (approximately 8 AM, 12 PM, and 4 PM) of 0.5 hour at 21% O₂.11,12 In the first group, identified as early-onset oxygen exposure, rats were exposed from birth to postnatal day 6 (0–6, n = 8), 9 (0–9, n = 7), 12 (0–12, n = 5), or 14 (0–14, n = 8), whereas in the second group, identified as late-onset oxygen exposure, they were exposed from postnatal day 6 (6–14, n = 7), 9 (9–14, n = 7), or 12 (12–14, n = 5) to postnatal day 14. After a preliminary analysis of the data, two new sets of rats were included. A first set, which included rats exposed from postnatal days 9 to 12 (9–12, n = 8) and a second one, which included rats exposed from birth until postnatal day 9. After a brief return to normoxia from postnatal days 9 to 12, the latter group were returned to the hyperoxic environment from postnatal days 12 to 14. This last group of rats was identified as (0–9)+(12–14) (n = 9).

Retinal Histology

Sections of the retinas were obtained according to the method previously reported.11,12 Briefly, immediately after death, the eyes were enucleated and the retinas were fixed in glutaraldehyde. After postfixation in OsO₄, the samples were dehydrated in ethanol and infiltrated with propylene oxide before being embedded in Epon resin. Semithin (0.7-μm) sections of the central retina were stained with toluidine blue.

Data Analysis

ERG measurements included only b-wave and OPs, because the a-wave has been shown not to be significantly affected by postnatal hyperoxia.11,12 The amplitude of the b-wave was measured from the trough of the a-wave to the peak of the b-wave and the amplitude of each of the major oscillatory potentials (i.e., OP₂, OP₃, OP₄, OP₅) was measured from the preceding trough to peak. Furthermore, the amplitudes of each OP of a given response (photopic and scotopic) were added to create the Sum OPs variable (i.e., SOPs = OP₂ + OP₃ + OP₄ + OP₅). Peak times were measured from onset of the flash to the peak of the wave under evaluation. Data analysis also included a sigmoidal dose-response regression curve (Prism 2.01 software; GraphPad, San Diego, CA) to measure the effect of the various oxygen regimens on the amplitude of the different ERG components. Structural analysis included only the measurement of the thickness of the OPL and the number of horizontal cells measured on cross sections of the central retina obtained after exposure to the 9 to 12 and (0–9)+(12–14) regimens, because our studies have shown that these were the only two retinal structures significantly altered by postnatal hyperoxia.11,12 All data (ERG and histology) were compared with the data obtained from normal age-matched rats. Each data point represents the mean ± 1 SD. Statistical analyses were performed with one-way ANOVA (P < 0.05) and the Tukey (honest significant difference) test as a post hoc pair-wise comparison.

RESULTS

The Effect of Postnatal Hyperoxia on the Photopic ERG

Representative photopic ERGs (left column) and OPs (right column) obtained from a control rat (Control) and rats exposed to the different hyperoxic regimens (given at the left of each tracing) described in the Methods section are shown in Figure 1. Group data on amplitudes and peak times are reported in Tables 1 and 2. Data analysis compared the effect of postnatal hyperoxia after early- and late-onset exposures.

Early-Onset Oxygen Exposure. The b-wave and the corresponding OPs all demonstrated a gradual decrease in amplitude with an increase in duration of oxygen exposure (Fig. 1, top panels). This is best illustrated in Figure 1 (bottom left) where the respective amplitudes (mean values) were fitted to sigmoidal dose-response curves. It is interesting to note that the amplitude of the b-wave as well as that of most of the OPs followed more or less the same biphasic regression curve, where oxygen exposure taking place within the first 6 days of life had no significant impact on the shape, amplitude, and timing of the resultant retinal signal, whereas progressively longer exposures caused rapid deterioration in the responses. The only exceptions were OP₂ and OP₅, which demonstrated a significant reduction in amplitude as early as the 0– to 6-day exposure (Table 1), resulting in a more linear relationship. At maximal effect, obtained after the 0– to 14-day exposure, the amplitudes of the b-wave and the OPs were reduced to 25% and 15% of normal, respectively. It should be noted that despite the differential effect between early and late OPs seen after the 0– to 6-day exposure, this distinction was no longer observed after the 0– to 14-day exposure, because all the OPs were equally attenuated (Fig. 1, Table 1).

Late-Onset Oxygen Exposure. Similar dose-effect relationships for the b-wave and OPs were observed when the onset of oxygen exposure was initiated at a later age, as shown in Figure 1. However, the functional consequences of delaying the hyperoxic regimen was more pronounced, compared with that observed after the early onset. This is best exemplified when the data gathered after exposure from birth to postnatal day 9 (nine consecutive days of exposure) and that after exposure from postnatal days 6 to 14 (eight consecutive days of exposure) are compared. For an almost equivalent total day of oxygen exposure, the former regimen reduced the ERG and OP components to approximately 70% of control compared with less than 30% of control after the latter (Fig. 1, bottom; Table 1).
Photopic ERG and oscillatory potentials responses obtained at 30 days of age from a control rat, rats subjected to postnatal hyperoxia during early-onset oxygen exposure (0–6, 0–9, 0–12, and 0–14 days) and during the late-onset oxygen exposure (6–14, 9–14, and 12–14 days). Duration (in days) of exposure to oxygen is indicated on the left of each waveform. Oscillatory potentials are identified in numerical order in the control recording. Calibrations: horizontal, 20 ms; vertical, 150 and 20 μV for ERG and OPs, respectively. All tracings include a 20-ms prestimulus baseline. Vertical arrows indicate flash onset. Sigmoidal dose-response correlations between the amplitude of photopic b-wave, SOPs, and individual oscillatory potentials OP2 to OP5 and age (days) of rats at cessation (bottom left) or at beginning (bottom right) of hyperoxia. Amplitude is presented as a percentage (mean of all subjects) of normal control values.
Table 1. Summary of Amplitude (μV) Measurements for 30-day-old Rats

<table>
<thead>
<tr>
<th>Amplitude (μV)</th>
<th>Control (0)</th>
<th>0–6 (6)</th>
<th>0–9 (9)</th>
<th>0–12 (12)</th>
<th>0–14 (14)</th>
<th>6–14 (8)</th>
<th>9–14 (5)</th>
<th>12–14 (2)</th>
<th>9–12 (3)</th>
<th>0–9 + 12–14 (11)</th>
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<td>Photopic</td>
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<tr>
<td>b-Wave</td>
<td>261.6 ± 31.4</td>
<td>228.1 ± 29.8</td>
<td>175.6 ± 44.7</td>
<td>103.3 ± 45.7</td>
<td>66.4 ± 25.3</td>
<td>101.2 ± 29.4</td>
<td>124.4 ± 58.8</td>
<td>207.3 ± 45.9</td>
<td>182.0 ± 36.6</td>
<td>139.3 ± 43.2</td>
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<tr>
<td>OP_2</td>
<td>19.4 ± 4.8</td>
<td>14.8 ± 2.6*</td>
<td>10.7 ± 2.9*</td>
<td>7.7 ± 2.7*</td>
<td>3.4 ± 2.4*</td>
<td>5.5 ± 1.9*</td>
<td>8.6 ± 4.7*</td>
<td>14.2 ± 3.5*</td>
<td>11.2 ± 3.9*</td>
<td>9.4 ± 3.2*</td>
</tr>
<tr>
<td>OP_3</td>
<td>45.9 ± 9.6</td>
<td>31.7 ± 6.7*</td>
<td>26.8 ± 7.3*</td>
<td>16.9 ± 8.9*</td>
<td>5.9 ± 3.6*</td>
<td>11.4 ± 7.0*</td>
<td>16.9 ± 13.4*</td>
<td>31.5 ± 8.7*</td>
<td>22.1 ± 6.7*</td>
<td>15.6 ± 4.7*</td>
</tr>
<tr>
<td>OP_4</td>
<td>33.7 ± 7.8</td>
<td>30.1 ± 5.9</td>
<td>25.0 ± 4.1*</td>
<td>15.8 ± 7.2*</td>
<td>5.4 ± 2.8*</td>
<td>10.4 ± 5.3*</td>
<td>13.9 ± 9.3*</td>
<td>26.1 ± 9.5</td>
<td>20.6 ± 5.9*</td>
<td>13.5 ± 4.2*</td>
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<tr>
<td>OP_5</td>
<td>20.6 ± 7.3</td>
<td>20.1 ± 5.5</td>
<td>15.3 ± 2.4</td>
<td>10.7 ± 4.8*</td>
<td>3.8 ± 1.8*</td>
<td>7.3 ± 3.7*</td>
<td>10.4 ± 7.0*</td>
<td>15.5 ± 7.4</td>
<td>9.6 ± 2.6*</td>
<td>7.6 ± 2.8*</td>
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<tr>
<td>SOPs</td>
<td>117.6 ± 26.1</td>
<td>96.8 ± 19.6</td>
<td>77.7 ± 13.7*</td>
<td>51.1 ± 22.7*</td>
<td>18.5 ± 8.8*</td>
<td>34.6 ± 17.6*</td>
<td>49.7 ± 34*</td>
<td>87.4 ± 28.2*</td>
<td>65.5 ± 18.0*</td>
<td>46.1 ± 13.4*</td>
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<td>Scotopic</td>
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<tr>
<td>b-wave</td>
<td>1122.1 ± 174.5</td>
<td>1064.8 ± 119.2</td>
<td>871.2 ± 129.3*</td>
<td>601.4 ± 145.4*</td>
<td>445.4 ± 155.4*</td>
<td>531.2 ± 113.8*</td>
<td>577.8 ± 175.8*</td>
<td>855.8 ± 150.7*</td>
<td>893.6 ± 128.1*</td>
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<tr>
<td>OP_2</td>
<td>50.1 ± 18.4</td>
<td>45.1 ± 22.5</td>
<td>25.2 ± 11.9*</td>
<td>13.4 ± 10.4*</td>
<td>5.1 ± 6.3*</td>
<td>15.0 ± 6.2*</td>
<td>13.9 ± 8.1*</td>
<td>25.9 ± 11.4*</td>
<td>40.7 ± 13.8</td>
<td>26.8 ± 12.4*</td>
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<tr>
<td>OP_3</td>
<td>189.5 ± 22.1</td>
<td>176.7 ± 32.3</td>
<td>157.5 ± 28.9*</td>
<td>87.0 ± 51.1*</td>
<td>33.2 ± 27.5*</td>
<td>81.4 ± 34.1*</td>
<td>81.5 ± 31.8*</td>
<td>131.1 ± 36.4*</td>
<td>160.7 ± 38.3</td>
<td>118.8 ± 41.1*</td>
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<tr>
<td>OP_4</td>
<td>169.2 ± 19.3</td>
<td>158.9 ± 15.0</td>
<td>121.9 ± 29.2*</td>
<td>70.2 ± 25.2*</td>
<td>38.4 ± 28.4*</td>
<td>74.0 ± 35.8*</td>
<td>83.9 ± 35.3*</td>
<td>122.7 ± 26.4</td>
<td>159.1 ± 28.0</td>
<td>129.0 ± 39.9*</td>
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<tr>
<td>OP_5</td>
<td>57.8 ± 18.0</td>
<td>50.1 ± 14.4</td>
<td>32.8 ± 10.5*</td>
<td>22.2 ± 7.6*</td>
<td>15.9 ± 10.5*</td>
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<td>27.9 ± 10.1*</td>
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<tr>
<td>SOPs</td>
<td>466.7 ± 63.6</td>
<td>430.8 ± 65.8</td>
<td>317.4 ± 77.1*</td>
<td>192.8 ± 84.8*</td>
<td>92.6 ± 71.5*</td>
<td>195.4 ± 79.5*</td>
<td>207.3 ± 74.3*</td>
<td>327.6 ± 70.5*</td>
<td>410.8 ± 78.2</td>
<td>332.5 ± 110.7†</td>
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</table>

Column headings show regimens, with number of days in parentheses.
* Significantly different from Control.
† 9–12 Significantly different from (0–9) + (12–14).
TABLE 2.

Oxygen Hypersensitivity in Rat Retina

<table>
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<th>Window</th>
<th>Peak Time (days)</th>
<th>Control</th>
<th>0-6 (G)</th>
<th>0-9 (J)</th>
<th>0-12 (12)</th>
<th>0-14 (14)</th>
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<tr>
<td>9–12 (3)</td>
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<td>12–14 (2)</td>
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<td>9–14 (5)</td>
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<td>0–14 (14)</td>
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</table>

Early-Onset Oxygen Exposure

As shown for the photopic responses, the amplitudes of the scotopic b-wave and OPs were progressively reduced as the duration of hyperoxia gradually increased (Fig. 2, top), resulting in a sigmoidal dose-response correlation similar to that observed with the photopic ERG and OPs (Table 1, Fig. 2, bottom left). However, unlike the photopic responses, none of the scotopic ERG components was significantly altered after the 0- to 6-day exposure regimen (Table 1). At maximal effect, obtained after oxygen exposure from birth to postnatal day 14, the amplitude of the b-wave was attenuated to 40% of normal value, whereas that of the OPs (SOPs variable) was reduced to less than 20% of control. Analysis of individual OPs revealed that the short-latency OP2 and OP3 were more severely affected by postnatal hyperoxia, the former being almost extinguished after the 0- to 14-day exposure. As estimated from the data reported at Table 1, after the 0- to 14-day exposure, OP2 and OP3 reached an average amplitude of 15% of control compared with an average 25% of control for OP1 and OP4.

Late-Onset Oxygen Exposure

When initiated at a later age, progressively longer exposures also gradually reduced the amplitude of the ERG components (Fig. 2, Table 1). However, as previously demonstrated for the photopic responses, for an equivalent duration, oxygen exposures initiated at a later age had a greater impact than those started earlier. This is best exemplified when the data obtained after the 0- to 9- and 6- to 14-day exposure regimens are compared. For an almost equivalent duration of exposure, the former reduced the amplitudes of the b-wave and OPs to 60% of normal amplitudes compared with less than 40% of control after the 1-day shorter regimen. Finally, contrasting the amplitude measurements, it is of interest to note that none of the peak time parameters was modified in any predictable fashion after exposures to the different hyperoxic regimens (Table 2).

The data presented in Figures 1 and 2 clearly indicate that when initiated at a later postnatal age, the hyperoxic shock affected retinal function more than when initiated at an early postnatal age, even if the duration of the oxygen exposure was markedly shorter. To explore this unique feature further, we combined in Figure 3 the early- and late-onset regression curves, which were obtained with the photopic and scotopic measurements shown in Figures 1 and 2. The data points numerically identified 1 to 6 in the photopic graph (Fig. 3A) and 1 to 7 in the scotopic graph (Fig. 3B) identify the intersection of the early- and late-onset regression curves obtained with each of the ERG and OP parameters considered. It was assumed that combining these intersections would help identify the postnatal age range that had to be included in our exposure regimen to yield an optimal effect (i.e., the maximal effect obtained after the shortest duration of exposure). We therefore defined the "temporal window of enhanced oxygen susceptibility" as that delimited by the postnatal ages at which the intersections accounting for the minimal and maximal effects were observed. What stood out from this analysis was that whereas both photopic and scotopic responses had an identical upper limit (minimal effect) of 35% of amplitude attenu-

The Effect of Postnatal Hyperoxia on the Scotopic (Mixed Cone-Rod) ERG

Similar dose-effect correlations were obtained with the scotopic responses. Representative scotopic ERGs (Fig. 1, top left) and OPs (Fig. 1, top right) obtained from a control rat (Control) and rats exposed to the different hyperoxic regimen (given at the left of each tracings) described in the Methods section are shown in Figure 2. Group data on amplitudes and peak times are reported in Tables 1 and 2. Data analysis compares the effect of postnatal hyperoxia after early- and late-onset exposures.

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Late-Onset Oxygen Exposure. When initiated at a later age, progressively longer exposures also gradually reduced the amplitude of the ERG components (Fig. 2, Table 1). However, as previously demonstrated for the photopic responses, for an equivalent duration, oxygen exposures initiated at a later age had a greater impact than those started earlier. This is best exemplified when the data obtained after the 0- to 9- and 6- to 14-day exposure regimens are compared. For an almost equivalent duration of exposure, the former reduced the amplitudes of the b-wave and OPs to 60% of normal amplitudes compared with less than 40% of control after the 1-day shorter regimen. Finally, contrasting the amplitude measurements, it is of interest to note that none of the peak time parameters was modified in any predictable fashion after exposures to the different hyperoxic regimens (Table 2).

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* Significantly different from Control.
† Significantly different from (0-9 + 12-14).**
FIGURE 2. Representative scotopic mixed (rod–cone) ERGs (top right) and oscillatory potentials (top left) responses obtained at age of 30 days from a control rat, rats subjected to postnatal hyperoxia during early-onset oxygen exposure (0–6, 0–9, 0–12, and 0–14 days) and during late-onset oxygen exposures (6–14, 9–14, and 12–14 days). Absolute numbers of days of exposure to oxygen are indicated on the left of each waveform. Oscillatory potentials are identified in numerical order in the control recording. Calibrations: horizontal, 20 ms; vertical, 350 and 100 μV for ERG and OPs, respectively. All tracings include a 20-ms prestimulus baseline. Arrows: flash onset. Sigmoidal dose–response correlations between the amplitude of photopic b-wave, SOPs, and individual oscillatory potentials OP2 to OP6 and age (days) of rat at cessation (bottom left) or at beginning (bottom right) of hyperoxia (bottom right). Amplitude is presented as a percentage (mean of all subjects) of control value.
tion, the lower limit (maximal effect) was slightly more pronounced for the photopic signals (approximately 50% of control amplitude) compared with the scotopic ones (approximately 45% of control amplitude). Similarly, whereas in photopic conditions, the boundaries for minimal and maximal amplitude attenuation were delimited by OP5 and OP3 respectively, in scotopic conditions they were delimited by b-wave and OP5 measurements. Comparing the age range at which the photopic and scotopic temporal windows of enhanced oxygen susceptibility take place, it is notable that although both windows end at the same postnatal age (calculated to be at postnatal day 10.84 according to linear equations) the onset is earlier for the scotopic responses (postnatal day 9.26) than the photopic ones (postnatal day 9.9). Thus, the temporal window of enhanced oxygen susceptibility, as evidenced with the photopic responses, is shorter (calculated duration: 23 hours) and yields more severe amplitude attenuation than does its scotopic counterpart (calculated duration, 38 hours).

The purpose of the data analysis shown in Figure 3 was to identify the postnatal age that had to be included within our exposure regimen to yield the optimal effect—that is, the maximal amplitude attenuation obtained after the shortest duration of exposure. Should we interpret the above findings as an indication that limiting exposure to the hypoxia from postnatal day 9 to 12 (that is, within the temporal window of enhanced oxygen susceptibility) and the results of this exposure were compared with normal rats as well as rats exposed to hyperoxia from birth to postnatal day 9 and from postnatal day 12 to 14—thus, an exposure regimen which avoided the window. Figure 4 compares representative photopic and scotopic ERG and OP responses obtained after exposure to the two new hyperoxic regimens with those obtained from an age-matched control rat. On first analysis, the results illustrated in Figure 4 suggest that exposure to hyperoxia outside the window was more detrimental to the function of the retina than exposure within the identified window. This is confirmed with the group data analysis, the results of which are given in Tables 1 and 2 and are graphically reported in Figure 3. Thus, whereas exposure within the window resulted in an average reduction in amplitude of 40% and 15% of the photopic and scotopic signals, respectively (all ERG and OP measurements considered), exposure outside the window yielded amplitude attenuations of 55% and 35% for the photopic and scotopic signals, respectively. However, when the significant difference in the duration of oxygen exposure for within (3 days) compared with the outside (11 days) the window regimens is considered, exposure within the window proportionally had a more profound impact on the retinal function than did the significantly longer exposure in the outside-the-window regi-

To verify this, a cohort of rats was exposed to hyperoxia from postnatal days 9 to 12 (that is, within the temporal window of enhanced oxygen susceptibility) and the results of this exposure were compared with normal rats as well as rats exposed to hyperoxia from birth to postnatal day 9 and from postnatal day 12 to 14—thus, an exposure regimen which avoided the window. Figure 4 compares representative photopic and scotopic ERG and OP responses obtained after exposure to the two new hyperoxic regimens with those obtained from an age-matched control rat. On first analysis, the results illustrated in Figure 4 suggest that exposure to hyperoxia outside the window was more detrimental to the function of the retina than exposure within the identified window. This is confirmed with the group data analysis, the results of which are given in Tables 1 and 2 and are graphically reported in Figure 3. Thus, whereas exposure within the window resulted in an average reduction in amplitude of 40% and 15% of the photopic and scotopic signals, respectively (all ERG and OP measurements considered), exposure outside the window yielded amplitude attenuations of 55% and 35% for the photopic and scotopic signals, respectively. However, when the significant difference in the duration of oxygen exposure for within (3 days) compared with the outside (11 days) the window regimens is considered, exposure within the window proportionally had a more profound impact on the retinal function than did the significantly longer exposure in the outside-the-window regi-
men. Again, as shown, the photopic responses were more affected by postnatal hyperoxia, whether it took place within or outside the temporal window of optimal effect. Similarly, as shown earlier, postnatal hyperoxia, whether within or outside the window had a minimal impact on the timing of the retinal potentials. Although the peak times obtained after exposure outside the window were slightly delayed compared with those measured after exposure within, this difference reached significance with measures of the scotopic OP2 only (Table 2).

Finally, as shown in Figure 4 (bottom), postnatal exposure to hyperoxia, whether within or outside the window also modified the retinal structure. In a previous study, we showed that the OPL was the retinal structure most sensitive to postnatal hyperoxia. After exposure within the window, the OPL’s thickness was 12.8 ± 0.09 μm compared with 9.35 ± 3.19 μm after exposure outside the window. Although there were a trend for a thinner OPL after exposure outside the window, these measurements are not significantly different.

**FIGURE 4.** Top: Representative examples of photopic and scotopic ERGs and OPs recorded at 30 days from control, 9- to 12- and 0- to 9- to 12- to 14-day exposures. Calibrations: horizontal, 20 ms; vertical, 100 and 350 μV for photopic and scotopic ERG, respectively, and 20 and 60 μV for photopic and scotopic OPs, respectively. All tracings include a 20-ms prestimulus baseline; vertical arrows: flash onset. Bottom: corresponding photomicrograph of cross sections of the central retinas from 60-day-old control rats and rats exposed to hyperoxia from 9- to 12- and from 0- to 9- to 12- to 14-days as indicated in the top panels. R, photoreceptor layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar, 10 μm.
from control (13.14 ± 0.12 μm). The latter result contrasts with the horizontal cell counts, which were 1.89 ± 0.49 and 1.45 ± 0.69 for the within and outside the window measurements, respectively (control, 5.77 ± 0.64), corresponding to 30% of normal.

**Discussion**

In a previous study we reported that postnatal hyperoxia, especially when applied during the second week of life, significantly modifies the amplitude of the rod mediated b-wave and markedly alters the retinal architecture in rats. Results reported in the present study not only extend our initial observation to the photopic responses but also to the OPs—results that suggest that exposure to high levels of oxygen during this critical postnatal period causes a severe panretinal disorder.

**Differential Effect of Postnatal Hyperoxia on Cone and Rod Responses**

It has been shown in rats that the cone function, as evaluated with the horizontal cell counts, which were 1.89 ± 0.49 and 1.45 ± 0.69 for the within and outside the window measurements, respectively (control, 5.77 ± 0.64), corresponding to 30% of normal. The Temporal Window of Enhanced Oxygen Susceptibility

The results presented also allowed us to demonstrate a temporal window of enhanced oxygen susceptibility, situated around postnatal day 10. Exposure regimens that included this very short temporal window were found to be proportionally more detrimental to the retinal function, especially that mediated by the cones. For instance, after exposure to hyperoxia from postnatal day 9 to 12, the amplitude of the cone b-wave showed a 40% reduction compared with 15% for the mixed rod-cone b-wave. Similarly, neither regimen had a significant impact on the thickness of the OPL. This suggests that the horizontal cells make a minimal contribution to the synapses of the OPL or that only a minimal number of horizontal cells is necessary to maintain the normal synaptic density of the OPL. It therefore appears that the alleged “window” can be demonstrated only functionally.

Another aspect of our results that must be taken into consideration is the period of oxygen hypersensitivity that we observed takes place just before the rat pups open their eyes (postnatal day 14)—that is, when all the retinal layers are already well established and the synaptic connections between the outer and inner nuclear layers are made. In rats, the OPL begins to be distinguishable at postnatal day 4 and is clearly demarcated by postnatal day 7. Furthermore, in rats, pyknotic activity in the bipolar cell layer reaches a maxi-

**Oxygen Hypersensitivity in Rat Retina**

These results thus clearly indicate that, despite the reported delay in the maturation of the cone function, postnatal hyperoxia was significantly more detrimental to the photopic signal than to the scotopic signal. Results obtained with the oscillatory potentials suggest a possible mechanism to explain the enhanced susceptibility of the cone function to postnatal hyperoxia.

**Differential Effect of Postnatal Hyperoxia on the b-Wave and OPs**

We have shown, with the scotopic response, that compared with the b-wave, the a-wave was minimally altered by postnatal exposure to hyperoxia. However, compared with the b-wave, the OPs appeared to be even more affected by the high levels of oxygen. At maximal effect, the photopic SOPs parameter (Photopic SOPs: Table 1, Fig. 3) was attenuated to approximately 15% of control amplitude compared with 25% for the b-wave. Similarly, after the same exposure regimen, the scotopic SOPs parameter (Scotopic SOPs: Table 1, Fig. 3) was attenuated to 20% of control amplitude compared with 40% for the b-wave. Even more interesting is that, after maximal exposure (0- to 14-day regimen) all photopic OPs appeared to be equally attenuated at a mean value of approximately 15% of control (Fig. 1, bottom left; Table 1), whereas scotopic OPs demonstrated a differential effect, in which the short-latency OP2 and OP3 were more affected by postnatal hyperoxia than the longer-latency OP4 and OP5. The short-latency OP2 and OP3 were also the only ERG components of the photopic response to be significantly affected after oxygen exposure from birth to postnatal day 6 (Fig. 1, bottom left; Table 1). It has been suggested that, in dark-adapted signals recorded from human subjects, the short-latency OP2 and OP3 signal the activation of the cone pathway, whereas the longer-latency OP4 and OP5 most probably reflect the activation of the rod pathways. It is also of interest to remember that in congenital stationary night blindness (CSNB) with myopia, there is a specific abolition of OP3 and OP4 from the photopic ERG response. This disorder is believed to result from a synaptic malfunction of the ON-depolarizing bipolar cells (ON-DBCs) which receive synaptic contacts from both types of photoreceptors, unlike the OFF-hyperpolarizing bipolar cells (OFF-HBCs) which receive inputs only from the cones. Given these facts, and assuming that the latter results can be transposed to dark-adapted signals recorded from rats, this not only further supports the claim that we have presented that the cone function is significantly more susceptible to postnatal hyperoxia than is the rod function, but also identifies the ON-DBCs as one of the retinal sites most susceptible to oxygen toxicity. This also explains the significant reduction in thickness of the OPL previously noted to occur after postnatal hyperoxia because it is at this level that the synaptic contacts between the photoreceptors and bipolar cells take place.
mum at approximately postnatal day 10, suggesting that postnatal hyperoxia could also interfere with the normal synaptic activity of the bipolar cells.²⁴ This also explains our findings with the OPs reported earlier.

A 7-day-old rat retina is said to correspond to a human retina at 5.5 months of gestation (24 weeks). At approximately postnatal day 10, the rat OPL is almost adultlike, whereas in humans, an equivalent development is reached after 6 to 7 months of gestation or approximately 30 weeks.²⁵ Consequently, because in rats the period of retinal hypersensitivity to oxygen that we have described appears to be situated between the postnatal days 9 and 12, in humans, the corresponding period appears after some 30 weeks of gestation. Flynn et al.²⁶ reported that in the very young infants born after 24 to 27 weeks of gestation, ROP is first diagnosed at approximately 7 weeks after birth, compared with 5 weeks in infants born after 28 to 29 weeks of gestation, and at 3 weeks in infants born after 30 to 34 weeks of gestation. It therefore appears that the younger in gestational age the premature infants are at birth, the older in postnatal age they will be when the first signs of ROP appear, should this event occur—as though the premature retina has to reach a predetermined level of maturity before the ROP can develop.²⁶ This suggests that ROP, as characterized by vascular changes, occurs at a certain developmental stage and explains why there is only a weak correlation between the incidence and severity of ROP and the amount of oxygen delivered to the infant.²⁶ Similarly, it has been reported that premature infants who are without ROP, despite postnatal exposure to hyperoxia, have a larger than normal incidence of visual problems,²⁷ suggesting that either the prematurity itself is an aggravating factor or the vascular assessment (funduscopy) is not a good enough clinical tool to rule out possible structural (inner retinal layer) and/or functional consequences of postnatal hyperoxia.

In summary, our study clearly demonstrates that OIR (and possibly ROP) is a panretinal disorder affecting the rod and the cone pathways. Structurally, the hyperoxic shock appears to be specific to the horizontal cells, although there is also some suggestion (from the OPs) that the ON-DBCs could also be impaired—if not structurally, at least functionally. Added together, these changes in the neuronal architecture of the retina most probably cause the gradual thinning of the OPL that we report. Data analysis further reveals that in rats, postnatal day 10 is critical to the development of OIR’s pathophysiological features for reasons that are speculative at this time. Clearly, should our rat model continue to be representative of the other animal models of OIR, including human ROP, an equivalent period of increased oxygen susceptibility of the retinal tissue must be postulated and taken into consideration when developing new therapeutic strategies.

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