# The Effect of Atropine on Human Global Flash mfERG Responses to Retinal Defocus

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**Purpose.** To investigate the action of atropine on global flash multifocal electroretinogram (gmfERG) responses to retinal defocus.

**Method.** gmfERG recordings were made monocularly in 19 healthy adults under three lens-imposed defocus conditions (2 diopters myopic, 2 diopters hyperopic, and no defocus) before and 24 hours after instillation of 1 drop of 0.1% atropine. Signals reflecting activity from the outer and inner retina (direct [DC] and induced [IC] components respectively) were analyzed. Responses were grouped into either a central (0°–6°) or peripheral (6°–24°) retinal zone. The gmfERG responses were compared relative to the no defocus, no atropine condition.

**Results.** Within the central zone, atropine had no effect on the amplitudes and peak times of DC or IC responses to defocus. For IC responses in the peripheral zone, there was a significant interaction effect of atropine and defocus ($F_{2,36} = 6.050$, $P = 0.011$) with greater post-atropine amplitudes under myopic defocus (mean increase = 15.5%, 95% confidence interval [CI] = 5.6%–25.4%, $P = 0.004$). Atropine also had a significant main effect of increasing IC peak times ($F_{1,18} = 9.722$, $P = 0.006$). For DC responses, atropine had a significant main effect of increasing DC amplitudes ($F_{1,18} = 7.821$, $P = 0.012$) and peak times ($F_{1,18} = 15.406$, $P = 0.001$) regardless of sign of defocus.

**Conclusions.** Our results imply that atropine acts in the inner layers of the peripheral retina to affect neuronal responses to myopic defocus, raising the possibility that atropine may potentiate the effects of myopic defocus in inhibiting eye growth.

Keywords: atropine, electrophysiology, myopic defocus, hyperopic defocus, retina

The worldwide prevalence of myopia (short-sightedness) has risen alarmingly,[1] and the World Health Organization has identified myopia as a major public health concern because it increases the risk of sight-threatening ocular comorbidities, such as myopic maculopathy, cataract, glaucoma, and retinal detachment.[2,3] Measures to prevent the development of myopia in children are starting to be implemented,[4] and there are several strategies for reducing the rate of progression.[5] Atropine eye drops, at least at higher doses, are the most effective treatment for reducing myopia progression,[5–7] with an efficacy of approximately 50%,[6] although how atropine acts to control myopia remains largely unanswered.[8] Evidence from animal studies indicates that atropine acts via a non-accommodative mechanism,[9] and in humans atropine eye drops cause thickening of the choroid[10] and also abolish the choroidal thinning normally associated with exposing the retina to hyperopic defocus.[11] Atropine also reduces the refractive error changes associated with myopia progression in concentrations as low as 0.01% (which produce negligible mydriasis and cycloplegia).[6]

Support for a retinal site of action comes from the finding that retinal dopamine levels, which are reduced in experimentally induced myopia in chicks, are increased with intravitreal injection of atropine that subsequently triggers spreading depression effects in the retina.[12] Furthermore, it has been proposed that the light-adaptive signaling molecule nitric oxide mediates the inhibition of myopia by atropine in form-deprived chicks, and it is thought that this occurs in the retina.[13] The exact retinal location remains elusive, as atropine can still block myopia development in the chick model of myopia despite the destruction of retinal amacrine cells containing muscarinic receptors.[14] At least in vitro, there is support for non-muscarinic targets for atropine, including α2A-adrenoceptors.[15]

Neural activity in the retina can be quantified using global flash multifocal electroretinography (gmfERG).[16] In gmfERG, a full-field (i.e., global) flash stimulus is inserted between successive focal flashes of a standard mfERG stimulus, allowing two response components to be isolated: the direct (DC) and the induced (IC) responses. The DC response is the conventional output from a standard mfERG, which approximates the a- and b-waves of an ERG, though, with mfERG, this represents a pooled response that is predominantly derived from the photoreceptor and bipolar cell activity.[17] The gmfERG IC component is derived from cells within the inner retina (ganglion and amacrine cells),[16] which is present because of the nonlinear adapted retinal state induced in response to successive stimuli from both the global flash and mfERG frames of the stimulus. This ability to isolate the inner retina is of particular interest to myopia researchers, as amacrine cells have been shown to modulate ZENK synthesis in the signaling of defocus,[18] and experimental myopia causes a reduction in

retinal concentrations of dopamine and its metabolites, with retinal amacrine cells being one of the main sources.[19,20] Previous research in humans has shown that gmfERG responses are altered under conditions of retinal defocus, increasing with myopic defocus and decreasing with hyperopic defocus, with the effects most pronounced in the mid-peripheral retina.[21,22]

The aim of this study was to investigate the effect of atropine on neural activity in the human retina under short-term imposed defocus. We hypothesized that if the antimyopia effects of atropine are based on a retinal site of action, then atropine may modify the above gmfERG responses to retinal defocus.

## Methods

### Subjects

A total of 19 adult subjects aged between 18 and 35 years (mean: 23.0 ± 3.5 years, 6 females) with emmetropia or low to moderate myopic refractive errors (spherical equivalent refraction in the experimental eye: range +0.25 to −4.50 diopters (D); mean: −1.85 ± 1.53 D) participated in the study. Each subject underwent an initial optometric examination to ensure that they were eligible to participate. The exclusion criteria were: age outside 18 to 35 years, visual acuity poorer than 6/9, SER > +5.00 D or < −5.00 D, astigmatism ≥1.00 D, amblyopia, ocular pathologies, or ocular anomalies (e.g., surgery, trauma), systemic disorders (e.g., neurologic conditions), and subjects undergoing optical or pharmacologic myopia control treatment (e.g., atropine eye drops, orthokeratology). Ethical approval was obtained from the University of Auckland Human Participants Ethics committee (reference: 017982) and written informed consent was provided by all subjects. All procedures were conducted in accordance with the tenets of the Declaration of Helsinki.

### Experimental Protocol

All subjects underwent gmfERG testing (RETIScan, Roland Consult, Germany) at the same time of day on 2 consecutive days (days 1 and 2). On each day, gmfERG responses were recorded monocularly under three defocus conditions: no defocus, 2D myopic defocus and 2D hyperopic defocus, in random order by using the random permutation function in a computing environment (MATLAB; MathWorks, Natick, MA, USA). Thirty minutes prior to gmfERG recording on each day, one drop of tropicamide 1% was instilled into the experimental (nondominant) eye as a cycloplegic.[23] At the end of the recording session on day 1, one drop of atropine sulphate 0.1% was instilled into the experimental eye, so that recordings on day 2 were made approximately 24 hours after atropine instillation.

The gmfERG recordings were made monocularly, with the fellow eye covered. As the use of a negative 2D lens to induce hyperopic defocus could stimulate accommodation and result in less than the desired 2D defocus under the hyperopic defocus condition, the residual accommodation was measured and compensated for. Approximately 25 minutes after the instillation of the cycloplegic drop, five consecutive autorefractor measures were made with an open-field autorefractor (NVision-K 5001; Shin Nippon, Hiroshima, Japan) to confirm the distance prescription of the experimental eye. A target line of letters (corresponding to their best visual acuity) was viewed monocularly at 50 cm through the distance prescription, with a +2.00 D add. The target was moved slowly toward the eye until the subject reported the first sustained blur. The residual accommodation was calculated as the dioptric difference between 50 cm and the blur point. This procedure was repeated three times to get a mean residual accommodation in diopters, which was then added to the negative lens power to ensure at least 2D of defocus was achieved during the hyperopic defocus condition. The mean residual accommodation for all subjects was 1.55 ± 0.59 D for day 1 and 1.50 ± 0.56 D for day 2. Defocus was induced using full aperture ophthalmic lenses which combined the power of the defocusing lens, the patient's refractive error and an add of +3.00 D to account for viewing the stimulus screen at 33 cm. The spectacle magnification (SM) induced by the defocusing lenses, as calculated from the formula $SM = 1/(1 - dF$; where $d$ = vertex distance + distance from corneal apex to entrance pupil, and $F$ = power of the lens) induced a ∼3% decrease or increase in retinal image size. To compensate, the screen viewing distance was adjusted by 1 cm (i.e., from 33 cm under no defocus to 32 cm for −2.00 D defocus and 34 cm for +2.00 D defocus).

### Electrophysiology Procedure

Subjects were light-adapted to 500 lux room lighting for a period of 30 minutes prior to gmfERG testing. The gmfERG stimulus pattern consisted of an array of 61 hexagons with a small red fixation cross (1° in size) in the center. Hexagon size progressively increased with eccentricity and was scaled to produce approximately equal mfERG amplitudes with eccentricity, so as to compensate for the reduction in retinal cell density with eccentricity. The stimulus was presented on a calibrated LCD monitor (38 × 30 cm in size) at 60 frames per second. The hexagons alternated black and white in a pseudorandom binary m-sequence.[24] The luminance of the gray background surrounding the stimulus was 108 cd/m$^2$ and that of the dark and bright hexagons were 2.8 and 184 cd/m$^2$, respectively. The global flash stimulus was inserted between successive focal flashes such that the stimulation sequence consisted of a multifocal flash frame, a dark frame, a full screen global flash and a dark frame in each cycle (Fig. 1A).[25] The gmfERG responses were measured across eight time segments, each of approximately 38 seconds duration, with a short break between each.

The gmfERG electrical responses were recorded using active, reference and ground electrodes. The reference and ground electrodes (grass gold cup) were applied following skin preparation using an exfoliant gel (NuPrep; Weaver and Company, Aurora, CO, USA) before applying the electrodes with a conductive paste (TEN20; Weaver and Company). The reference electrode was attached on the temple, 1 cm lateral to the outer canthus. The ground electrode was attached to the center of the forehead. Following corneal anesthesia with one drop of 0.4% oxybuprocaine, the active electrode (Dawson-Trick-Litzkow fine conductive thread) was positioned in the lower lid tear prism. Impedance was monitored throughout the test and maintained below 10 K Ohm by repositioning the electrodes as required. The contralateral eye was occluded with an eye pad and light pressure throughout the test period. Responses were band pass filtered (1 to 300 Hz, with a 50 Hz notch) and amplified by ×100,000. Using an inbuilt artefact rejection algorithm, the electrophysiology software automatically detected artefacts (e.g., from blinking), removed the corresponding responses and retested the sequence. Details of the measurement of the DC and the IC amplitudes in gmfERG have been well described elsewhere.[21] In brief, the DC amplitudes were measured from the first negative trough to the first positive peak, while the IC amplitudes were measured from the second positive peak to the second negative trough (Fig. 1C). The peak time of the DC component was measured

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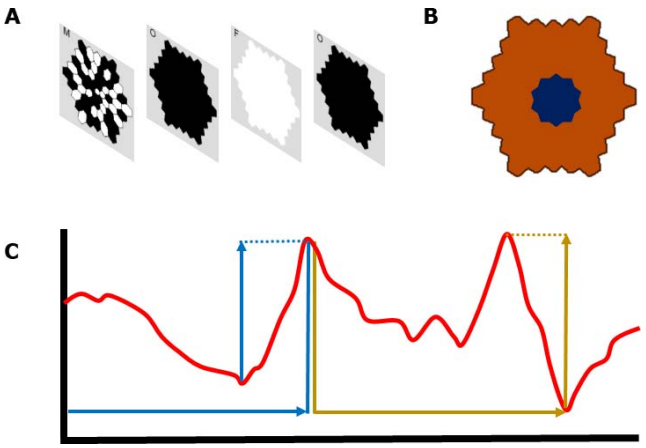


FIGURE 1. (**A**) The stimulus array for the gmfERG had four frames in each cycle: a multifocal flash frame (M), dark frame (O), global flash frame (F), and dark frame (O) with an interframe interval of 16.66 ms. The multifocal flash frame consisted of 61 light and dark hexagonal elements: approximately 50% of these elements were illuminated at each presentation with the pattern of light and dark hexagons changing over time according to a pseudo-random binary m-sequence. (**B**) Figure showing central retinal zone (*dark blue*, 0°–6° eccentricity), and peripheral retinal zone (*orange*, 6°–24° eccentricity). The sum of all responses within a zone was divided by the area of hexagons within the zone to give nV/degree$^2$. (**C**) The gmfERG response waveform demonstrated two main components: DC and IC. The *vertical arrows* represent the amplitude of the DC component measured from the first trough to the first peak (*light blue*) and the IC component measured from the second trough to the second peak (*yellow*). The *horizontal arrows* represent the peak times of DC (*light blue*) and IC (*yellow*).

from the presentation of the multifocal flash, while that of the IC component was measured from the presentation of the global flash (i.e., 33.3 ms after the multifocal flash). The DC component amplitudes of the gmfERG recordings were automatically detected using commercial software (RETIScan version 6.16.1.5; Roland Consult, Brandenburg an der Havel, Germany), and the raw values were exported for analysis. The IC component peaks and troughs were manually determined by repositioning the arrows along the trace using commercial software (Roland Consult) and then also exported for analysis. Response data corresponding to the hexagons within each of the two zones (central: 0° to 6° and peripheral: 6° to 24°; Fig. 1B) were averaged to give mean values for each zone.

### Statistical Analysis

Statistical analysis of the data was carried out using statistical software (SPSS 22.0; SPSS, Inc., Chicago, IL, USA, and MATLAB 2016b; MathWorks). Normality of the data was verified using graphical methods (histograms and P-P plots). The assumption of sphericity was tested with Mauchly's test and corrected with Greenhouse-Giesser estimates upon occasional violation. The gmfERG responses (amplitudes and peak times) under no defocus, no atropine condition was taken as the baseline. Changes in the DC and the IC amplitudes and peak times were assessed as the difference in responses from the baseline. A 2-way repeated-measures ANOVA was conducted using atropine (yes/no) and defocus (hyperopic/plano/myopic) as within-subject factors. Variables with significant within-subject effects were compared with pairwise comparisons using the Sidak correction for multiple comparisons. Results were considered significant at $P < 0.05$.

## RESULTS

The gmfERG DC and IC parameters (amplitudes and peak times) of 19 subjects were compared in the central and peripheral zones, across the three different defocus conditions. Examples for one subject are shown in Figure 2.

### Central Zone Amplitude

There was no significant interaction effect of atropine and defocus on DC amplitude ($F_{2,36} = 0.353$, $P = 0.705$; Fig. 3), nor an effect of defocus ($F_{2,36} = 0.757$, $P = 0.436$) or atropine ($F_{1,18} = 0.024$, $P = 0.879$). The same was true for the IC component, with no significant interaction effect of atropine and defocus on IC amplitude ($F_{2,36} = 0.487$, $P = 0.618$), and no effect of atropine ($F_{1,18} = 2.440$, $P = 0.136$). However, optical defocus had a significant effect on IC amplitude ($F_{2,36} = 4.041$, $P = 0.026$). Pairwise testing showed the changes in amplitudes were significantly greater for myopic defocus compared to the hyperopic defocus condition (mean difference = 11.3%, $P = 0.015$), but neither were different from the no defocus condition (myopic defocus versus no defocus: mean difference = 6.55%, $P = 0.468$; hyperopic defocus versus no defocus: mean difference = –4.75%, $P = 0.472$; Table 1).

### Peripheral Zone Amplitude

There was no significant interaction effect of atropine and optical defocus on the DC amplitude ($F_{2,36} = 0.908$, $P = 0.385$), but both atropine ($F_{1,18} = 7.821$, $P = 0.012$) and defocus ($F_{2,36} = 16.435$, $P < 0.001$) had significant effects (Fig. 3). The DC amplitude with myopic defocus was higher than that for hyperopic defocus (mean difference = 19.28%, 95% CI = 9.067–29.48%, $P < 0.001$) and for no defocus (mean difference = 12.63%, 95% CI = 4.334–20.921%, $P = 0.002$). However, there was no difference between the hyperopic defocus and no defocus conditions (mean difference = 6.65%, 95% CI = –1.667% to 14.970%, $P = 0.143$; Table 1). The DC amplitudes post-atropine were greater than that for pre-atropine (mean difference [post-minus preatropine] = 11.10%, 95% CI = 2.761 to 19.437%, $P = 0.012$) across all defocus conditions (Table 2).

There was a statistically significant main effect of optical defocus ($F_{2,36} = 26.172$, $P < 0.001$) on IC amplitudes, with amplitude increasing progressively from the hyperopic defocus condition through no defocus to the myopic defocus condition (Fig. 3). The IC amplitude under myopic defocus was higher than that for no defocus (mean difference = 14.67%, 95% CI = 4.42%–24.92%, $P = 0.004$) and hyperopic defocus (mean difference = 25.95%, 95% CI = 14.622%–37.268%, $P < 0.001$), and there was also a significant difference in the IC amplitudes between the hyperopic defocus and no defocus conditions (mean difference = 11.27%, 95% CI = 5.327%–17.218%, $P < 0.001$; Table 1). Although the main effect of atropine was not significant ($F_{1,18} = 4.365$, $P = 0.051$), there was an interaction effect of atropine and defocus on the IC amplitudes ($F_{2,36} = 6.050$, $P = 0.011$), implying a differential effect of atropine based on the sign of optical defocus. Compared to baseline, atropine resulted in a significant increase in IC amplitude under the myopic defocus condition (mean difference = 15.52%, 95% CI = 5.627%–25.421%, $P = 0.004$). However, atropine had no effect on the IC amplitude under either hyperopic defocus (mean difference = 1.77%, 95% CI = –6.54% to 10.09%, $P = 0.659$) or no defocus conditions (mean difference = 5.66%, 95% CI = –3.50% to 14.82%, $P = 0.211$).

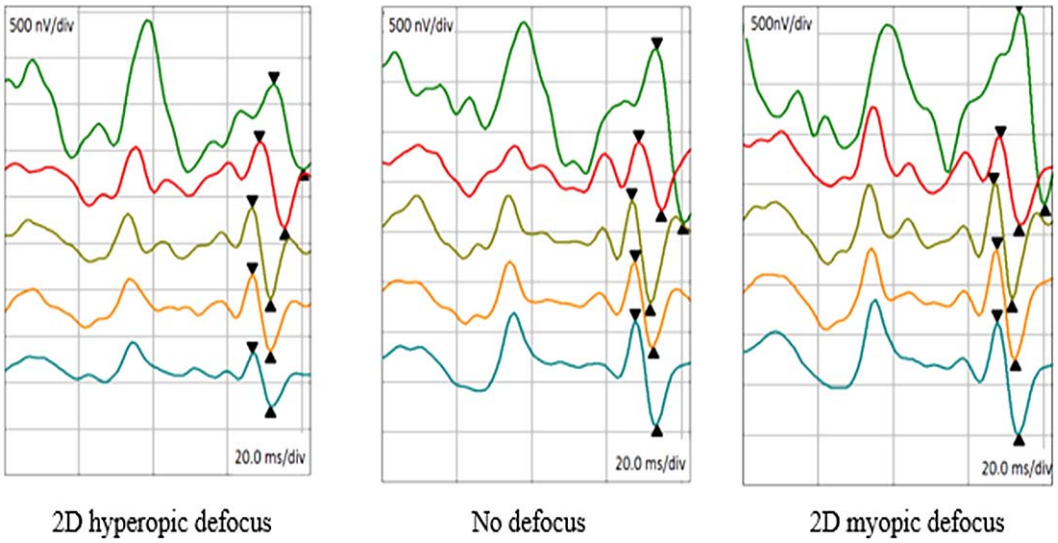


FIGURE 2. An example of the gmfERG waveforms from a single subject for hyperopic defocus (–2.00 D, *left*), no defocus (fully corrected, *center*), and myopic defocus (+2.00 D, *right*) prior to atropine instillation. The *black arrows* indicate the amplitude of the IC, which was measured from the second maximum peak to the second minimum trough. The five colored traces represent responses from different retinal eccentricities from the center (*top* trace, *green*) to the periphery (*bottom* trace, *blue*).

## Central Zone Peak Times

Atropine did not have a significant effect on the peak times of the DC responses ($F_{1,18} = 0.864$, $P = 0.365$; Fig. 4), but while there was a just significant effect of defocus ($F_{2,36} = 3.563$, $P = 0.039$), pairwise differences were not statistically significant on multiple comparisons. Furthermore, the interaction effect of atropine and defocus was also not significant ($F_{2,36} = 2.315$, $P =$

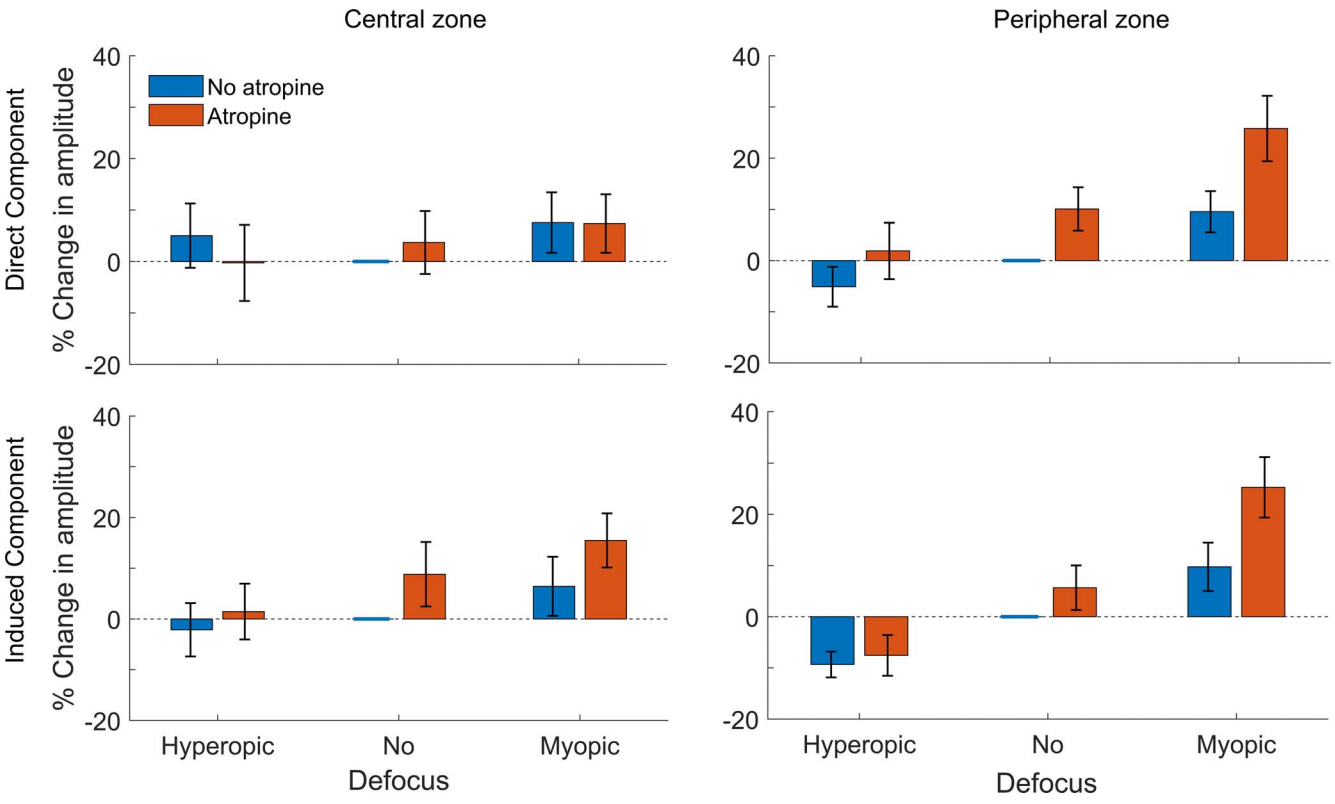


FIGURE 3. Percentage change in gmfERG signal amplitudes under myopic and hyperopic defocus relative to baseline (i.e., the no defocus, no atropine condition) for $n = 19$ subjects. *Blue*: prior to atropine, *Red*: 24 hours after instilling 0.1% atropine. *Top*: DC component for central (*left*) and peripheral (*right*) zones. *Bottom*: IC for central (*left*) and peripheral (*right*) zones. In the central zone, there was no difference in the amplitudes of either the DC or IC before and after atropine. In the peripheral zone, atropine had a significant main effect of increasing the DC amplitudes in response to defocus ($P < 0.012$). For the IC amplitude, atropine caused a significant increase under the myopic defocus condition only ($P = 0.004$). *Error bars* represent standard error of the mean.

**Table 1.** Main Effects of Defocus Reported as the Estimated Marginal Means for DC and IC Responses

| | Hyperopic Defocus | | No Defocus | | Myopic Defocus | | |
|---|---|---|---|---|---|---|---|
| | Mean, % | SE, % | Mean, % | SE, % | Mean, % | SE, % | *P* Value* |
| Central zone | | | | | | | |
| DC amplitudes | 2.38 | 5.63 | 1.85 | 3.06 | 7.47 | 4.29 | 0.436 |
| DC peak times | 1.16 | 0.74 | −0.42 | 0.71 | −0.98 | 1.10 | 0.039 |
| IC amplitudes | −0.34 | 4.43 | 4.41 | 3.18 | 10.96 | 5.02 | 0.026 |
| IC peak times | 0.27 | 0.93 | 0.37 | 0.82 | −0.26 | 0.72 | 0.678 |
| Peripheral zone | | | | | | | |
| DC amplitudes | −1.61 | 3.78 | 5.03 | 2.12 | 17.67 | 4.17 | <0.001 |
| DC peak times | 1.24 | 0.41 | 0.79 | 0.25 | 0.45 | 0.29 | 0.085 |
| IC amplitudes | −8.44 | 2.68 | 2.83 | 2.18 | 17.50 | 4.80 | <0.001 |
| IC peak times | 0.89 | 0.36 | 0.54 | 0.27 | 0.79 | 0.41 | 0.344 |

Estimated marginal means (i.e., adjusted for other factors in the model) are shown for central and peripheral retinal zones and the three different defocus conditions.

* Main effects analysis, 2-way RM-ANOVA.

0.129). For the peak times of the IC component, there were no effects of atropine ($F_{1,18} = 1.741$, $P = 0.204$) or defocus ($F_{2,36} = 0.283$, $P = 0.678$), and there was no interaction effect ($F_{2,36} = 0.004$, $P = 0.967$).

## Peripheral Zone Peak Times

There was no effect of defocus on DC peak times ($F_{2,36} = 2.886$, $P = 0.085$) and no interaction effect of atropine and defocus ($F_{2,36} = 0.159$, $P = 0.854$; Fig. 4). However, there was a significant effect of atropine ($F_{1,18} = 15.406$, $P = 0.001$). DC peak times without atropine were less than post-atropine peak times (mean difference [post-minus preatropine] = 1.45%, 95% CI = 0.672–2.221; Table 2). For the IC peak times, there was no significant interaction effect of atropine and defocus ($F_{2,36} = 0.226$, $P = 0.799$), although again there was a significant effect of atropine ($F_{1,18} = 9.722$, $P = 0.006$), with preatropine IC peak times shorter than postatropine IC peak times (mean difference = 1.00%, 95% CI = 0.327–1.678; Table 2). Optical defocus had no effect on the peak times ($F_{2,36} = 1.033$, $P = 0.344$).

## Discussion

Our results show that myopic retinal defocus causes an increase in IC amplitudes, whereas hyperopic defocus decreases IC amplitudes. This is consistent with previous reports of a sign-dependent change in IC response to short-term imposed optical defocus.[21,22] Our study extends these findings by demonstrating that the application of topical atropine modifies these inner retinal responses in the peripheral retina while having minimal impact on the central retinal responses. Specifically, atropine increased the peripheral retina IC amplitudes while under myopic defocus (by approximately 15%) but did not change the response amplitudes to no defocus or to hyperopic defocus. Atropine also increased the peak times of both the DC and IC components in the peripheral but not central retina. Together, these results suggest that one site of action of atropine may be the peripheral retina, and more specifically, the inner retinal layers, which contribute to the IC component of the gmfERG response.

**Table 2.** Main Effects of Atropine Reported as the Estimated Marginal Means for DC and IC Responses

| | Preatropine | | Postatropine | | |
|---|---|---|---|---|---|
| | Mean, % | SE, % | Mean, % | SE, % | *P* Value* |
| Central zone | | | | | |
| DC amplitudes | 4.19 | 3.45 | 3.60 | 4.24 | 0.879 |
| DC peak times | −0.41 | 0.50 | 0.25 | 1.02 | 0.365 |
| IC amplitudes | 1.44 | 3.41 | 8.58 | 4.98 | 0.136 |
| IC peak times | −0.25 | 0.54 | 0.51 | 0.84 | 0.204 |
| Peripheral zone | | | | | |
| DC amplitudes | 1.48 | 1.98 | 12.58 | 4.51 | 0.012 |
| DC peak times | 0.10 | 0.18 | 1.55 | 0.42 | 0.001 |
| IC amplitudes | 0.14 | 1.93 | 7.79 | 4.20 | 0.051 |
| IC peak times | 0.24 | 0.23 | 1.24 | 0.45 | 0.006 |

Estimated marginal means (i.e., adjusted for other factors in the model) are shown for central and peripheral retinal zones pre- and postatropine.

* Main effects analysis, 2-way RM-ANOVA.

There is increasing evidence that the peripheral and central areas of the retina serve a variety of different functions. They have different anatomic structures,[26,27] and distinct central and peripheral retinal cell domains arise during embryogenesis.[28] The peripheral retina appears to be the more important of the two in terms of eye growth control, as following laser photoablation of the fovea in infant monkeys, the eyes still grow to have normal refractions and if form-deprived, can still recover,[29] indicating that signals from the fovea are not necessary for visually guided eye growth. Moreover, when the fovea is present and monkeys are reared wearing diffusers which allow clear central vision but impose peripheral form deprivation, the eyes become axially myopic,[30] suggesting that signals from the peripheral retina are required for emmetropization. Similar conclusions have been reported from experiments in chicks[31] and marmosets[32] although a recent study has indicated that the central retina can also play a significant role.[33]

It has also been proposed that the peripheral retina is important in some optical methods for controlling myopia progression.[34] Overnight orthokeratology (corneal molding) is often employed as an effective myopia control strategy[35] on the premise that it changes peripheral hyperopic defocus to myopic defocus.[36] Similarly, soft contact lenses with peripheral plus power,[37,38] which are effective in slowing myopia progression, alter the refractive status of the peripheral retina to reduce hyperopic defocus or induce myopic defocus.[39,40]

Animal studies have also implicated the inner retina in signaling defocus-related changes in eye growth. In monkeys, the activity of ON-bipolar and amacrine cells is focus-dependent, with greater stimulation for in-focus or myopic defocus than that for hyperopic defocus.[41] In chicks, Fischer et

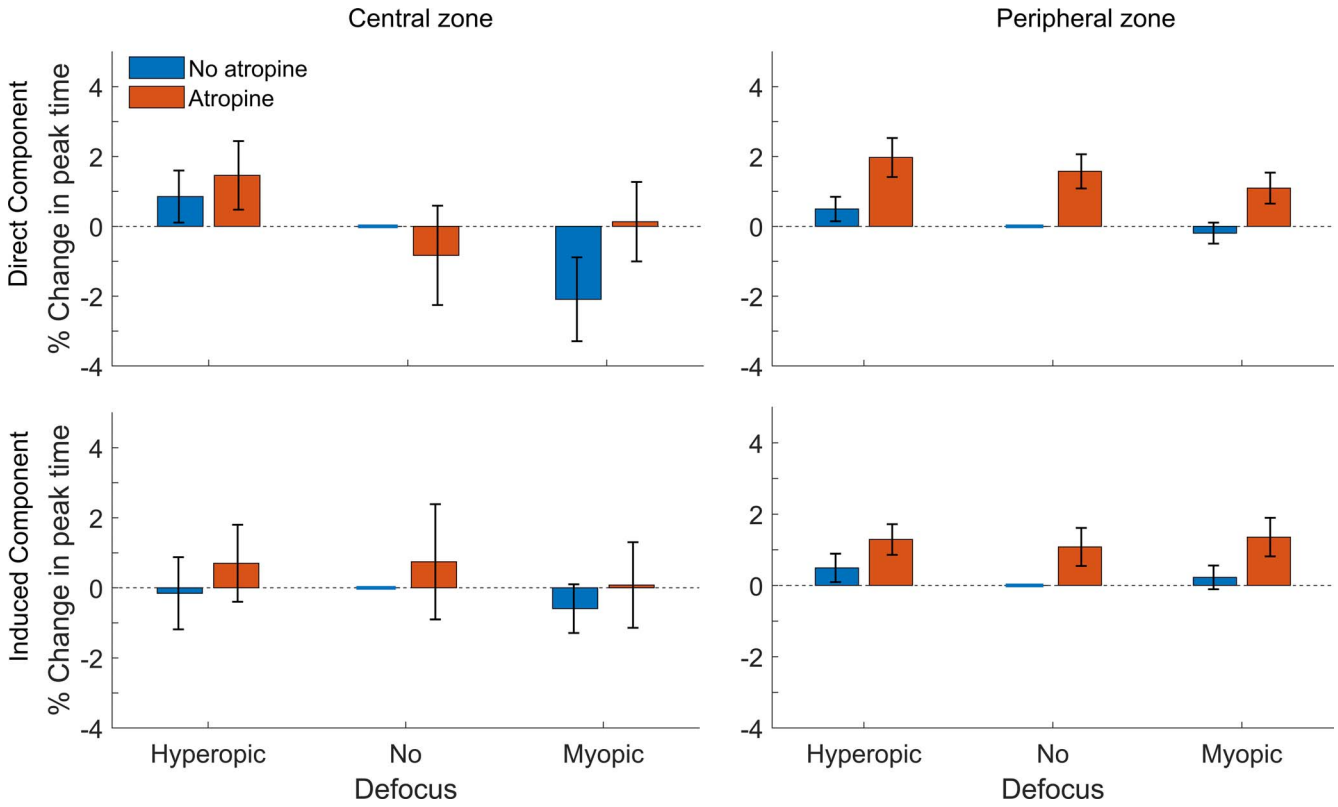


FIGURE 4. Percentage change in peak times of the gmfERG signals under myopic and hyperopic defocus relative to baseline (i.e., the no defocus, no atropine condition) from $n = 19$ subjects. *Blue*: prior to atropine. *Red*: 24 hours after instilling 0.1% atropine. *Top*: changes in the DC in the central (*left*) and peripheral (*right*) zones. *Bottom*: effect on IC in the central (*left*) and peripheral (*right*) zones expressed as percentages. In the central zone (*left*), there was no difference in the peak times of the DC or IC before or after the use of atropine. In the peripheral zone (*right*), atropine had a significant main effect of increasing the peak times of the DC ($P = 0.001$) and the IC ($P = 0.006$) in response to defocus. *Error bars* represent standard error of the mean.

al. showed that ZENK synthesis is modulated in a sign-dependent fashion in glucagon-containing amacrine cells, suggesting that the amacrine cells mediate defocus-induced changes in ocular growth and refraction.[18] By inducing defocus with the application of +7 D and −7 D lenses, the authors found that myopic defocus induced ZENK expression whereas hyperopic defocus suppressed it.[18] Similar bidirectional changes occur for levels of retinal dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in lens-induced refractive errors in chicks,[42] although the role of retinal dopamine in lens-induced myopia is still debated.[43] In addition, proteomic studies in mice suggest the involvement of γ-aminobutyric acid (GABA) transporters in the inhibition of lens-induced myopia by atropine.[44] In much the same way, several other biochemical messengers, including retinoic acid[45] and glucagon,[46] show predictable compensatory responses to defocus induced by positive and negative lens treatments. Together, these lens-rearing studies provide compelling evidence for a role of the inner retina in discerning the sign of defocus and initiating signals that ultimately modulate axial eye growth. More recently, it has been speculated that a newly identified retinal ganglion cell with unusual receptive field properties (i.e., field structure different from the classical center-surround pattern) may be involved in signaling image focus, at least in the mouse retina.[47] Our electrophysiology results support the evidence from these animal studies that the inner retina is primarily involved in modifying signals related to defocus and myopia.

We found that atropine enhanced the IC amplitude in response to myopic defocus, but not to hyperopic defocus or no defocus. Such asymmetrical interactions between atropine and defocus have been previously observed, as atropine abolishes choroidal thinning in response to hyperopic defocus but does not affect the thickening to myopic defocus.[11] These findings suggest that the response to hyperopic and myopic defocus could be mediated by different mechanisms in the retina and the choroid and that atropine is specifically targeting a downstream factor in the myopiagenic pathway. However, atropine also extended peak times in peripheral retina under all defocus conditions, with no sign dependence. The additive effect of myopic defocus and atropine on IC amplitudes, as observed in our study, further lends weight to the notion that a combination therapy of atropine and defocus is likely to provide more effective myopia control than either therapy alone, and indeed one-year results from a recent randomized controlled trial suggest a summative effect of orthokeratology and atropine in slowing axial elongation in children with myopia.[48]

There are several limitations to this study. Since atropine was administered topically to the cornea, it is possible that it might have reached the peripheral retina but not the posterior pole in sufficient concentration to affect central responses. In future studies, use of different concentrations or dosages of atropine may help determine the distribution of the drug. In addition, we measured and defined on-axis defocus, but then measured off-axis responses, so the amount of defocus experienced by each region may have been different. However, as our participants were largely myopes, who tend to have relative peripheral hyperopia, this would likely have diminished the amount of myopic defocus and exaggerated the

imposed hyperopic defocus delivered to the periphery. Another limitation relates to the effective pooling of emmetropic and myopic subjects. Refractions ranged from +0.25 to −4.50 D and included 4 nonmyopes (+0.25 D to −0.50 D), too few to reasonably perform subgroup analysis on our 19 participants. Additionally, we only investigated the effects of ±2.00 D lenses to induce myopic and hyperopic defocus and so we are unable to determine whether there is a dose-dependent or optimal response to the level of defocus. Furthermore, although the different retinal image sizes associated with plus and minus lenses were compensated for by moving the stimulus screen by a small amount, this will have resulted in slightly different degrees of brightness under the different defocus conditions. A further limitation is that measures with atropine were always made on the second day (i.e., there was no randomization in the order in which atropine was used between days). The reason was that the effects of atropine can be very long-lasting (~18 days)[49] so had atropine been instilled on the first day, then a long washout period (>2 weeks) would have been necessary before making the nonatropine measure. This would likely have allowed other, potentially confounding factors (e.g., changes in participant health etc.) to influence the results.

In conclusion, our results imply that 0.1% atropine enhances the neuronal responses to myopic defocus in the inner layers of the peripheral retina, raising the possibility that atropine may potentiate the effects of myopic defocus in inhibiting eye growth.

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