Local Neuroretinal Function during Acute Hypoxia in Healthy Older People

Beatrix Feigl,1,2 Ian B. Stewart,1,3 Brian Brown,1,2 and Andrew J. Zele1,2

PURPOSE. To measure the effect of acute, mild, systemic hypoxia on neuroretinal function in two different age groups.

METHODS. The slow-flash paradigm of the multifocal electoretinogram (mfERG) was measured in two different oxygenation levels. Twelve participants of two age groups (28 ± 4 and 55 ± 5 years) breathed room air (normoxia) or 12% oxygen (hypoxia).

RESULTS. During normoxia (SaO2 = 98%) there was a significantly different effect between the younger and older participants on the mfERG positive waveform components (N1P1 amplitudes and P1 implicit times; P < 0.001). Hypoxia (SaO2 = 90%) significantly decreased N1P1 response density amplitudes in the central retinal field in both groups, with a larger effect in the older group (P < 0.001). There was no significant change in function of the cellular generators of oscillatory potentials (OPs).

CONCLUSION. The results extend recent findings by showing a greater effect of hypoxia on ON- and OFF-bipolar cell function in healthy older persons than in younger persons, but no effect on OPs during moderate acute hypoxia in either age group.

Study supported by an Institute of Health and Biomedical Innovation Fellowship (BF) and Australian Research Council Discovery Project (Invest Ophthalmol Vis Sci. 2008;49:807–813) DOI:10.1167/iovs.07-09994

Ischemia and hypoxia are thought to have an important role in the development of retinal disease.1,2 Oxygen is delivered to the retina via two vascular systems: the retinal and choroidal blood supplies. The retinal circulation is characterized by a low level of blood flow relative to that of the choroid and high oxygen extraction. The retinal circulation provides oxygen to the inner two thirds of the retina, including the bipolar and ganglion cell layers.3 The choroid, on the other hand, has high blood flow and low oxygen extraction4 and supplies primarily the outer parts of the retina including retinal pigment epithelial cells, the photoreceptor layer, and also parts of the (anatomically inner retinal) bipolar cell layer.5 Parts of the bipolar cell layer may therefore be vulnerable to ischemic insult because it is located between the two blood supplies.2

Blood flow responses can be altered during flickering stimulation, or during experimental hypoxia, because these conditions impose stress and increase retinal metabolic challenge.6–8 Shakoor9 demonstrated increased retinal blood flow in response to flickering stimuli delivered from a modified slit lamp biomicroscope, whereas there was no effect on choroidal blood flow. Falsini et al.7 showed significant correlations between flicker-evoked changes in the human optic nerve blood flow and changes in the flicker electroretinogram (ERG). Riva et al.10 proposed the possibility that electrophysiology can be a valuable, indirect tool for the assessment of blood flow-dependent neural activity. Although substantial studies have investigated the effect of hypoxia on global neuroretinal function using the full-field flicker ERG in animal and human eyes,11-17 recent studies have identified localized changes using the multifocal ERG (mfERG).18-20 These studies demonstrate that central neuroretinal function is reduced in healthy young participants in response to breathing 10% to 14% oxygen (as opposed to 21% in normal air at sea level).18-19 Retinal areas less than 5° in diameter were selectively affected during reduced blood oxygenation. Depending on the level of hypoxia, most of the findings indicate that the outer neuronal cells of the retina (photoreceptors) are more resistant to hypoxia than are the inner neuronal cells (bipolar cells, ganglion cells).11,21

Studies have found a preferential ON- and OFF-bipolar cell deficit during hypoxia in young participants.18,19 No study, however, has considered the consequences of hypoxia in different age groups. In the present study, we investigated the effect of hypoxia on central retinal function in eyes of young participants in comparison with those of an older group.

MATERIALS AND METHODS

Participants

Twelve healthy participants were divided into two (younger and older) age groups. The younger and older age groups consisted of four women and two men with mean ages of 28 ± 4 and 55 ± 5 years, respectively. All participants had normal vision (6/6 or better), clear ocular media, and no ocular disease and were in good general health as assessed by an ophthalmologist (BF). None of the participants in the young group had a refractive error. All participants in the older group were presbyopic and had mild corrections for the distance (< ±6.0 D).22 The participants gave written informed consent. The study was conducted in accordance with the requirements of the Queensland University of Technology Human Research Ethics Committee and the tenets of the Declaration of Helsinki.

METHODS

All tests were performed under the instruction of the same researcher (BF). Visual acuity (VA) was assessed with Bailey-Lovie charts at a distance of 6 m. We evaluated neuroretinal activity with the slow-flash paradigm of the mfERG (VERIS; EDI, Redwood City, CA). This paradigm is sensitive in detecting neuroretinal deficits during hypoxia18,19 and in retinal disease.24,25 The visual stimulus of the slow-flash mfERG consisted of 103 scaled black and white hexagons (50° retinal field) displayed on a calibrated 9-in. CRT monitor. The CRT monitor screen was refreshed every 13.33 ms (75 Hz frame rate). Calibration was performed according to standardized procedures.26 During testing, participants were instructed to fixate on a cross at the center of the CRT monitor and were corrected for the test distance (35 cm) using

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Supported by an Institute of Health and Biomedical Innovation Fellowship (BF) and Australian Research Council Discovery Project DPO775544 (AJZ).

Submitted for publication August 3, 2007; revised September 17 and October 8, 2007; accepted December 12, 2007.

Disclosure: B. Feigl, None; I.B. Stewart, None; B. Brown, None; A.J. Zele, None.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

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the VERIS eye monitor and refraction unit. Pupils were dilated using tropicamide 0.5%. All pupil sizes were at least 7 mm during testing.

For the slow flash mfERG paradigm, each hexagonal patch flickered between white (200 cd/m²) and black (3 cd/m²) according to a pseudorandom m-sequence (2¹³ − 1 steps in length) and was then followed by three dark frames (3 cd/m²; mean luminance = 26 cd/m²). Recordings were divided into 16 segments resulting in a total recording time of approximately 7 minutes. The mfERG was recorded monocularly with a DTL thread electrode as the active electrode, placed across the lower bulbar conjunctiva. The reference and ground were Ag-AgCl cup electrodes placed at the outer canthus close to the tested eye and on the forehead, respectively. Retinal signals were band-pass filtered (10−300 Hz), sampled every 0.83 ms, and amplified (50,000×; Grass-Telefactor, West Warwick, RI). We recorded the signals in ambient room light conditions. An eye camera and the online signal were used to monitor fixation and blinks; signal segments were rejected and rerecorded if small eye movements or artifacts were detected during the recording.

We analyzed N1P1 peak to trough response densities (amplitudes per unit retinal area, in nV/deg²), peak N1 and P1 implicit times (in milliseconds, are defined from the onset of the stimulus to the first negative and positive peak) as shown in Figure 1A. Oscillatory potentials (OPs). The peak-to-trough amplitude and peak implicit times of the four major OPs were analyzed. Bottom right: the OPs, averaged into central and nasal and temporal hemifields, as shown in the stimulus array.

\[ s(t) = \sin(2\pi f t) + p. \]  

where \( g(t) \) defines a Gaussian envelope of amplitude \( a \) in nanovolts, width \( \sigma \) in milliseconds, and peak time \( n \) in milliseconds; \( s(t) \) defines a sinusoidal carrier as a function of time \( t \) in ms, with the frequency \( (b) \) in hertz and phase \( p \) in degrees specified relative to the start of the waveform.\(^{27}\) The Gabor envelope was fitted by minimizing the sum-of-square differences between the data and model by changing the free parameters using a Marquardt-Levenberg algorithm.

The mfERG responses were averaged into concentric rings (Fig. 1A) and central, nasal, and temporal waveform responses (Fig. 1B) due to nasal-temporal asymmetry found in the OPs.\(^{28–30}\) Before analyzing the first-order kernel (mean local responses to all stimuli in a stimulus cycle) amplitudes and implicit times, two iterations of spatial averaging procedure (with 17% of the response of its six neighbors) and two artifact removals were performed on the raw mfERG data, according to standard procedures.\(^{37}\) Although these procedures are suggested by the manufacturer, they should be used cautiously.\(^{31}\)

**Hypoxia Gas Delivery and Measurement**

The order of normoxic and hypoxic conditions for mfERG recording were determined randomly. The hypoxic gas (12% O₂ in balance with nitrogen) was delivered via a nonrebreathing mask, containing a reservoir, which was connected via small-bore tubing to a medical gas cylinder. The gas was delivered at a flow rate of 12 to 15 L/min. We monitored the heart rate and arterial oxygen saturation (SaO₂) with a pulse oximeter (model 3900; Datex-Ohmeda Inc, Madison, WI). The SaO₂ level quantified the level of hypoxia. Before the mfERG was recorded, participants breathed the gas mixture until an SaO₂ level of 92% was reached.

**Statistical Analysis**

For statistical analysis, the mean of the N1P1 amplitude response densities, N1 and P1 implicit time, and OP amplitudes and peak implicit times in normoxic and hypoxic conditions were compared. A repeated-measures ANOVA (SPSS ver. 14; SPSS, Chicago, IL) was used to identify the presence of significant (main) effects between the two oxygenation levels (normoxic and hypoxic), the two groups (young and older), and retinal locations (103 locations grouped in five rings or in central, temporal, and nasal areas as shown in Fig. 1). Post hoc analysis and Student’s t-tests (suitably adjusted for multiple comparisons) were performed when there were significant main effects and interactions, respectively.

**Results**

The SaO₂ level in both groups decreased significantly while breathing the hypoxic gas mixture; SaO₂ decreased from 98 ± 1% to 90% ± 2% in the young group and from 97% ± 1% to 91% ± 0.4% in the older group. The pulse rate increased from 78 ± 7.7 bpm during normoxia to 85 ± 13.7 bpm during hypoxia in the young group. In the older group, the pulse rate increased from 59 ± 13.0 bpm during normoxia to 67 ± 13.7 bpm during hypoxia, but these increases were not significant for either group.

Figure 2 demonstrates the trace array of a representative younger (top) and older (middle) participant during normoxia and hypoxia. The arrays at the bottom are those of the extracted first-order kernel multifocal OPs during normoxia and hypoxia of a representative older participant. The trace array for the slow-flash positive waveforms demonstrate lower amplitude responses during hypoxic conditions for both age groups. This was not evident for the OPs which were unaffected during hypoxia.
Figure 2. The trace arrays for a representative younger (top) participant and an older (middle) participant during the normoxic and hypoxic conditions. The older participant showed a generalized reduction in central and paracentral responses during hypoxia, whereas the young participant's responses were more affected only in the central areas. Bottom: extracted multifocal OPs of a representative older participant. The amplitude of the OPs did not differ significantly between normoxia and hypoxia in both the younger and older participants.

Figure 3 shows how normoxia and hypoxia altered the 103 local responses averaged over five retinal rings. There was a significant main effect for N1P1 response density amplitudes between the younger and older group ($F_{1,98} = 1251.6, P < 0.001$) and between the normoxic and hypoxic conditions ($F_{1,98} = 298.3, P < 0.001$) showing lower amplitudes with age.

Figure 3. The younger and older participants' amplitude response densities (±SD) during normoxia and hypoxia.
(Table 1) and during hypoxia. A significant group by condition interaction indicated that the effect of hypoxia on amplitudes was significantly larger in the older group than in the young group at all locations \((F_{1,98} = 134.4, P < 0.001)\). We also found a significant main effect of age on P1 implicit times \((F_{1,98} = 143.1, P < 0.001)\) but not on N1 implicit times, with longer implicit times in the older group. However, there was no main effect or interaction between P1 and N1 implicit times during hypoxia compared with normoxia.

Table 2 shows the N1P1 response density amplitudes and P1 and N1 peak implicit times for the younger group during normoxia and hypoxia. During hypoxia, there was a significant location interaction \((F_{1,98} = 3.7, P < 0.01)\) for the N1P1 response density amplitudes indicating significantly lower local response density amplitudes for ring 1 (central 0°–5°) compared with normoxic conditions in the young group \((P = 0.02; \text{Fig. 3})\). The older group (Table 2), however, had significantly lower amplitude responses in all 103 retinal locations in the five areas during hypoxia compared with normoxia \((P < 0.001)\).

The oscillatory potentials for a representative younger and an older participant during normoxia are shown in Figure 4A, left and right. The OPs are indicated by the symbols (dots) and the best-fitting model is indicated by the solid lines. During normoxia, there was a significant effect between the age groups for the averaged central, nasal, and temporal OP amplitude response densities \((F_{1,10} = 21.2, P = 0.001)\) and for the implicit times \((F_{1,10} = 3.9, P = 0.04; \text{Table 3})\). Post hoc analysis revealed significantly larger amplitudes in the younger group than in the older group for the central OP4 \((P = 0.05)\), nasal OP2 \((P = 0.05)\), and temporal OP2 and OP3 \((P = 0.03; \text{Table 3})\). Central OP1 \((P < 0.01)\), nasal OP1 \((P = 0.05)\), and OP2 \((P < 0.01)\) and temporal OP1 \((P = 0.01)\) and OP3 \((P = 0.05)\) were significantly delayed in the older group compared with the younger group. During hypoxia, however, the OPs were not significantly reduced in either group compared with the normoxic condition. The average (±SD) frequency of the oscillatory potentials for the young (square symbols) and old (diamond symbols) groups during normoxia (unfilled symbols) and hypoxia (filled symbols) are shown in Figure 4B. There were no significant differences in the oscillation frequency across age group or oxygenation level.

We can exclude the influence of optical factors on the functional results because the older participants had no lens opacification on slit lamp examination and lens grading. The pupil sizes of the young and older participants were also similar (≈7 mm). Taken together, the absence of preretinal optical changes and differences in pupil size, the retinal illumination would have been comparable in both age groups.

**DISCUSSION**

Central neuroretinal function was significantly reduced during hypoxia in the younger and older participants. We have shown for the first time that hypoxia affects the central and paracentral retina to a greater extent in the older eye than in the younger eye (Fig. 2). Our findings provide further support for a hypothesized decrease in bipolar cell activity in older eyes, consistent with data from the slow-flash mfERG paradigm\(^{25}\) and from histologic studies.\(^{32}\)

Histologic studies show that the density of foveal cone photoreceptors remains stable with ageing,\(^{33,34}\) but there is selective vulnerability of the paracentral rods.\(^{35}\) We can exclude rod contributions to our results because the overall mean luminance condition of our experiment (26 cd/m\(^2\)) saturates rod responses.\(^{36}\) The cone photoreceptors, however, undergo structural changes (i.e., abnormal orientation, cytological ab-

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**Table 1. The Concentric Ring-Averaged mfERG Amplitude and Implicit Time Parameters between the Younger and Older Group**

<table>
<thead>
<tr>
<th>Ring</th>
<th>Young</th>
<th>Older</th>
<th>Young</th>
<th>Older</th>
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<tbody>
<tr>
<td>Ring 1 (7 Hexagons)</td>
<td>13.7 ± 0.7</td>
<td>10.5 ± 0.9</td>
<td>1.3 ± 1.6</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>Ring 2 (12 Hexagons)</td>
<td>12.9 ± 1.6</td>
<td>9.3 ± 0.8</td>
<td>1.1 ± 1.2</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>Ring 3 (18 Hexagons)</td>
<td>12.3 ± 1.5</td>
<td>9.0 ± 0.6</td>
<td>0.9 ± 0.4</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Ring 4 (24 Hexagons)</td>
<td>12.2 ± 1.2</td>
<td>8.9 ± 0.4</td>
<td>0.8 ± 0.3</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Ring 5 (42 Hexagons)</td>
<td>12.1 ± 1.2</td>
<td>8.9 ± 0.4</td>
<td>0.8 ± 0.3</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

Data are the mean ± SD. *Statistically significant compared with the older group, \(P < 0.001\).
normalities) by the mid-50s that can result in reduced quantal catch, due to decreased photopigment density, and/or slowed photopigment regeneration due to ageing changes in Bruch’s membrane.

Recent immunohistochemical studies of the human retina indicate that there is extensive dendritic reorganization of ON cone bipolar cells and retinal interneurons, with the elongation of dendrites into the outer nuclear layer in the retinas of older compared with younger participants. Our findings of more impaired bipolar cell function in the older group than in the younger group could reflect such cellular reorganization. Although neuronal functional changes in the retinal cells in older eyes have previously been reported, these studies used the standard fast-flicker mfERG paradigm, which gives a clearer measure of bipolar cell contributions. The slow-flash mfERG paradigm better discriminates the cellular changes because it gives a mixed response that can mask isolated cell contributions.

There was a significant reduction in the amplitude and an increase in implicit time of the multifocal OPs in older eyes compared with younger eyes, consistent with observations by Kurtenbach and Weiss et al. However, this effect was not evident in all four OPs or in every location. This finding supports the proposal that OPs have their cellular origin in retinal inhibitory feedback circuits from amacrine to bipolar cells and/or from ganglion cells to amacrine cells. The average frequency of the OPs for the young (squares) and older (diamonds) groups during normoxia (filled) and hypoxia (unfilled) are outlined. There was a significant age effect but no change in frequency across the age groups and during hypoxia.

### Table 2. Concentric Ring-Averaged mfERG Amplitudes and Implicit Times in Normoxic and Hypoxic Conditions

<table>
<thead>
<tr>
<th>Ring 1 (7 Hexagons)</th>
<th>Ring 2 (12 Hexagons)</th>
<th>Ring 3 (18 Hexagons)</th>
<th>Ring 4 (24 Hexagons)</th>
<th>Ring 5 (32 Hexagons)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normoxic</td>
<td>Hypoxic</td>
<td>Normoxic</td>
<td>Hypoxic</td>
</tr>
<tr>
<td>N1P1 (nV/deg²)</td>
<td>13.7 ± 0.7</td>
<td>12.8 ± 0.2</td>
<td>12.9 ± 1.0</td>
<td>12.3 ± 0.5</td>
</tr>
<tr>
<td>P1-IT (ms)</td>
<td>17.6 ± 0.4</td>
<td>17.3 ± 1.0</td>
<td>18.1 ± 0.4</td>
<td>17.9 ± 1.3</td>
</tr>
<tr>
<td>N1-IT (ms)</td>
<td>36.7 ± 0.6</td>
<td>37.9 ± 1.0</td>
<td>35.1 ± 0.4</td>
<td>34.8 ± 0.7</td>
</tr>
<tr>
<td>Older group</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N1P1 (nV/deg²)</td>
<td>11.3 ± 0.6†</td>
<td>8.3 ± 0.6</td>
<td>10.7 ± 0.7†</td>
<td>8.3 ± 0.6</td>
</tr>
<tr>
<td>P1-IT (ms)</td>
<td>17.3 ± 1.0</td>
<td>17.8 ± 1.4</td>
<td>17.7 ± 1.1</td>
<td>17.2 ± 1.0</td>
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</tbody>
</table>
| Data are the mean ± SD. † Statistically significant compared with hypoxic condition, \( P < 0.001 \).
may be differently affected in the older eye. Age-related deficits in multifocal OPs have been suggested to involve changes in postreceptoral processing. The frequency of the OPs, however, was unaffected in older and younger eyes or by alteration of oxygenation level in our study (Fig. 4B). Neuronal oscillations are thought to be involved in the control of the temporal precision of action potential discharges. The finding that the amplitude reduction is unaccompanied by changes in the oscillation frequency suggests that, in the presence of increased neuronal spike thresholds, neuronal impedance remains constant. Therefore, in the older eye, neurons can maintain temporal precision, but require greater stimulus energy to generate a response. We did not show an effect of hypoxia on OP amplitude and latency, as had been found in previous studies. Although a small sample size and greater variability could have influenced the OP data, the differing findings may also be due to the magnitude of hypoxia induced in the other studies (average SaO2 between 69% and 82%, as opposed to our average of 90%). OPs are known to be strongly dependent on retinal circulation and lower oxygen levels may have reproduced the findings of Klemp at al. and Janaky et al.

We extend previous findings in the young person to an older sample by showing further reduction in central visual function in the older observers during acute hypoxia. Although this may reflect neuronal reorganization in the older eye, our results may also be related to vascular changes. Blum et al. demonstrated that retinal arterioles have a lower contractility in older eyes, and reduced vessel stability has been observed during ageing in an animal model. Changes in the pericyte-endothelial cell contacts have been suggested, consistent with impaired autoregulation and higher susceptibility to vascular endothelial cell loss. Although we have not measured vascular contractility, our results of impaired neuroretinal function may reflect reduced vessel stability in the older eye.

Our previous and current results demonstrate that bipolar cell deficits occur at systemic oxygenation levels of ~ 90%, a level with occurs naturally at an altitude of approximately 4000 m. These altitudes and levels of hypoxia are becoming more frequently encountered due to the increased popularity of commercial mountain trekking tours, as well as with reduced cabin pressure during air travel. Although these oxygenation levels are well tolerated in healthy humans, they can cause discomfort; individuals with a predisposition to ischemic disease such as the elderly, may be more likely to experience harmful effects in the central retina.

In conclusion, we found a significant effect of older age on central neuroretinal function during experimental hypoxia, which suggests that older people may be more susceptible to retinal ischemia and hypoxia. The investigation of different levels of experimental hypoxia in healthy older humans and its effect on retinal neurons may be important in a human model of retinal diseases such as age-related macular degeneration (AMD) where chronic hypoxia and ischemia may be causative factors. Hypoxia and ischemia dependent changes in retinal function may help in the understanding of the pathomechanisms of AMD and the new treatments for AMD which target the growth factors that are likely to be released in the retina in response to hypoxia.

References


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**TABLE 3. Amplitudes and Peak Implicit Times of OPs in the Younger and Older Participants for the Averaged Visual Fields during Normoxia**

<table>
<thead>
<tr>
<th></th>
<th>Younger</th>
<th>Older</th>
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</thead>
<tbody>
<tr>
<td><strong>Amplitudes (nV/deg²)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP 1</td>
<td>2.7 ± 0.5</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>OP 2</td>
<td>2.7 ± 1.3</td>
<td>2.3 ± 0.7</td>
</tr>
<tr>
<td>OP 3</td>
<td>3.9 ± 1.3</td>
<td>2.6 ± 1.2</td>
</tr>
<tr>
<td>OP 4</td>
<td>3.8 ± 1.1*</td>
<td>2.1 ± 1.2</td>
</tr>
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<table>
<thead>
<tr>
<th></th>
<th>Younger</th>
<th>Older</th>
</tr>
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<tbody>
<tr>
<td><strong>Implicit times (ms)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OP 1</td>
<td>16.0 ± 1.2*</td>
<td>19.3 ± 1.7</td>
</tr>
<tr>
<td>OP 2</td>
<td>26.0 ± 2.6</td>
<td>27.0 ± 2.3</td>
</tr>
<tr>
<td>OP 3</td>
<td>34.9 ± 1.0</td>
<td>34.2 ± 2.2</td>
</tr>
<tr>
<td>OP 4</td>
<td>43.3 ± 1.0</td>
<td>41.8 ± 2.1</td>
</tr>
</tbody>
</table>

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Data are the mean ± SD.

* Statistically significant compared with the older group, *P ≤ 0.05.
Local Neuroretinal Function during Acute Hypoxia


