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Ozone Induced Effects on the Mammalian Visual System

Jordan Wetz
University of the Incarnate Word

OZONE INDUCED EFFECTS ON THE MAMMALIAN VISUAL SYSTEM

by

JORDAN WETZ

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DEDICATION

Grandma Mary Ann, this thesis is dedicated to you. This degree wouldn't be possible if it weren't for you. You told me to go for it, and I went for it.

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Jordan Wetz

University of the Incarnate Word, 2019

Research Focus. Millions of people who suffer from retinal disease live in air-polluted environments. The impact of the gaseous air pollutant ozone (O_3), a strong oxidant, on the retina is unknown. The aim of the present study is to compare the electroretinographic responses between control (no O_3 exposure) and O_3 -exposed rats and to better understand the effects of O_3 on retinal function.

Materials and Methods. Age-matched adult female rats were separated into two groups ($N = 6$), 3 control and 3 experimental. The experimental group was exposed to 0.4 ppm O_3 for 4 hours a day for 7 days in an environmental chamber. Control rats were not exposed to O_3. Rats were dark-adapted for a minimum of one hour. ERG recordings were performed under general anesthesia (ketamine 70 mg/kg, xylazine 2.5 kg/mg IP). Active electrodes, which are designed to be used in rats, were placed on each cornea. Reference and ground electrodes were placed subcutaneously in the rat's forehead and scruff, respectively. Pupils were dilated and a topical anesthetic applied to each eye (2.5% phenylephrine and 1% tropicamide). Lubrication and proper conductance of the active electrodes were maintained with lubricating eye drops (Refresh). Atipamezole 0.8 mg/kg was administered to reverse the effects of the xylazine.

Research Results/Findings. Experimental data indicates, in the scotopic ERG, a sub-chronic exposure to O_3 significantly ($p < 0.05$) decreased V_{max} from 361.2 μV in the control group to

323.6 μV after an acute O_3 exposure. A 7-day O_3 exposure further decreased V_{max} to 224 μV ($p < 0.05$). Amplitudes of the a-wave decreased significantly ($p < 0.05$) from – 221 μV in the control group to – 145 μV in the 7-day O_3-exposed group.

Conclusions from Research. This present study has demonstrated that an acute and sub-chronic exposure to the air pollutant, O_3, disrupts retinal function as demonstrated by changes in the ERG response. The rod dominated dark-adapted system appears to be altered as evidenced by the reduced a-wave and b-wave ERG amplitudes. The peak a-wave amplitude mostly reflects rod photocurrents plus postreceptoral contributions, thus O_3 exposure appears to decrease rod function. The decreased peak of the b-wave amplitude suggests ozone lowers the currents from depolarizing rod bipolar cells. This clinically significant work demonstrates O_3-induced oxidative stress aggravates retinopathies and contributes to vision deficits in sensitive populations living in air-polluted environments.

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Ozone and Air Pollution

Millions of people live in air-polluted environments. Air pollution results from the release of contaminants into the air potentially detrimental to human health and the planet as a whole (Mackenzie, 2016). Ozone (O_3), one of the main air pollutants is a colorless, unstable toxic gas with a pungent odor and powerful oxidizing properties. Ozone occurs naturally in the Earth's stratosphere where it is a shield that protects biological systems from harmful ultraviolet radiation emitted by the sun. Ground level or tropospheric ozone is created by a chemical reaction between volatile organic compounds (VOC) and oxides of nitrogen (NOx). This happens when pollutants emitted by cars, factories, airplanes, lawn mowers, and many of other sources chemically react in the presence of sunlight. Ozone is the main ingredient in smog (US EPA).

Health Effects of Ozone

Ozone exposure has long been linked to adverse health effects in humans and laboratory animals (Kona & Mancuso, 2017). The 21st century is fraught with dangers like climate change and pollution, which impact human health and mortality (Jung et al. 2018). Medical use of ozone (O_3) has been explored during the last decade leading to applications in a variety of fields such as in otitis media treatment and obstetrics and gynecology. However, some unexpected complications, such as one reported death, suggest the necessity of investigating adverse effects of medical O_3 use (Alfaro-Rodríguez & González-Piña, 2003).

Previous studies have shown that ozone affects the heart. When it comes to ozone affecting the cardiovascular system, daily fluctuations in pollution have been associated with acute changes in subclinical measures of disease (e.g., systemic inflammation, endothelial dysfunction, and vasoconstriction), and increases in risk of overt cardiovascular events (e.g.,

myocardial infarction, stroke, and mortality), as well. Chronic ozone exposure has been linked to enhanced sensitivity to reperfusion after an ischemic event due to promoting levels of oxidative stress and inflammatory mediators in rat hearts (Perepu et al., 2009). Additional work in healthy rat hearts further established a link between increased myocardial TNF-α levels and lipid peroxidation resulting in diminished myocardial function due to ozone exposure (Perepu et al., 2012). Sethi et al., 2012 hypothesized and presented evidence that a signaling pathway disrupting the balance between caveolin-1 and caveolin-3 may be involved in ozone-mediated cardiac toxicity. Moreover, living in a more polluted area over a long period of time has been shown to elevate risks of cardiovascular morbidity and mortality (Adar, 2012).

In Mexico City, investigators explored the effect of ozone exposure on the nervous system (Alfaro-Rodríguez & González-Piña, 2005). Their work demonstrated a decrease in paradoxical sleep tied to an increase in slow-wave sleep in ozone-exposed rats. The circadian disruptions were linked to decreases in the release of the catecholamine acetylcholine in the medial preoptic area of the rat brain. The impact of ozone air pollution on the visual system is largely unknown.

Ozone and the Eye

Dopamine, a major central nervous system catecholamine, decreases in the retinas of ozone-exposed rats (Stephens et al., 2011). Additionally, the expression of the core circadian clock genes is altered in the rat retinas of ozone-exposed rats (Stephens & Garcia, 2012). Less understood are surface of the eye-related complaints, which are commonly associated with increasing pollution. Affected people may complain of irritation, redness, foreign body sensation, tearing, and blurring of the vision. Sources of pollution are varied, ranging from gases (such as

ozone and NOx) and particulate matter produced from traffic. Mechanisms causing ocular surface disease involve toxicity, oxidative stress, and inflammation (Jung et al., 2018).

Retinal Layers

The retina is a very thin layer of tissue located on the back of the eye. The human retina is made up of nine layers. These layers, starting with the most internal, are the internal limiting membrane, the stratum opticum, the ganglion cell layer, the inner plexiform later, the inner nuclear layer, the outer plexiform later, the outer nuclear layer, the external limiting membrane and the photoreceptors (Hart, 1992). The inner retina includes the ganglion cell layer, which includes a subset of intrinsically photosensitive retinal ganglion cells (pRGCs) containing the photopigment melanopsin (Hart, 1992).

Retinal Visual Processing

Photoreceptors, rods and cones, convert light into electrical signals that are translated to the brain via the optic nerve. Photoreceptors communicate with bipolar cells, which in turn have synapse to ganglion cells (Seeley, Stephens, & Tate, 1992). Horizontal cells are interconnecting neurons that extend across the outer retina between the photoreceptors and bipolar cells. Amacrine cells are interneurons found where bipolar cells communicate with ganglion cells. The amacrine and horizontal cells can speed-up or slow down communication between photoreceptors and the ganglion facilitating visual sensitivity in the retina (Stephens & Garcia, 2012). Rods facilitate dark-adapted or night vision. There are over 100 million rod cells in each eye. Rods, which are very light sensitive, are situated mainly in the peripheral vision. In the center of the eye, there are approximately 5 million cone cells in each eye, not near as many as rods. Cones mediate sharper resolution and increased color sensitivity. This is what allows us to see during the day and pick up all different light intensities. As stated before, the optic nerve is

what transmits electric signals from the retina to the brain and allows vision perception. It is made up of a bundle of nerve fibers. The optic nerve creates a blind spot in the eye.

Electroretinogram

The electroretinogram **(**ERG) is an electrophysiological test used worldwide to assess the status of the neurons in the retina of humans and laboratory animals used as models of retinal disease (Creel, 1985). During an ERG examination, an electrode is placed on the cornea to measure the retina's different electrical responses to light. The ERG responses evoked by relatively bright flashes are recorded in the dark-adapted state. A plot of the resulting b-wave amplitude as a function of the a-wave amplitude describes the functional integrity of the retina. A normally functioning retina should elicit an ERG response that fits the normal curve (Perlman, 1983) (Figure 1). In general, when retinal function deteriorates, the light-induced electrical activity in the retina reduces. The currents I_A (a-wave) and I_B (b-wave) will be smaller and the ERG will be smaller too thus, indicating retinal pathology (Perlman, 1983). In this study, the scotopic, photopic negative response (PhNR), and the oscillatory potential will be studied closely and examined to see if ozone has a negative effect on the retina.

Hypothesis

The hypothesis and overarching objective of this research is to determine if air pollution, more specifically, ozone, negatively impacts the visual system as measured electrophysiologically by the ERG. Dark-adapted rats will be exposed to ozone (0.4 ppm) for four hours, seven days in a row. Under a general anesthetic, ERG recordings will be measured from each rat eye every day after ozone exposure and closely reviewed and compared to the control group (no ozone exposure).

Materials and Methods

Long Evans Rats (IACUC IS0112)

Age-matched adult Female Long Evans rats ($N = 6$) were purchased from Charles Rivers Laboratories and maintained at the vivarium at the University of Texas at San Antonio (UTSA). The rats were maintained on a 12-hour light/12-hour dark photoperiod in a temperature and humidity controlled environment.

Ozone Chamber

The ozone chamber is a 5' x 3' x 4' plexiglass box that contains a fan (which allows constant air flow), a humidity sensor, thermostat, ozone generator (which provides a constant flow of ozone), and an ozone sensor. The ozone generator is pre-set to 0.4 parts per million (ppm) before the start of each experiment. Once the ozone chamber stabilizes at the 0.4 ppm, a sensor modulates the generation of ozone to lower the production of ozone so that the chamber does not get any higher than 0.4ppm. Prior to each run, the ozone generator is calibrated according to manufacturer guidelines to make sure it is functioning correctly.

Ozone Exposure

The rats were separated into two groups; three control (no ozone exposure) and three experimental (ozone exposed). The experimental group was exposed to 0.4 ppm of O_3 for 4 hours, seven days in a row, in an environmental chamber, while control rats were not exposed to O_3. Prior to the experiment, each rat had the tail marked with different colors with a sharpie to identify each animal. Rats were dark adapted prior to measuring the ERGs. Animals in the experimental group were exposed to ozone in the chamber for four hours. Control rats were not exposed to ozone.

Administering Anesthetic

Prior to measuring the ERG and after ozone exposure, each rat was given an anesthetic (ketamine 70 mg/kg, xylazine 2.5 mg/kg) by intraperitoneal injection. This part of the experiment was done in total darkness, with each investigator only using a headlight that produces red light and does not stimulate rod vision. A waiting time of approximately thirty minutes was given to each rat to make sure that they were completely under anesthesia and comfortable. The rat's pupils were dilated (2.5% phenylephrine, 1% tropicamide) and a topical anesthetic (proparacaine hydrochloride ophthalmic solution USP, 0.5%) applied to each eye.

Using the Electroretinogram

The rats were placed on a heating plate to maintain core body temperature and administered Refresh Lubricating eye drops (carboxymethylcellulose sodium 0.5%, glycerin 0.9%)) to prevent corneal dryness and irritation during the experiment. Active electrodes, which are designed for rats, were placed on each cornea. Reference and ground electrodes were placed subcutaneously in the rat's forehead and scruff, respectively. The dome of the ERG machine, which is used to control light intensity and color, was lowered over the rat. Animals were monitored via a camera located inside of the ERG dome. Scotopic, oscillatory potential, and photopic negative response (PhNR) ERGs were then run concurrently.

ERG Recordings

ERG experiments were performed on dark-adapted rats under dim red illumination (wavelength > 650 nm sourced by the investigator's lamp). Light stimulation was provided by a commercially available system designed to measure rodent ERGs (Diagnosys LLC, diagnosysllc.com).

Administering the Antecedent

Once the ERG recordings were complete, atipamezole hydrochloride (0.8 mg/kg) was administered to each rat to reverse the effect of the anesthetic. Once administered, arousal begins within minutes. Rats are monitored afterwards for thirty minutes to make sure they are completely awake. A team member from the UTSA animal facility picked up the rats and returned the animals to the UTSA vivarium where they are housed.

ERG Analysis

Analysis of variance (ANOVA) of the mean amplitudes and implicit times of the a-wave and b-wave was used to determine differences in the ERGs among the control, acute, and sub-chronic exposed groups. Statistical analyses were performed using JMP software (SAS Institute Inc., Cary, NC). Data are represented as the mean among all the eyes tested in each group. Differences between groups were regarded as significant if $p < 0.05$.

To examine the changes in retinal visual parameters, using the samples of b-wave amplitudes at each luminance from the 6 test subjects, a model of the average b-wave amplitude as a function of luminance using the Naka-Rushton function, also known as the three-parameter sigmoidal Hill equation.

$$V = \frac{V_{\max} L^n}{(L^n + K_m^n)},$$

V_{max} is the upper limit of possible voltage (in microvolts), K_m is the semi-saturation point (i.e. the L at which $V=.5V_{max}$), and n is consider to be the power of the function and controls the rate at which V ascends to V_{max}. The three parameters were estimated using maximum likelihood with the help of SigmaPlot software.

Results

A-Wave

Acute and 7-day ozone exposure (0.4 ppm) for four hours per day altered the a-wave amplitude and implicit time at each of the luminance levels tested (0.001 to 4 cd.s/M^2)(Table 1). In the control condition, the smallest a-wave amplitude, -21.4 μV was measured at the 0.001 cd.s/M^2. The largest a -wave amplitude was measured at the 4 cd.s/M^2 luminance level where the amplitude reached -221.5 μV (Figure 5). In the acute ozone-exposed condition, the smallest a-wave amplitude recorded was -9.6 μV at the lowest light intensity, while the largest a-wave amplitude was measured as -196.2 μV at the highest light intensity (Figure 6). Similarly, in the 7-day ozone exposed condition, the smallest a-wave, -21.3 μV was measured at the lowest luminance level (Figure 7). The peak a-wave amplitude of -196.2 μV was recorded at the same luminance level as the control and in the sub-chronic ozone-exposed condition, 4 cd.s/M^2. The trough of the a-wave amplitude only reached -145.2 μV at that luminance level.

At the lowest luminance tested, 0.001 cd.s/M^2, the control implicit time was 21.8 ms, which was similar, 20.8 ms in the acute exposed rats and increased to 26.0 ms in the 7-day ozone-exposed animals (Table 2). Similarly, at the higher luminance level tested, 4 cd.s/M^2 the implicit time only increased a little from the control implicit time of 17.6ms (Figure 2) to the acute implicit time of 18.6 ms (Figure 3) and dropped slightly to 16.2 ms in the 7-day exposed ERG (Figure 4). Since the changes were very minor when comparing the control to chronic O_3 exposure at 4 cd.s/M_2, we speculate retinal adaptation. Overall, a closer examination by performing a repeated-measures ANOVA with luminance as the repeated factor, no statistically significant difference in mean a-wave implicit time was detected between the three days (F = 0.527, p = 0.6.09).

Table 1. *Trough of A-Wave Being Impacted Due To O_3 Exposure*

Trough of a-wave			
Luminance	Control	Acute	Chronic
0.001 cd.s/M^2	-21.4 ms	-9.6 ms	-21.3 ms
0.0025 cd.s/M^2	-16.8 ms	-70.1 ms	-25.8 ms
0.006 cd.s/M^2	-13.3 ms	-70.3 ms	-39.5 ms
0.016 cd.s/M^2	-63.2 ms	-43.1 ms	-28.6 ms
0.04 cd.s/M^2	-75.9 ms	-82.5 ms	-51.2 ms
0.1 cd.s/M^2	-106.9 ms	-97.5 ms	-66.5 ms
0.25 cd.s/M^2	-137.1 ms	-144.2 ms	-50.4 ms
0.63 cd.s/M^2	-180.9 ms	-151.6 ms	-123.1 ms
4 cd.s/M^2	-221.5ms	-196.2 ms	-145.3 ms

Table 2. *Altered A-Wave Amplitude and Implicit Time at Each Luminance Level*

	a-wave		
Luminance	Control	Acute	Chronic
0.001 cd.s/M^2	21.8 ms	20.8 ms	26.0 ms
0.0025 cd.s/M^2	21.2 ms	34.8 ms	26.0 ms
0.006 cd.s/M^2	29.6 ms	31.0 ms	24.8 ms
0.016 cd.s/M^2	27.2 ms	28.0 ms	24.6 ms
0.04 cd.s/M^2	25.2 ms	26.0 ms	25.0 ms
0.1 cd.s/M^2	24.0 ms	24.6 ms	23.4 ms
0.25 cd.s/M^2	22.2 ms	22.6 ms	21.2 ms
0.63 cd.s/M^2	19.6 ms	21.2 ms	18.2 ms
4 cd.s/M^2	17.6 ms	18.6 ms	16.2 ms

B-Wave

Acute and 7-day ozone exposure (0.4 ppm) for four hours per day altered the b-wave amplitude and implicit time at each of the luminance levels tested (0.001to 4 cd.s/M^2)(Table 3).

In the control condition, the peak b-wave amplitude was measured at the 0.1 cd.s/M^2 luminance level where the amplitude reached 953 μV (Figure 8). In the acute ozone-exposed condition, the peak b-wave amplitude of 946 μV shifted to the 0.63 cd.s/M^2 and in the sub-chronic ozone-exposed condition, the peak of the b-wave amplitude was lower, 670 μV at the same luminance level as the chronic exposure recording, 0.63 cd.s/M^2. At the lowest luminance tested, 0.001 cd.s/M^2, the control implicit time was 102 ms, which increased to 105 ms in the acute exposed rats and appeared at 99.6 ms in the 7-day ozone-exposed animals. Similarly, at the

higher luminance level tested, 4 cd.s/M^2 the implicit time changed from 76 ms in the control condition increased to 77 ms in the acute exposure and returned to 73.6 ms in the 7-day exposed ERG. Overall, a closer examination by performing a repeated-measures ANOVA with luminance as the repeated factor, no statistically significant difference in mean b-wave implicit time was detected ($F = 2.271$, $p = .166$).

Table 3. *Altered B-Wave Amplitude and Implicit Time at Each Luminance Level*

Implicit time of b-wave			
Luminance	Control	Acute	Chronic
0.001 cd.s/M^2	102.0 ms	105.0 ms	99.6 ms
0.0025 cd.s/M^2	103.0 ms	104.2 ms	107.2 ms
0.006 cd.s/M^2	97.4 ms	102.8 ms	99.6 ms
0.016 cd.s/M^2	100.8 ms	99.2 ms	95.8 ms
0.04 cd.s/M^2	91.8 ms	94.4 ms	96.8 ms
0.1 cd.s/M^2	84.0 ms	87.8 ms	83.4 ms
0.25 cd.s/M^2	79.8 ms	81.0 ms	80.8 ms
0.63 cd.s/M^2	79.4 ms	78.0 ms	77.6 ms
4 cd.s/M^2	76.0 ms	77.0 ms	73.6 ms

Naka-Rushton

At the control, no ozone exposure level of treatment, V_{max}, K_m, and n were estimated to be 1170.72, 0.0056 and 0.4905, respectively (Table 4). The 95% confidence intervals computed for each of these parameters are (1069.78, 1271.66), (0.323, 0.658), and (0.0023, 0.0089), respectively. At the acute ozone-exposure level, the estimated values of V_{max}, K_m, and n to be 1077.12, 0.0052, and 0.4467, respectively. The 95% confidence intervals computed for each of

these parameters are (993.11, 1161.13), (0.310, 0.583), and (.0025, .0079), respectively. At the 7-day, sub-chronic ozone-exposure level of treatment, the estimated V_{max}, K_m, and n are 811.13, 0.0065, and 0.3924, respectively. The 95% confidence intervals computed for each of these parameters are (625.19, 999.07), (0.089, 0.696), and (0, 0.017), respectively. The confidence intervals for K_m, and n in the sub-chronic ozone-exposure case are particularly wide due to greater variation in the data. By comparing the initial confidence interval results, there is evidence for a significant decrease in V_{max} from the control case to the sub-chronic case.

Discussion and Conclusion

Exposure to air pollutants can affect human health in various ways, leading to increased mortality and morbidity. Epidemiological evidence on the health effects of air pollution is growing and evolving quickly. Today, air pollution is the largest environmental risk factor (Brauer et al., 2015). The photochemical oxidant ozone (O_3) is a major gas component of urban air pollution, which upon inhalation reaches the central nervous system. A number of O_3-mediated neuronal dysfunctions have been reported, including decreased motor activity, neurochemical alterations, headache, cellular degeneration, and impaired mental performance (Pereyra-Munoz, Rugerio-Vargas, Angoa-Pérez, Borgonio-Pérez, & Rivas-Arancibia, 2006). This study investigated how air pollution, more specifically ozone, leads to increased morbidity focused on the nervous system. Furthermore, this preliminary work led to an analysis of the hypothesis that O_3 can produce physiological and biochemical changes in the central nervous system (Alfaro-Rodríguez & González-Piña, 2005). In the present study, the exposure protocol of 4 hours of 0.4ppm of O_3 per day for 1 and 7-day exposure was designed to investigate acute and sub-chronic responses to the oxidant in the retina. The results demonstrate that exposure to

ozone is decreasing the a-wave and b-wave amplitudes in the retina. Because of this decrease, photoreceptor pathways contribute to changes in visual sensitivity.

Retinal Visual Processing

The visual system operates over a remarkable range of lighting conditions through transduction by two classes of photoreceptor cells, rods and cones. In photopic conditions, three types of cone photoreceptors with overlapping spectral sensitivities produce spatial acuity and color vision. In scotopic conditions, visual performance relies on rod photoreceptors, resulting in a reduction of spatial resolution of approximately 1200:1 in exchange for increased light detection sensitivity (Bartholomew, Lad, Cao, Bach, & Cirulli, 2016). The photopic negative response (PhNR) is a slow negative component of a flash photopic full-field ERG that has been shown to be specific for retinal ganglion cell activity (Karanjia et al., 2017). Retinal ganglion cells bear the sole responsibility of propagating visual stimuli to the brain. Their axons, which make up the optic nerve, project from the retina to the brain through the lamina cribrosa and in rodents, decussate almost entirely at the optic chiasm before synapsing at the superior colliculus. For many traumatic and degenerative ocular conditions, the dysfunction and/or loss of retinal ganglion cells is the primary determinant of visual loss (Mead & Tomarev, 2016).

The oscillatory potential of the human ERG is present most prominently when an intense white stimulus is used (Jacobson, Hirose, & Popkin, 1967). Oscillatory potentials are different from the a- and b-waves, the major components of the ERG. The oscillatory potentials are most easily recorded in mesopic adaptational conditions and reflect rapid changes of adaptation. They represent photopic and scotopic processes, probably an interaction between cone and rod activity in the retina. The oscillatory potentials are sensitive to disruption of inhibitory (dopamine, GABA-, and glycine-mediated) neuronal pathways (Wachtmeister, 1998). This study reports that

the scotopic, photopic negative response (PhNR), and oscillatory potential are all being affected when being exposed to O_3, thus affecting retinal amacrine and ganglion cells.

Exposure Differences

The results of the present study revealed a decrease in the a-wave and b-wave amplitudes, indicating O_3 is causing oxidative stress in the retina. For the acute exposure (4 hours), only slight decreases in the a-wave and b-wave amplitudes were observed. When comparing the control and acute exposure to the chronic exposure (7 day), there is a significant decrease in both the a-wave and b-wave amplitudes. These changes in amplitudes could be due to O_3 triggering abnormal responses by retinal cells disrupting photoreceptor and bipolar cell responses resulting in night and day visual deficits.

Ozone and Oxidative Stress

A possible mechanism of action resulting in the alterations of the a-wave and b-wave expression mediated by ozone exposure will likely include oxidative stress. Studies have established a link between air pollution and cardiovascular and inflammatory neurodegenerative disorders in polluted cities (Henrotin, Besancenot, Benatru, & Giriud, 2005). Oxidative stress can be easily defined as the condition arising from the imbalance between toxic reactive oxygen species (ROS) and the antioxidant systems (Balmus, Ciobica, Antioch, Dobrin, & Timofte, 2016). Inhaled O_3 produces reactive oxygen species (ROS), which can reach the central nervous system via the bloodstream resulting in increased oxidative stress (Dorado-Martinez, Paredes-Carbajal, Mascher, Borgonio-Perez, & Rivas-Arancibia, 2001). Aging is a process characterized by the progressive loss of tissue and organ function. The oxidative stress theory of aging is based on the hypothesis that age-associated functional losses are due to the accumulation of ROS-induced damages (Liguori et al., 2018). Oxidative stress is implicated in the pathogenesis of

many neurodegenerative diseases, including age-related macular degeneration (Kaarmiranta, Salminen, & Kopits, 2009) and triggering neuronal death in several retinal neurodegenerative diseases (Abrahan et al., 2008). At the same time, oxidative stress is involved in several age-related conditions (i.e. cardiovascular diseases, chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases, and cancer), including sarcopenia and frailty (Liguori et al., 2018).

This clinically significant work begins to shed light on the complex relationship among ozone air pollution toxicity and cone and rod function in the retina. There are millions of people that live and work in air-polluted environments and many of them suffer from macular edema, macular degeneration, diabetic retinopathy and other retinal degenerative disorders. This study provides useful data in helping to establish air quality standards to better protect sensitive people that live in areas most commonly affected with heavy air pollution. Another important factor is to help prevent oxidative stress that too often occurs in people who live in these large metropolitan cities.

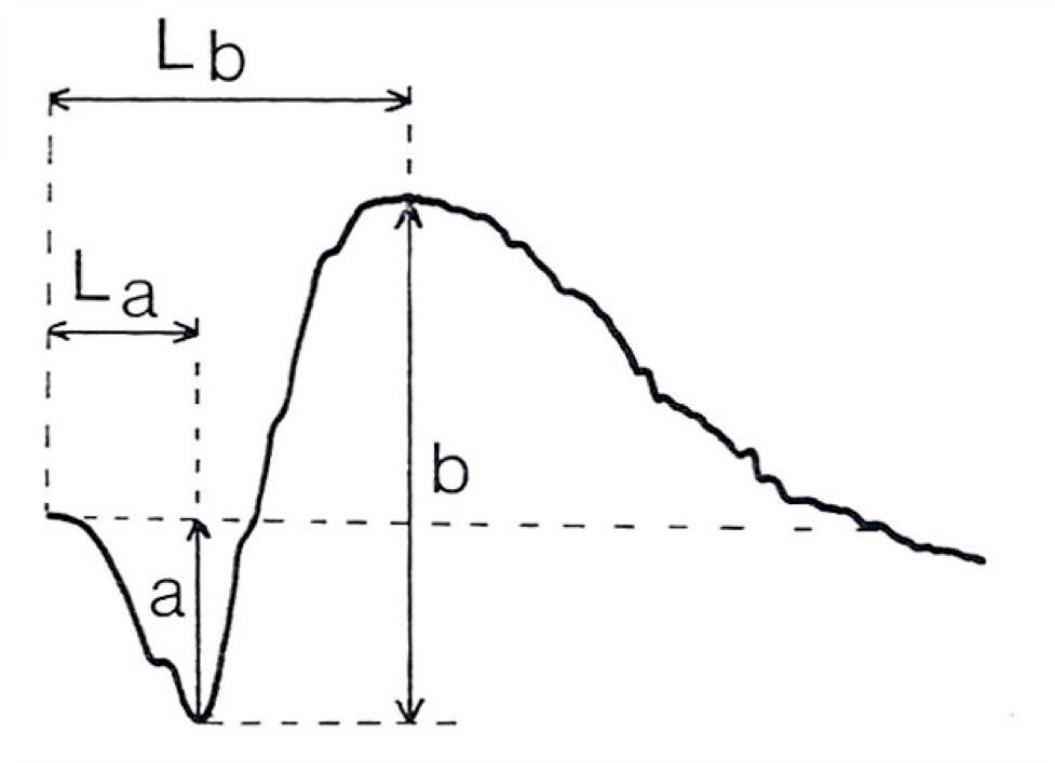

Figure 1. Normal ERG parameters. These are measured in the ophthalmic clinic. The a-wave is measured from the baseline to the trough of the wave. The size of the b-wave is measured from the trough of the a-wave to the peak of the b-wave. L_a and L_b, the time it took for both waves to peak, is measured from the onset of the stimulus to the trough or peak of the waves.

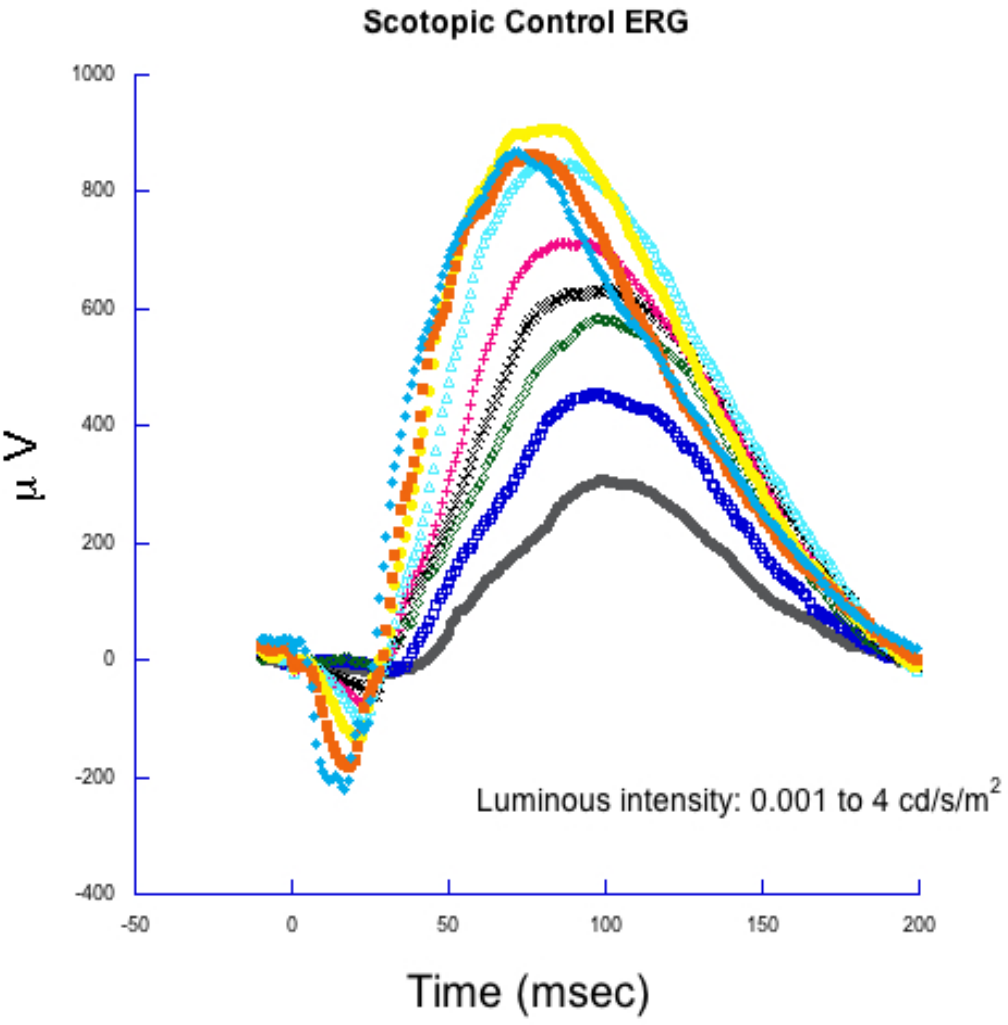

Figure 2. Scotopic control ERG. This figure represents no ozone exposure and what the acute and 7-day ERG are compared to.

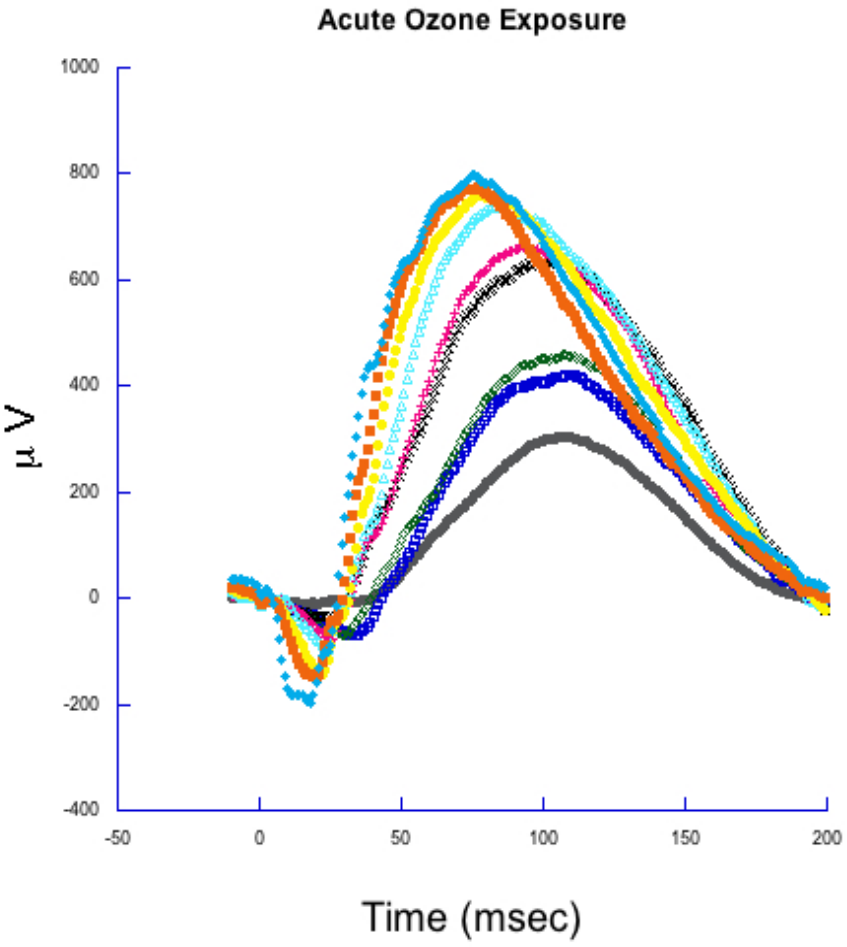

Figure 3. Acute ozone exposure. This figure represent a one day, 4 hour O_3 exposure. When compared to the control ERG (Figure 2), a slight decrease is detected in the trough of the a-wave and the peak of the b-wave due to the ozone exposure.

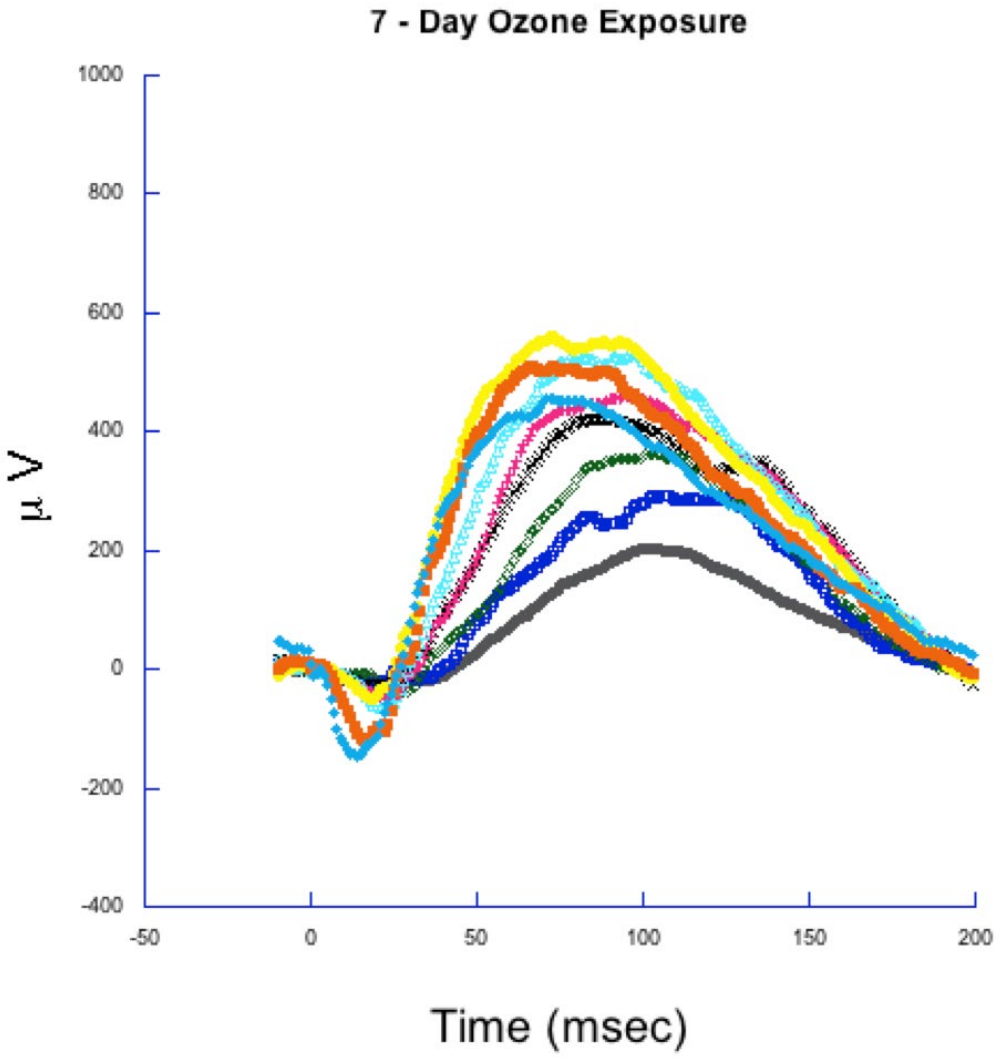

Figure 4. 7-day ozone exposure. This figure represents how the trough of the a-wave and the peak of the b-wave have both drastically decreased when being compared to the control (Figure 2) and acute exposure (Figure 3). This shows that O_3 induced oxidative stress aggravates retinopathies and contributes to vision deficits.

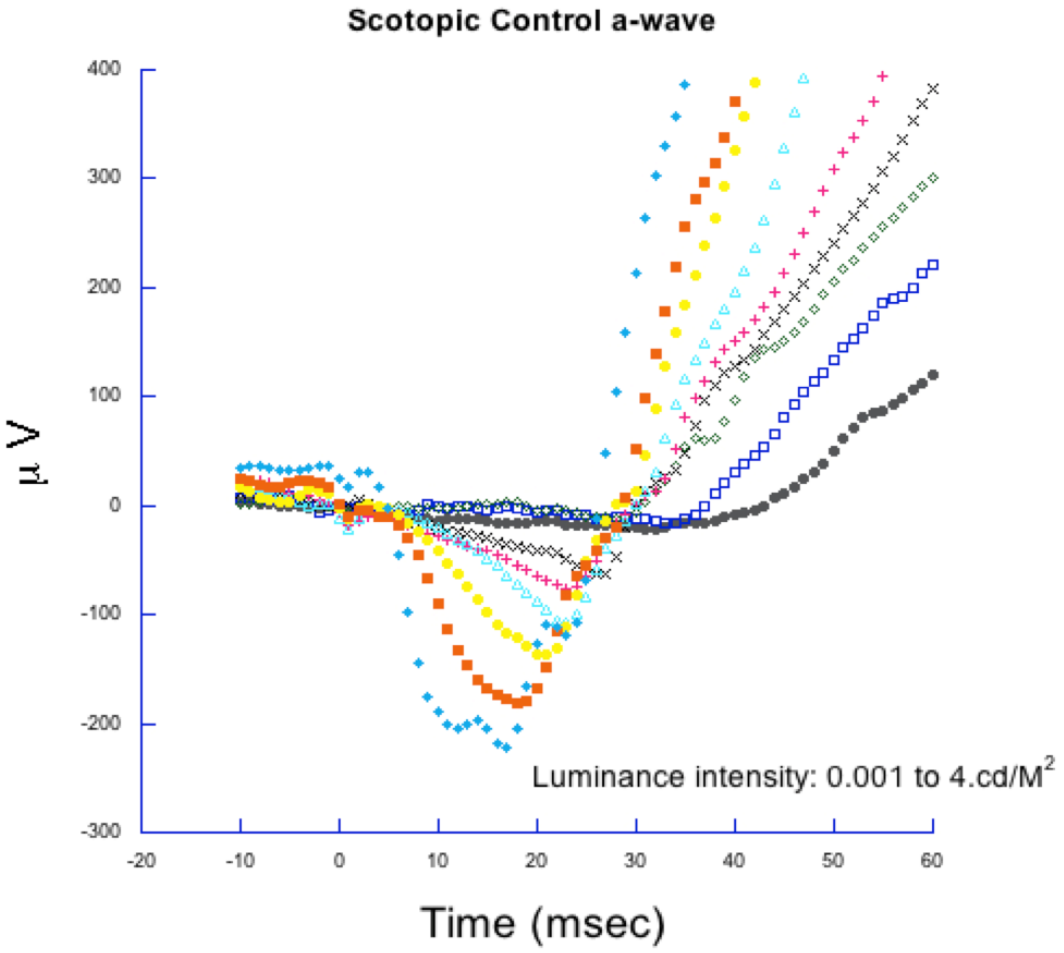

Figure 5. Scotopic control a-wave. This figure represents a no exposure ERG. This figure was used as a starting baseline when comparing the acute and 7-day a-wave amplitudes.

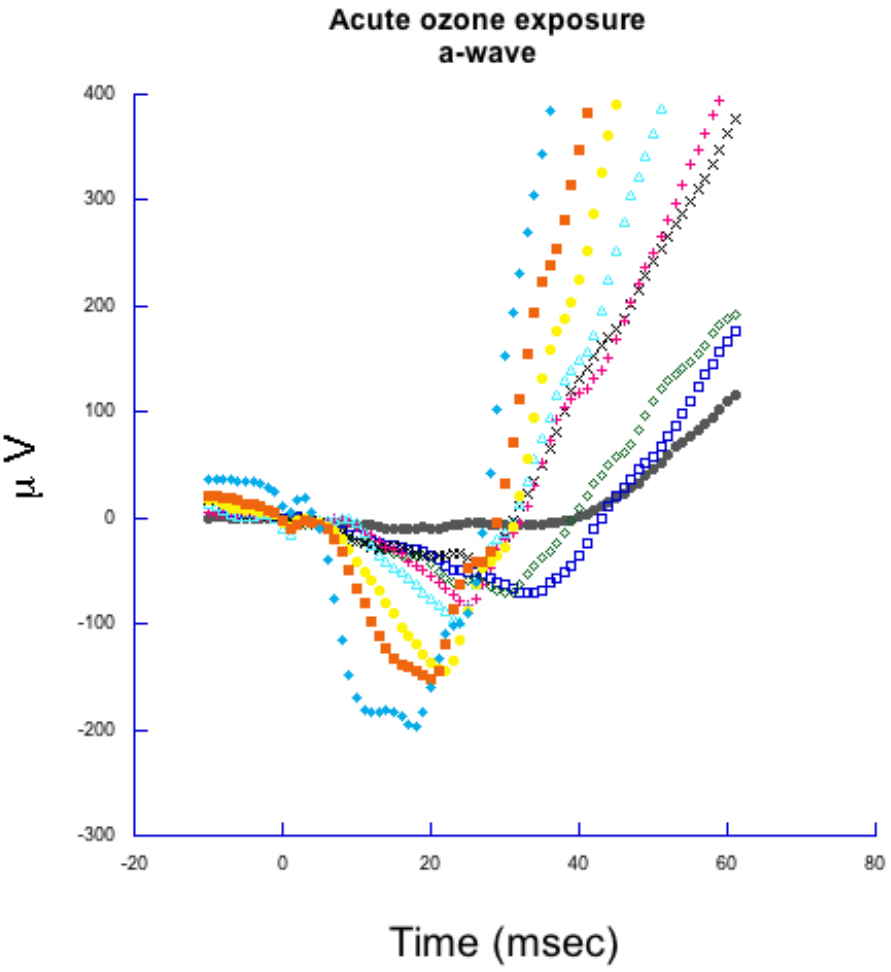

Figure 6. Control ozone exposure: a-wave. This figure represents a one day, 4-hour ozone exposure. When compared to the control ERG (Figure 5), there is a decrease in the highest light intensity (baby blue line) from ~225 μV to ~200 μV. This shows that rod function is being impacted.

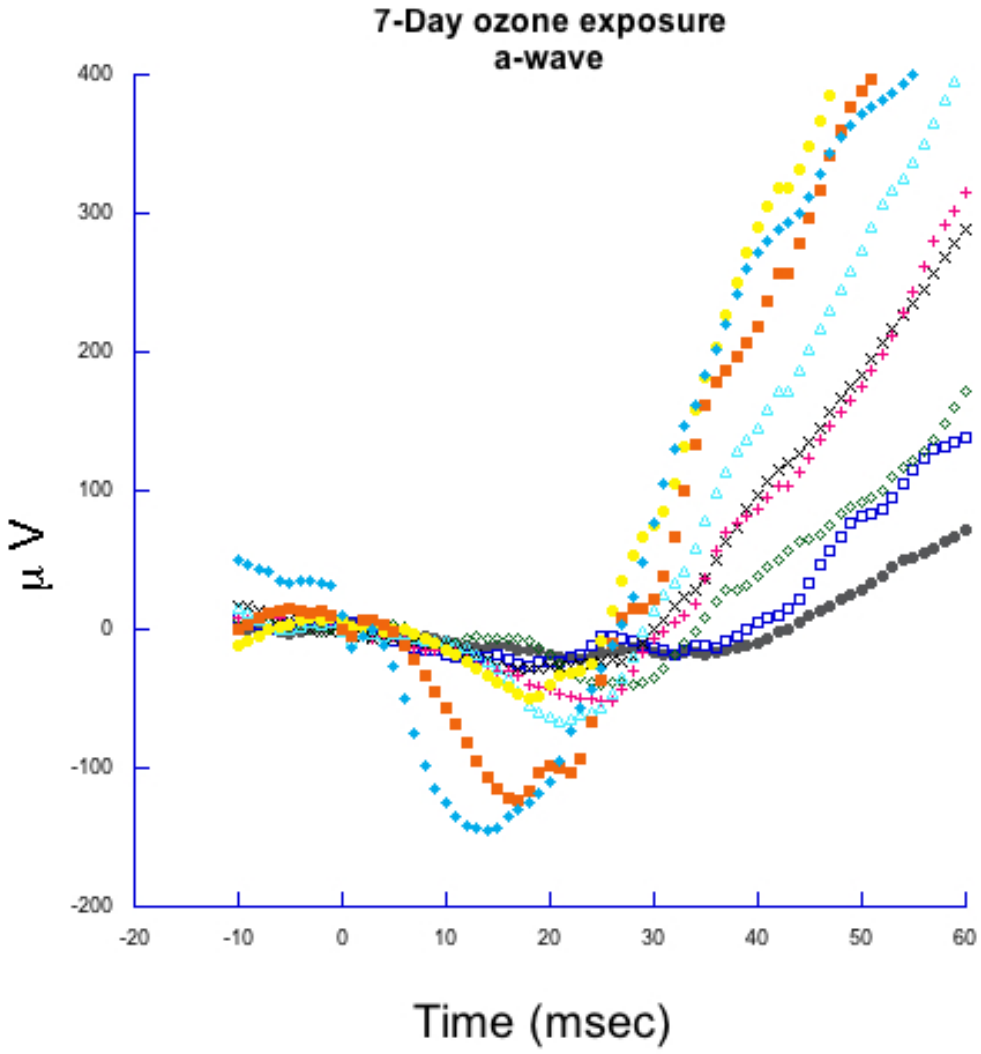

Figure 7. 7-day ozone exposure: a-wave. The peak a-wave amplitude mostly reflect rod photocurrents plus post-receptor contributions, thus O_3 exposure appears to decrease rod function. When comparing the control (Figure 5) and acute (Figure 6) exposure to the 7-day, the peak of the a-wave decreased each time when being exposed to O_3, showing that rod function is being impacted.

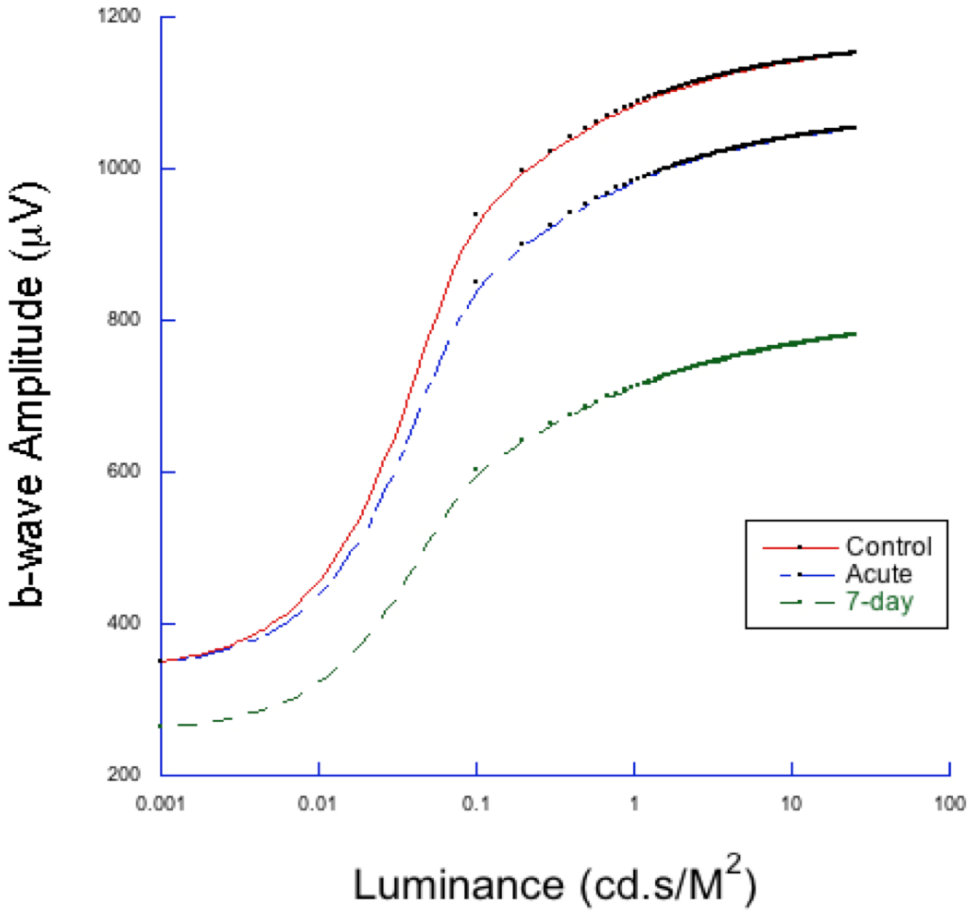

Figure 8. Naka-Rushton: b-wave function. This figure is derived from Naka-Rushton parameters and shows the study of the b-wave function. There was a large decrease in the b-wave amplitude in the control (~280μV) versus the 7-day O_3 (~360μV) exposure. There was also another large decrease when comparing the control, acute and 7-day exposure at the 10 cd.s/M^2 luminance levels. There was only a small decrease from the control (~1120μV) to the acute (~1060μV) ozone exposure, but then a drastic decrease when comparing the 7-day exposure (~780μV). This proves that the b-wave amplitude is being negatively impacted due to the O_3 exposure.

Table 4. *Naka-Rushton Parameters*

Naka – Rushton Parameters			
	V_{Max}	K_m	***n***
Control	1170	0.0056	0.49
Acute O_3 Exposed	1077	0.0052	0.45
7 – day O_3 Exposed	811	0.0065	0.39

Note. The decrease in V_{MAX} , as shown above, indicates a drop in retinal responsiveness in both the acute (1077) and 7-day (811) O_3 exposure. K_m represents changes in retinal sensitivity, particularly in the 7-day O_3 exposure. In the final column, *n* represents how ozone alters retinal homogeneity responses. The decrease from the control (0.49) to the 7-day (0.39) proves that ozone is having a negative impact on retinal responses.

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