CHICK CHROMATIC AND LUMINANCE RESPONSE TO FLICKER ERG

"A thesis presented to the graduate faculty of New England College of Optometry in partial fulfillment of the requirements for the degree of Master of Science."

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December 2021

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ABSTRACT

CHICK CHROMATIC AND LUMINANCE RESPONSE TO FLICKER ERG

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Purpose: ERG has long been a valuable tool to study visual pathways and characterize the chromatic and luminance processing of humans. ERG luminance responses have been studied in chicks, however, little is known about the chromatic responses of the chick's retina and the contribution of the long- (L), middle- (M), and double- (DC) cones. In this study, we quantified the chick's temporal frequency response to chromatic flicker while minimizing luminance input to show similarities between chick and human visual processing.

Methods: Full-field photopic flicker ERGs were recorded monocularly through a dilated pupil from 2-week old White Leghorn chicks using a four primary, full-field, Ganzfeld stimulator (Diagnosys, LLC). Luminance flicker ERGs were measured at a range of temporal frequencies to find the frequency corresponding to the maximal (36 Hz) and minimal (12 Hz) response amplitude. 36 Hz and 12 Hz were used as the high and low temporal frequencies respectively. The chicks were then exposed to a stimulus of varying ratios of red and green sine-wave luminances in counterphase with constant mean luminance for each LED (215 cd/m^2). Red-green (R/(R+G)) ratios ranged from zero where the red LED was not modulated and the green was maximally modulated, to one, where the red was maximally modulated and the green was held constant. A similar experiment was performed with the DC silenced using silent substitution.

The minimum response amplitude from both experiments were then compared across a range of temporal frequencies

Results: Our results showed that chicks have a minimum response amplitude at a R/(R+G) ratio of 0.04. In terms of cone contrast, this was achieved with 7% L-cone contrast, 93% M-cone contrast, and 40% DC contrast. When the DC was silenced (0% contrast), the minimum response amplitude was achieved with L-cone contrast of 23% and M-cone contrast of 19%. Both of these conditions showed similar low pass temporal frequency response amplitude, similar to the human chromatic response.

Conclusions: With an active DC, the minimum response amplitude is strongly influenced by the long-wavelength sensitive photopigment in the DC and L-cone. When the DC is silenced, the L- and M-cone contribution to the chick chromatic flicker ERG is more balanced and more similar to that found in humans. This indicates similarities in the chick and human visual processing. It also shows the DC has minimal input to the temporal frequency response of the chromatic system. This can be applied to future myopia control studies using chicks as a model for human eye growth by testing chromatic content of a stimulus while limiting the response of the luminance system.

ACKNOWLEDGEMENTS

I would like to express my deepest and most sincere gratitude to Dr. Thanasis Panorgias for his continued support and patience over the past 5 years. Without his encouragement and guidance, this project would not have been possible. I have benefited greatly from his extensive knowledge

Thank you to Dr. Francis Rucker for providing a valuable education during this process and the resources needed to complete my research.

I am greatly appreciative to the members of my thesis committee for their time and feedback especially Dr. Jane Gwiazda who deserves special thanks for keeping us organized and providing countless revisions.

I am grateful to NECO for their flexibility and for providing me the opportunity to complete my doctor of optometry degree while simultaneously pursing my master's.

TABLE OF CONTENTS

ABSTRACT ..... **iii**

ACKNOWLEDGEMENTS ..... **v**

TABLE OF CONTENTS ..... **vi**

LIST OF FIGURES ..... **viii**

LIST OF TABLES ..... **ix**

INTRODUCTION **9**

Chick Anatomy / Physiology: *3*

Human Anatomy/Physiology: *7*

Differences between Chick and Human: *8*

Rationale: *10*

METHODS **10**

Stimuli: *10*

Animal Protocol: *13*

ERG Recording Protocols: *14*

Experiment 1: Luminance flicker ERGs in chick: *15*

Experiment 2: Flicker ERGs with varying R/(R+G) ratio: *15*

Experiment 3: 36 Hz flicker ERG without DC modulation: *16*

Experiment 4: Flicker ERGs of the chick chromatic system with varying temporal frequency: *18*

RESULTS **18**

Experiment 1: Luminance flicker ERGs in chick: *18*

Experiment 2: Flicker ERGs with varying R/(R+G) ratio: *19*

Experiment 3: 36 Hz flicker ERG without DC modulation: *21*

Experiment 4: Flicker ERGs of the chick chromatic system with varying temporal frequency: *23*

DISCUSSION **25**

Luminance Flicker ERGs in Chicks: *25*

Flicker ERGs with varying R/(R+G) ratio: *27*

36 Hz flicker ERG without DC modulation: *30*

Flicker ERGs of the chick chromatic system with varying temporal frequency: *32*

CONCLUSIONS.....**34**

REFERENCES.....**35**

LIST OF FIGURES

Figure 1: Spectral sensitivity function for chick cone photoreceptors……...5

Figure 2: Amplitude and phase of Flicker ERG in humans…….13

Figure 3: R/(R+G) Stimuli……16

Figure 4: Luminance ERG waveform for 12 Hz and 36 Hz.……..18

Figure 5: Luminance ERG amplitude and phase……..……19

Figure 6: R/(R+G) ERG waveform for 12 Hz and 36 Hz ……20

Figure 7: R/(R+G) ERG amplitude and phase……..21

Figure 8: DC silenced ERG waveform…….22

Figure 9: DC silenced ERG amplitude and phase……..…23

Figure 10: Luminance and flicker ERG amplitude and phase …….……23

Figure 11: Luminance ERG amplitude and phase……30

LIST OF TABLES

Table 1: Cone-contrast calculations and degrees correspondace……17

Table 2: Signal to noise ratio for each temporal frequency for the DC condition……..24

INTRODUCTION

Animal models have been instrumental in the study of myopia and the chick has provided information critical for our understanding of emmetropization. This is the process with which the eye will naturally increase or decrease the rate of growth to match the axial length to the eye's optical power so that the unaccommodated eye is in focus at optical infinity. Studies in chicks have shown that emmetropization is a visually guided experience (Wallman, 1993) that takes place at a retinal level (Wildsoet & Pettigrew, 1988). The chick's eye is able to very quickly determine the sign of defocus of a stimulus and modify its axial length to compensate.

There have been many studies of how various light conditions affect emmetropization but how each visual cue is weighted by the visual system, as well as the underlying physiological mechanisms that drive emmetropization, are still unknown. There are several theories of the signals used by the chick to guide emmetropization.

One theory suggests chromatic signals are used by the retina as the sign of defocus. In accordance with longitudinal chromatic aberration (LCA), the longer wavelength component of the retinal image will be more or less in focus on the retina compared to the shorter wavelength component, depending if the image is falling in front of or behind the retina. The eye can detect the difference in focal plane of each wavelength giving instantaneous feedback on the sign of defocus, which in turn might drive the emmetropization process. This wavelength defocus is one method LCA is used as a signal for eye growth but it may also function as a chromatic signal. The sign of defocus of the eye will affect the relative defocus of light, causing a change in the color contrast of the retinal image: meaning the same light will have a different chromatic blur on the retina of a myopic eye compared to a hyperopic eye (Rucker., 2013). This is likely only part of the equation as studies have shown emmetropization occurs with monochromatic stimuli,

despite containing one wavelength with one focal plane (Liu et al., 2014). These monochromatic tests indicate the eye may rely solely on changes in luminance contrast to guide emmetropization (Rohrer et al., 1992; Rucker & Wallman, 2008; Schaeffel & Howland, 1991; Seidemann & Schaeffel, 2002; Wildsoet et al., 1993). However, the improved accuracy of emmetropization in white light compared to monochromatic light does show that although it is not essential, LCA does provide cues beneficial for emmetropization (Wildsoet et al., 1993). Further cues for the sign of defocus may be from comparisons of relative cone contrast across multiple receptors or from a comparison of changes in luminance and color contrast (Rucker & Kruger., 2004). Regardless of the type of stimulus and the luminance or chromatic content, high contrast enhances the ability of the eye to pick up on cues needed to direct eye growth (Gottlieb et al., 1987; Schwahn & Schaeffel, 1997; Rucker et al., 2015; Tran et al., 2008).

The structural and functional similarities that exist between chick and human vision indicate that the chick is a reliable animal model for studying the emmetropization process, and better characterization of the chick's visual processing will increase the confidence with which the chick can be used as a model for myopia. The current study hopes to increase the knowledge of how the chick's retina processes luminance and chromatic information, shown to play a role in emmetropization, by utilizing flicker ERGs to assess postreceptoral retinal signals.

Electroretinogram or ERG is a device that records changes in electrical potentials in the eye created in response to visual stimulation. It has been used to measure the activity and specificity of photoreceptors and their postreceptoral pathways. The results are recorded in a waveform and from that waveform we can extract information about the amplitude and timing characteristics of the processing pathways. The amplitude refers to the difference in electrical impulse elicited by the stimulus measured in microvolts and the phase is the timing characteristic

of when the impulse is elicited. Shifts in phase can indicate a change in the visual processing mechanism responding to the stimulus.

Chick Anatomy / Physiology:

The organization of the chick visual system is very similar to that of humans. There are functionally distinct layers beginning in the retina with the ganglion cell layer (GCL), inner and outer nuclear layers (INL, ONL), retinal nerve fiber layer (RNFL), and inner and outer plexiform layers (IPL, OPL). Visual information travels in vertical and horizontal pathways through these layers beginning with photon absorption by the photoreceptors and then transmission via bipolar cells to retinal ganglion cells while being modulated by horizontal and amacrine cells (Masland, 2001).

The chick retina contains six different photoreceptors, four single cones (L-, M-, S-, and UV-), one double cone (DC), and rods (R-). Each of the single cones contains a different opsin (Bowmaker, 2008; Hart, 2001; Okano et al., 1992; Yokoyama, 2008, 2002) while the DC contains two cells which both express the same opsin as the L-cone (Smith et al., 1985). Each cone type also contains an oil droplet that alters sensitivity. The UV- cone contains an unpigmented oil droplet while the other single cones as well as the DC contain a carotenoid pigmented oil droplet that acts as a long pass filter to attenuate the spectral sensitivity of the cone.(Stavenga & Wilts, 2014; Wilby et al., 2015; Wilby & Roberts, 2017). The spectral sensitivity of chick cones is a function of both the absorption of the opsin and the transmission of the carotenoid pigment oil droplets. Despite the DC and the L-cone containing the same opsin, the different carotenoid pigments in their oil droplets causes a difference in spectral sensitivity. The DCs have a much broader spectral sensitivity than any other single cone type and it overlaps with the sensitivity of the L-, S-, UV-, and M- cones (Figure 1).

The function of the DC has been studied through operant studies focused on the function of cone types in pattern texture recognition. Chicks were able to distinguish line orientation when the stimulus contained luminance contrast but were unable to distinguish the orientation when the stimulus was isoluminant for the DC (Jones & Osorio, 2004). This implies that orientation selective mechanisms of the chick rely on luminance input and supports the theory that the DC is responsible for that luminance input. Another study using isodipole textures found that chicks can discriminate textures displaying an achromatic contrast but not when the texture is equiluminant for the DC and only displays chromatic contrast to the L- and M- cones (Osorio & Miklosi, et al., 1999). These studies do not rule out the possibility that a summed output from L- and M-cones could be contributing to the chick luminance signal but the prevailing theory states the chick single cones function only in color vision (Osorio, Vorobyev, & Jones, 1999). Previous studies suggest chicks encode color using at least 3 separate opponency mechanisms L/M, S/UV, and a combination L+M / S. These studies have not ruled out the possibility of opponency from other combinations of cone types or some chromatic input from the DC but with achromatic encoding mechanisms it does support the theory chicks possess tetrachromatic color vision with sensitivity to wavelengths of light between 370-700nm (Kelber, 2019; Olsson et al., 2015; Osorio et al., 1999).

The chick retina also contains rods that use rhodopsin as their visual pigment (Okano et al., 1992; Bruhn et al., 1996) and can be found in relatively uniform density across the chick retina in a cone to rod ratio of 6:1 (Morris et al., 1970). The rods exhibit diurnal activation and are endogenously turned off during the day (Schaeffel et al., 1991).

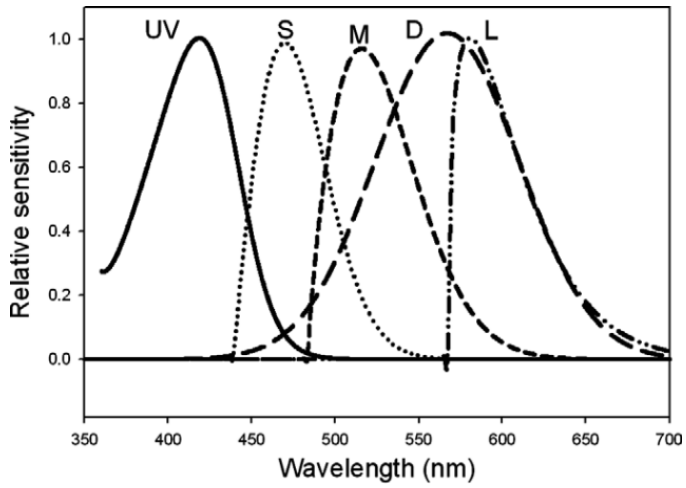


Figure 1: Spectral sensitivity function for chick ultra-violet (UV), short (S), medium (M), long (L) and double (D) wavelength-sensitive cones (Figure adapted from Rucker & Wallman, 2008).

The chick photoreceptors span several retinal layers with somata in the ONL, outer segments that interdigitate with apical processes in the retinal pigment epithelium, and segments that synapse with horizontal and bipolar cells in the OPL. The photoreceptors project to one of the three strata of the OPL (Wahlin et al., 2010). Here horizontal cells provide feedback and lateral inhibition between photoreceptors and bipolar cells. The input from photoreceptors and horizontal cells are summed by the bipolar cells and transmitted to ganglion cells in the IPL with lateral inhibition via amacrine cells. There are as many as 15 different types of bipolar cells and most are either bi- or tri- stratified which leads to complex connections within the plexiform layers. (Quesada et al., 1988, Gunther et al., 2022). There are likely 4 types of ganglion cells (Fischer et al., 2007; Sun & Crossland, 2000; Wahlin et al., 2010) that receive input from the bipolar cells and create axons that form the optic nerve. The relative density of retinal ganglion cells (RGCs) in the chick retina is high compared to other vertebrates and the size of their receptive field varies with retinal eccentricity (Naito & Chen, 2004b). The smallest receptive fields correspond to the area centralis, the retinal area with the highest density of RGCs and cone photoreceptors that is free from rods (da Silva & Cepko, 2017; Morris, 1970; Weller et al., 2009). There is also a second area of increased receptor density in the dorsal retina which corresponds to the visual field used

to view the ground in front of the bird, crucial to ground-foraging for food (Evans & Evans, 1999).

Postreceptoral pathways are not as clearly defined in the chick but several studies have looked at retinal processing. Operant condition techniques have been used to study photopic flicker sensitivity in chicks. The temporal resolution of chicks has been well documented as many studies have focused on the chick critical flicker frequency (CFF) due to ethical concerns of housing domestic poultry in fluorescent light with a flicker rate between 100-120Hz. The temporal resolution is dependent on the intensity and wavelength of light that comprises the stimulus as well as the age and breed of the chick and even the time of day to account for diurnal activation of chick rods. Many studies fail to account for all of these factors and as a result, there are varying descriptions of chick temporal resolution with average CFFs between 75-87 Hz (Jarvis et al., 2002; Lisney et al., 2011; Prescott et al., 2003; Railton et al., 2009; Rubene et al., 2010). Linsey et. al, (2011) and Linsey et. al, (2012) found CFF using both psychophysical methods and ERGs and came to the conclusion that chicks are able to consciously perceive flicker till approximately 90 Hz but ERG responses indicate a higher maximum flicker detection above 100 Hz. Ultimately the curve of photopic flicker sensitivity shows a bandpass curve with high and low frequency drop off and a peak around 15 Hz. This is comparable to other mammals, which is likely due to the similarities in lateral inhibition mechanisms (Jarvis et al., 2002).

Pattern ERG (pERG) has been used to quantify the spatial frequency response curve of the chick. This showed a bandpass filter with a peak spatial resolution of approximately 2.2 cycles/degree (Ostrin et al., 2016). Previous psychophysical studies using optokinetic nystagmus estimated the chick visual acuity to be between 7.7-8.6 cycles/degree (Schmid & Wildsoet,

1998). The same study also found chicks with transected optic nerves were able to form pERG responses indicating that RGCs do not meaningfully contribute to pERGs. The origin of the pERG signals is still unknown but likely differs from mammals where the surgical transection of the optic nerve decreases or eliminates the pERG response (Harrison et al., 1987; Miura et al., 2009; Berardi et al., 1990).

Human Anatomy/Physiology:

By comparison, the structure of the human retina differs slightly with four photoreceptors; long- (L), middle- (M), and short- (S) wavelength sensitive cones and rods-(R). The human visual process is also better defined with separate pathways for chromatic and luminance processing. The parvocellular system is the main substrate for red-green color vision, the koniocellular pathway is responsible for blue yellow color signals, and the magnocellular is responsible for an achromatic luminance response (Merigan & Maunsell 1993; Shapley & Perry, 1986). These postreceptoral afferent pathways begin with retinal ganglion cells; but their functional differentiation begins at the level of the bipolar cells (Kaplan & Shapley, 1990). At the cone level, the response generated by the photoreceptors is undifferentiated and is used as input for all three pathways. The koniocellular pathway is less of a focus in this study but uses bistratified ganglion cells while L- and M- cones connect selectively with midget bipolar cells which input to midget ganglion cells of the parvocellular pathway. This creates an opponent response with low-pass temporal frequency characteristics (Hicks et al. 1983; Derrington et al. 1984; Derrington & Lennie, 1984; Lee et al. 1987, 1989c; Schiller et al. 1990). L- and M- cones synapse non-selectively with diffuse bipolar cells, which input to parasol ganglion cells of the magnocellular pathway. This is an additive input and it forms the substrate for a non-opponent

response with bandpass temporal characteristics (De Monasterio & Gouras, 1975; Dreher et al., 1976; Derrington et al., 1984; Kaplan et al., 1986; Shapley et al., 1986; Lee et al., 1990; Dacey, 2000; Valberg et al., 1992; Penn & Hagins, 1969). The visual information processed by both pathways is segregated through the visual pathway and integrated in the primary visual cortex (Livingston & Hubel, 1988).

Differences between Chick and Human:

There are some differences between chick and human vision:

- Both humans and chicks possess a central retinal area with high cone densities and no rods which corresponds to central vision, however chicks area centralis contains 20,000 cones/mm^2 while human fovea contain closer to 200,000 cones/mm^2 (da Silva and Cepko, 2017; Morris, 1970; Weller et al., 2009;Bueno et al., 2011; Chui et al., 2008; Kram et al., 2010, JB Jonas et al., 1995).
- Despite having a significantly smaller retina, the chick has a much higher density of retinal ganglion cells; more than double what is found in the human eye. This results in the chick having higher degree of stratification through the plexiform layers of the retina but does not translate to better visual acuity as there are no chick retinal ganglion cells analogous to the human midget ganglion cell with connectivity to a single bipolar cell (Seifert et al., 2020).
- The structure and number of chick photoreceptors differ from humans where humans are limited to three cone types and have trichromatic color vision with chromatic processing mechanisms in red-green and blue-yellow cone opponency (Jameson & Hurvich, 1955; Wyszecki & Stiles, 1982; Lennie & D'Zmura, 1988).

- The chick lacks a human-like visual cortex dominated by orientation selective neurons and the higher order processing of spatial frequency is more scattered (Wang & Frost, 1991). The function of the DC seems analogous to the human luminance signal in its role in motion detection which may indicate convergent evolution in the separate processing of luminance and chromatic information (Mollon, 1989; Osorio & Miklosi, et al., 1999).
- The physiological differences in the chick retina can be seen as a benefit to their color vision and the pigmented oil droplet found in the photoreceptors causes an attenuation to the spectral sensitivity but color discrimination and constancy are enhanced (M. Vorobyev, 2003). This gives chicks comparable color discrimination to humans (A. Kelber & D. Osorio, 2010).
- Chick visual acuity was estimated to be between 2.2 and 8.6 cycles/degree depending on which techniques was used to measure. (Schmid & Wildsoet, 1998).
- The chick temporal frequency sensitivity curve as measured by psychophysics is similar in shape to human but with a lower response at every frequency and a peak sensitivity shifted to a slightly higher temporal frequency (15 Hz), but showing a bandpass filter with low frequency fall off, and slightly higher critical flicker fusion values.
- CFF in chicks was estimated to be between 87-105 Hz by psychophysiology and ERGs (Lindsey et al., 2011,2012) and in humans, between 53 and 87 Hz as measured with ERGs (Bowles & Kraft, 2012). This suggests a similar underlying visual mechanism.
- A model for human flicker sensitivity applied to the chick data indicates the chick visual system has faster signal processing compared to humans (Jarvis, 2002). This was also seen in a study of pupil dynamics with chicks demonstrating response latency roughly 4x shorter than humans (Barbur et al., 2001).

Rationale:

The activity of human photoreceptors and their postreceptoral pathways have been quantified extensively using ERGs. Initial studies focused on the spectral sensitivities of human cone types and their distribution within the retina (Jacobs & Neitz, 1993; Usui et al., 1998; Kremers & Meierkord, 1999; Murray et al., 2004). As knowledge and technology advanced, ERGs were also used to determine the function of the inner layers of the retina as well as the individual cell types that make up these layers and form the basis for postreceptoral visual processing pathways (Bush & Sieving, 1996; Nassi & Callaway 2009; Wassle, 2004). However, there is limited information regarding the postreceptoral processing when it comes to the chick retina. For this study, we used ERG techniques that have been successfully used to characterize the human retina, in chicks to better understand the postreceptoral luminance and chromatic processing.

METHODS

Stimuli:

To characterize the chicks chromatic and luminance pathways we followed a method used by Kremers et. al., (2010). Below we briefly describe their methods that we used for our experiments.

Kremers & Link (2008) attempted to find visual processes represented in the ERG waveform to increase the practical use of ERG in the diagnosis of ocular disease. They used several flickering red and green stimuli that activated L- and M-cones either selectively or simultaneously at high (30 Hz) and low (12 Hz) temporal frequencies. Their results showed the ERG response could not be explained solely by photoreceptor activity, indicating postreceptoral

pathways must be involved and second: an increase in phase difference with decreasing temporal frequency, signifying different post- receptoral mechanisms were driving the ERG response at high and low temporal frequencies.

A follow up paper by Kremers et. al., (2010) expanded this research, again testing the hypothesis that ERG responses would reflect the activity of the luminance channel when measured at high temporal frequencies (36 Hz) and the chromatic channel when measured at low temporal frequencies (12 Hz). This time an LED stimulator was used capable of generating a larger range of wavelengths with higher luminances than the CRT screens used previously, and control experiments were included to rule out photoreceptor-driven responses. The experimental setup involved conducting ERGs on five trichromatic human subjects using a stimulus that alternated from red to green. Each stimulus had varying luminance contrast and constant chromatic contrast (the total contrast arising from the sum of the red and green remained constant) that varied in the amount of red or green modulation. The ratio of red to green (R/(G+R) in the stimulus ranged from 0 to 1 with a red-green ratio of 0 being maximal green modulation and no red, and a red-green ratio of 1 being maximal red modulation with no green. The red-green stimulus was tested at seven temporal frequencies ranging from a low temporal frequency of 12 Hz to a high temporal frequency of 36 Hz. A control experiment was included using a deuteranopic subject who lacked M-cones and therefore lacked a normal physiological red-green postreceptoral channel. The difference between this subject and the healthy trichromats showed the low temporal frequency responses were governed by the chromatic channel. A second control experiment was also conducted using a stimulus with the same ratios of L- and M-cone contrasts but with the activity of the rods and S-cones silenced by silent

substitution. The response between the control and the main experiment showed similar behavior indicating little to no input from the rods and S-cones during normal testing.

Below is a figure (Figure 2) adapted from Kremers et. al., (2010). It shows the amplitude of the fundamental harmonic components (at 12 and 36 Hz) of the ERG response after Fourier Transform. This represents the ideal response from a healthy human trichromat to the modulating red and green stimulus. The amplitude of the ERG is shown in the graph on the left and the phase of the ERG is shown on the graph on the right. These graphs show the response amplitude at 36 Hz reaches a minimum at a red green ratio of 0.50 which is accompanied by a 180-degree phase shift. This reflects a model of luminance activity, which is what is expected at high temporal frequency with a stimulus of constant chromatic contrast. The 12 Hz response amplitude is overall lower with a flat slope and no phase shift. This reflects the activity of the chromatic system in response to the constant chromatic contrast without luminance interference. These results suggest that the ERG response to a stimulus with mixed luminance and chromatic content is dependent on the temporal frequency, with the sensitivity to chromatic contrast dominating at low frequencies and the sensitivity to luminance contrast dominating high temporal frequencies.

The minimum response amplitude achieved with the 36 Hz stimulus represents a range of stimuli where the visual system perceives the modulating stimulus as changing only in chromatic contrast but not luminance contrast. At the higher temporal frequency, the ERG response reflects the activity of the luminance channel, receiving non-opponent input from both L- and M- cones, and at the lower temporal frequency, the ERG response reflects the activity of the chromatic channel, with an opponent input from L- and M-cones. When the luminance of the red and green stimulus is equated on the retina, the cones will produce a minimal response. The minimum

amplitude of the first harmonic also corresponds with a 180-degree change in the phase, which reflects the change in dominance from chromatic to luminance signal.

For healthy human trichromats, the red-green chromatic pathway is balanced close to a R/(R+G) ratio of 0.50. This can differ slightly among subjects due to varying numbers of L- and M- cones. It is not uncommon for some trichromats with otherwise normal color vision to have a higher proportion of L-cones. This shifts the minimum response amplitude towards shorter wavelength light and requires a higher component of green in the stimulus or lower R/(R+G) ratio to achieve a balance within the visual system (Brainard et al., 2000; Kremers et al., 2000).

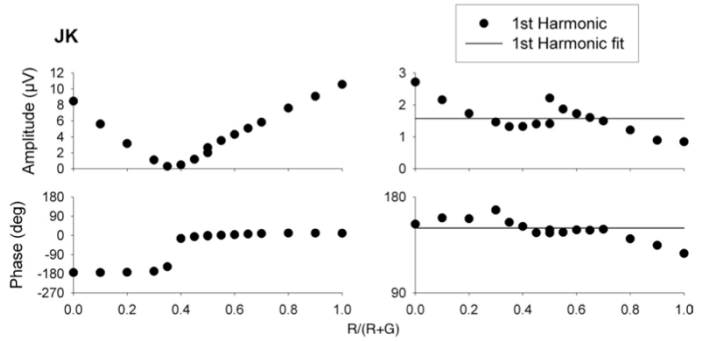


Figure 2: Amplitude (Top) and phase (Bottom) of the fundamental harmonic of Fourier Transformed ERG response of a single subject to red-green modulated stimuli at 36 Hz (Left) and 12 Hz (Right). Figure adapted from Kremers et. al., (2010).

Animal Protocol:

The chicks used in these experiments were White Leghorn chicks (*Gallus gallus domesticus*, Cornell K strain; Cornell University, Ithaca, NY, USA). Before inclusion in the study, chicks were raised in 12-hour cycles of light and dark, with food and water supplied ad libitum. Illumination in the chicks' housing ranged from 50 to 350 lux depending on the chick's proximity to the light source. Care and use of the animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the CCNY Institutional Animal Care and Use Committee.

The chicks, ages 2-3 weeks, were randomly selected for testing. All recordings were done through the right eye with a pupil dilated with 1mg/mL vecuronium bromide. 5 drops were instilled at 2 minute intervals in the eye 1 hour before testing with another 5 drops added 30 minutes before testing. The chicks were sedated approximately 15 minutes before testing using intramuscular injections of 100mg/kg ketamine and 5mg/kg xylazine. Anesthetized birds were kept on a heated pad. The lid of the right eye was held open with a speculum and an electrode was placed in contact with cornea. Position of the speculum and level of sedation allowed for the continued action of the nictitating membrane and a small amount of Celluvisc was added to the eye to prevent corneal surface drying and unnecessary irritation. The reference electrode was placed subcutaneously along the top of the bird's head and the ground electrode was placed subcutaneously in the right thigh.

ERG Recording Protocols:

Full field photopic flicker ERGs were recorded monocularly in each chick using the ColorDome (Diagnosys LLC, Lowell, MA) four primary Ganzfeld stimulator with blue (454 nm), green (524 nm), amber (596 nm), and red (636 nm) LEDs. The spectral characteristics, chromaticities, and luminances of each class of LED were measured and calibrated using a spectrophotometer (PhotoResearch SpectraScan PR-670, USA). Protocols for ERG use were adapted from the guidelines of the International Society for clinical Electrophysiology of Vision (Bach et al., 2013; McCulloch et al., 2015). Data was collected, interpolated, and submitted to Fourier Transform using MatLab to extract the amplitude and phase of the first fundamental.

Experiment 1: Luminance flicker ERGs in chick:

To find the temporal frequency at which the eye showed maximum sensitivity to luminance flicker, six birds were tested using a sine-wave modulated white light stimulus at temporal frequencies ranging from 2Hz to 20Hz in steps of 2Hz, from 20 Hz to 60 Hz in steps of 5Hz, and from 60 to 100Hz in steps of 10Hz. We used a contrast of 80% and a mean luminance level of 350 cd/m^2.

Experiment 2: Flicker ERGs with varying R/(R+G) ratio:

A counter-phase, sinusoidally modulated, red and green stimulus was used, while mean luminance of both the red and the green LED were kept constant. Testing was done at two frequencies, 12 Hz and 36 Hz. The 36 Hz high-frequency stimulus corresponds to the peak ERG amplitude as determined in Experiment 1. 12 Hz was the low frequency used by Kremers et. al., (2010), but also, and it was selected because it was shown to stimulate the red / green chromatic pathway in humans. However, 12 Hz is also close to the temporal frequency for which the luminance ERGs recorded in Experiment 1 show a minimum amplitude.

The R/(R+G) ratios were sampled from 0 to 1 in steps of .1 with finer steps of .02 near the minimum ERG response amplitude. In total 14 conditions were used for the 36 Hz testing. The low temporal frequency 12 Hz testing only required 10 conditions from 0 to 1 using steps of 0.1. Both the red and the green LEDs had an average luminance of 215 cd/m^2 as measured by a spectrophotometer (PhotoResearch SpectraScan PR-670, USA).

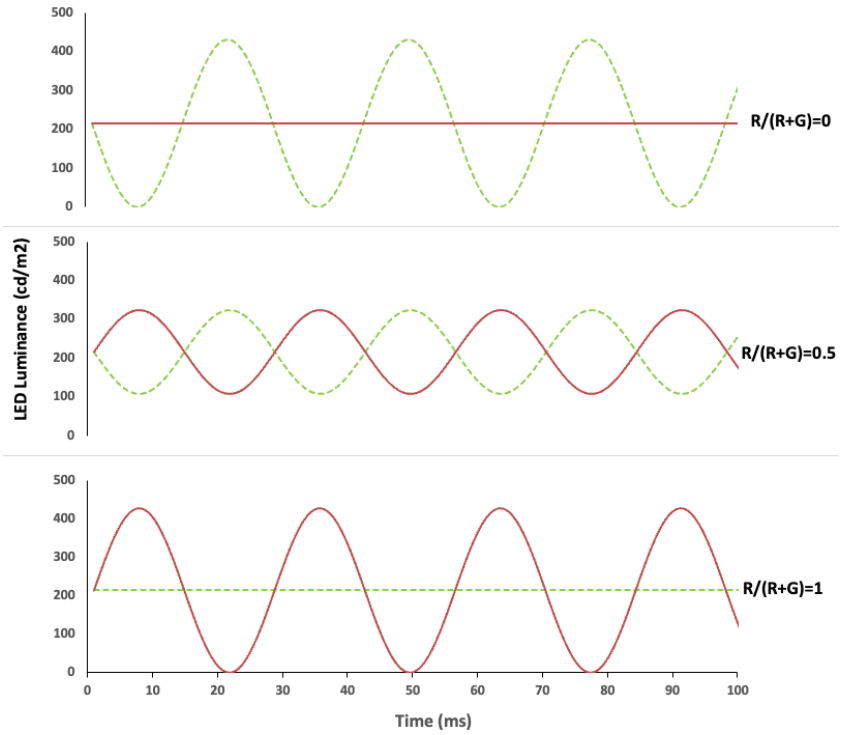


Figure 3: Graphic representation of the sinusoidally modulated red and green stimuli used in Experiment 2. The amount of red or green modulation in the stimulus is varied from all green and no red at a R/(R+G) = 0 to no green and all red at R/(R+G) = 1.

Experiment 3: 36 Hz flicker ERG without DC modulation:

To control for the effect of the DC in the chick flicker ERG, stimuli were developed that produced no DC modulation (n=20 chicks). This was done using the silent substitution method, which uses three stimuli of different wavelengths and intensity to excite a single cone type by the same extent, so that there is no change in cone excitation over time (Estevez & Spekreijse, 1974, 1982; Sawusch et al., 1987). In this case, the DC will have constant excitation and zero contrast, while the stimuli that modulate the L- and M-cones are presented in counter phase. This method allows an isolated ERG response to be obtained from the other cone classes without the interference of the DC. The photoreceptor excitations were calculated by multiplying the emission spectra of the LEDs by the spectral sensitivity of the cones and then integrated. This information was used to find the spectral and intensity combinations that produced no changes in net excitation for the DCs. To repeat Experiment 2 (in general terms), but without DC

stimulation, eight stimuli were created with different ratios of L- and M-cone contrast, ranging from 30% L-cone and 5% M-cone contrast to 5% L-cone and 30% M-cone contrast. The DC contrast was always kept below 1%.

Stimuli were represented on a Polar plot with 100 degrees representing 5% L-cone and 30% M-cone contrast, and 170 degrees representing 5% M-cone and 30% L-cone contrast. These conditions are described in terms of both degrees and cone contrast in Table 1. We were unable to create stimuli with higher contrast levels because of the limitations of stimulator and constraints required to keep the DC contrast at zero. Stimuli were created at 10 degrees interval from 100 degrees to 170 degrees, and with 2 degrees interval between 130 degrees and 140 degrees. The mean light level for each individual LED had to be modified to create the stimuli but the sum of combined sources was approximately constant at 4000 cd/m^2 for all the different angles tested.

Degrees	L/(L+M) Cone Ratios	L/M Cone Ratios	L- Cone Contrast	M- Cone Contrast
170	0.8571	5.6713	0.2954	0.0521
160	0.7419	2.7475	0.2819	0.1026
150	0.6341	1.7321	0.2598	0.1500
148	0.6100	1.6634	0.2590	0.1557
146	0.5900	1.5322	0.2529	0.1650
144	0.5800	1.4156	0.2464	0.1741
142	0.5600	1.3110	0.2397	0.1828
140	0.5472	1.2042	0.2298	0.1908
138	0.5260	1.1301	0.2255	0.1995
136	0.5100	1.0510	0.2180	0.2074
134	0.4900	0.9779	0.2103	0.2151
132	0.4700	0.9101	0.2024	0.2224
130	0.4591	0.8391	0.1928	0.2298
120	0.3659	0.5774	0.1500	0.2598
110	0.2632	0.3640	0.1026	0.2819
100	0.1429	0.1763	0.0521	0.2954

Table 1: The stimulus conditions for Experiment 3 expressed in degrees, L/(L+M) cone ratio, L/M cone ratio, as well as L- and M- cone contrast.

Experiment 4: Flicker ERGs of the chick chromatic system with varying temporal frequency:

In Experiments 2 and 3, the point at which the flicker ERG amplitude is minimized was found both with the DC being stimulated (experiment 2) and with the DC silenced (experiment 3). In order to determine how the DC might affect the ERG responses in experiment 2, we recorded ERGs for both the R/(R+G) = 0.04 and the 142-degree angle conditions for a range of temporal frequencies from 2-30 Hz.

RESULTS

Experiment 1: Luminance flicker ERGs in chick:

Figure 4 shows two representative ERG waveforms recorded from the chick's eye using a 12 Hz and 36 Hz luminance temporal modulation, as described in the methods. The waveforms were analyzed using a fast Fourier transform to extract the amplitude and phase of the responses.

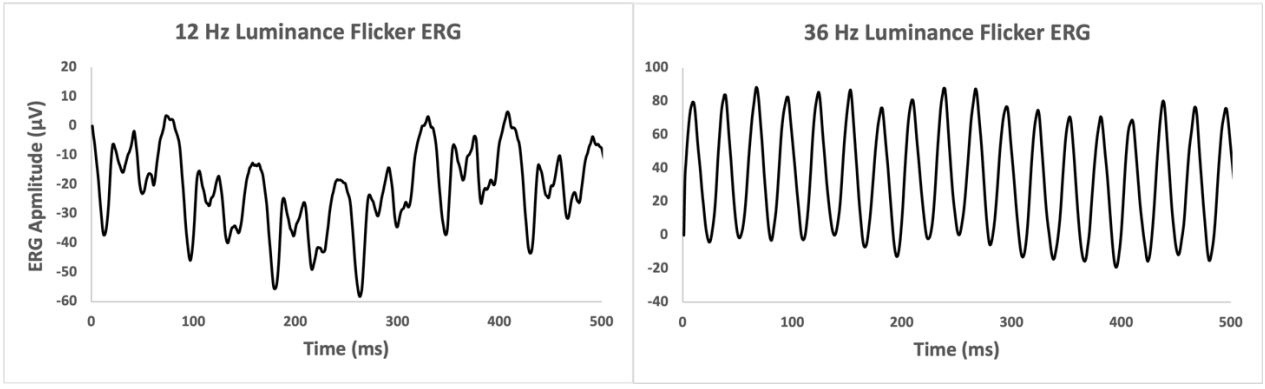


Figure 4. A 12 Hz (left) and 36 Hz (right) luminance ERG waveforms were recorded from one chick's eye.

Figure 5 (left) depicts the results of the luminance ERGs as a function of temporal frequency from 2 Hz to 100 Hz. We can see from the amplitude results that the chick's retina responds maximally to flickering light at intermediate frequencies approximately between 30 and 40 Hz, with a peak ERG amplitude at 36 Hz. On either side of this peak, the ERG amplitude decreases; however, a local minimum is formed at approximately 10-12 Hz since the ERG amplitude increases for lower temporal frequencies.

The phase results of the luminance ERG (Figure 5, right) show a polyphasic function, with phase changing abruptly for ERGs below and above ~10 Hz, and then for ERGs above 20 Hz until there is another sudden change in phase for ERGs above 70 Hz.

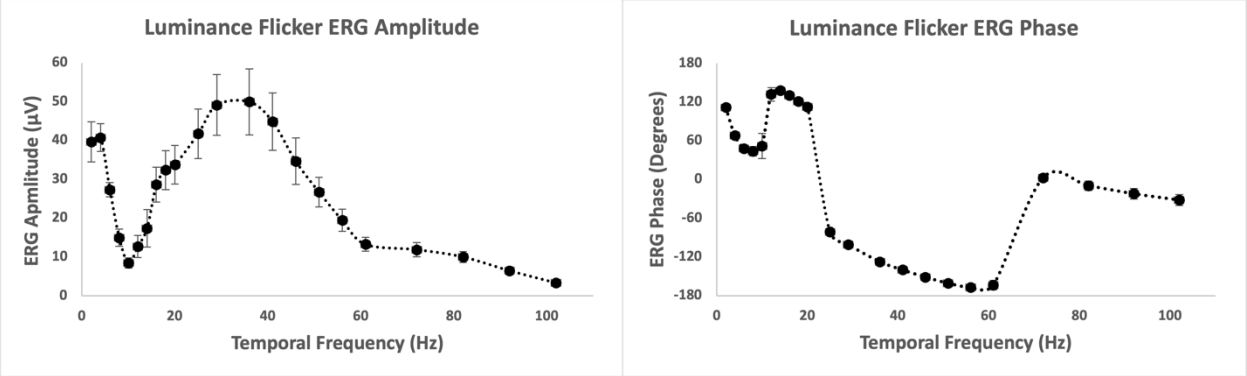


Figure 5. The ERG amplitude (left) and phase (right) of the luminance ERG. The error bars depict ±1 Standard Error (SE).

Experiment 2: Flicker ERGs with varying R/(R+G) ratio:

The 36 Hz temporal frequency is selected to use in the R/(R+G) ratio experiment as it corresponds to the maximal luminance response of the chick's retina. The 12 Hz temporal frequency was used for this experiment because the luminance response function as measured

with flicker ERGs in experiment 1 is minimized at approximately this temporal frequency. Figure 6 shows the raw data recorded from one chick with 36 Hz (left) and 12 Hz (right) stimulation for different R/(R+G) ratios (see figure legend).

Figure 7 shows the average ERG amplitude and phase to R/(R+G) ratio testing at 36 Hz and 12 Hz. A V-shaped function forms for the 36 Hz amplitude data. The minimum ERG amplitude is formed at the R/(R+G) ratio of 0.04, meaning that at that ratio, the luminance response of the chick's retina is minimized. The shift in phase is gradual and close to a 130-degrees change. Note the logarithmic scale used for the amplitude data to better visualize the V-shape but also to highlight the significant increase in amplitude found with increasing R/(R+G) ratio (i.e., with increasing R).

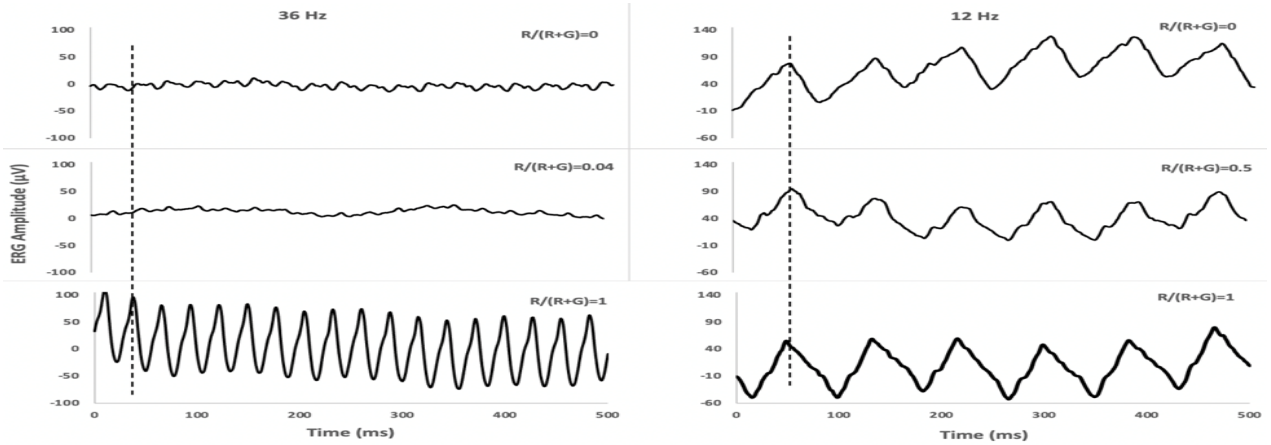


Figure 6. Raw ERG data as recorded from one chick for the 36 Hz (left) stimulation for R/(R+G) ratios of 0 (top), 0.04 (middle), and 1 (bottom), and for the 12 Hz (right) stimulation for R/(R+G) ratios of 0 (top), 0.5 (middle), and 1 (bottom). The dashed vertical lines are plotted to highlight the changes (or not) in phase.

The 12 Hz data in Figure 7 (bottom) do not follow a V-shape, like with the 36 Hz data, but show a gradual increase in the response amplitude as the amount of red in the stimulus increased but

several orders of magnitude lower than what was seen with the 36 Hz testing. The 12 Hz phase data show no shift and are nearly flat.

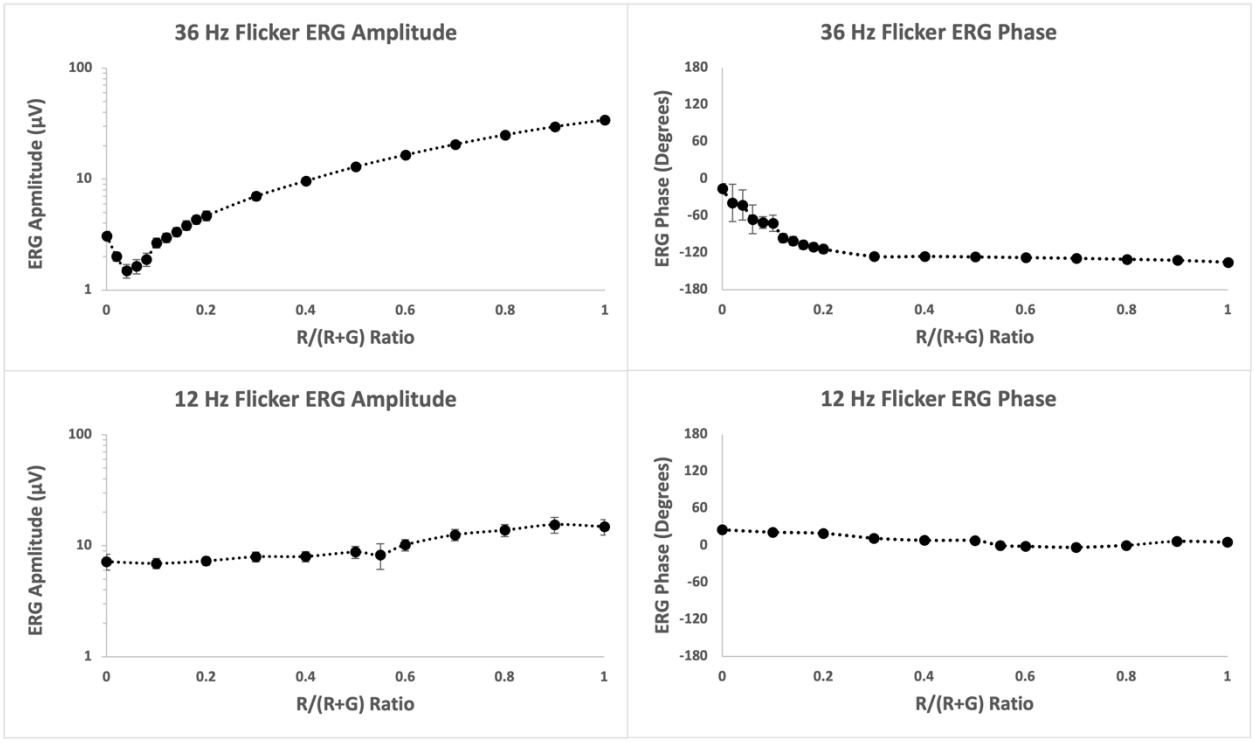


Figure 7: Average amplitude (left) and phase (right) of the first harmonic of the Fourier transform of the ERG response to sinusoidal modulation of stimuli with R/(R+G) ranging from 0 to 1 measured at 36 (top) and 12 Hz (bottom) respectively. The error bars show ±1 SE.

Experiment 3: 36 Hz flicker ERG without DC modulation:

The data recorded in experiment 2 contain responses from all cone types, mainly the L-, M- and DC with minimal modulation of the S- and UV-cones. In order to isolate the L-, M-cone responses from the responses of the DC, we recorded ERGs with stimuli that elicited no DC modulation but only L-, and M-cone modulation (the S-cone contrast was less than 1%).

Figure 8 shows the raw data recorded from one chick with 36 Hz stimulation for different L- and M-cone contrasts (100, 142, and 170 degrees).

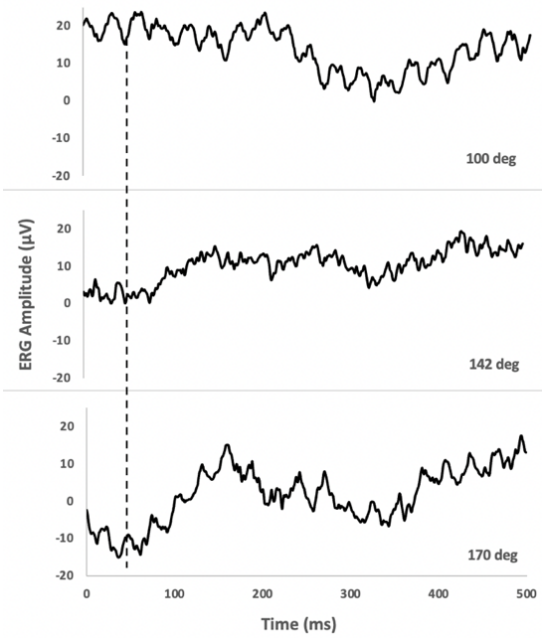


Figure 8. Raw ERG data for 36 Hz stimulation for three different L- and M-cone contrast ratios, 100 (top), 142 (middle) and 170 degrees (bottom). The dashed vertical lines are plotted to highlight the changes (or not) in phase.

Figure 9 plots the ERG amplitude and phase for the DC-isolating ERGs using 36 Hz flicker. The data show that there is a minimum in the ERG amplitude for an angle of 142 degrees, with the amplitude increasing on either side of that minimum. The minimum point is another stimulation for which the luminance ERG response of the chick retina is minimized (similar to the R/(R+G) ratio). The phase data show again a change in phase at the minimum point. Note that the ERG amplitude is significantly lower compared to experiments 1 and 2.

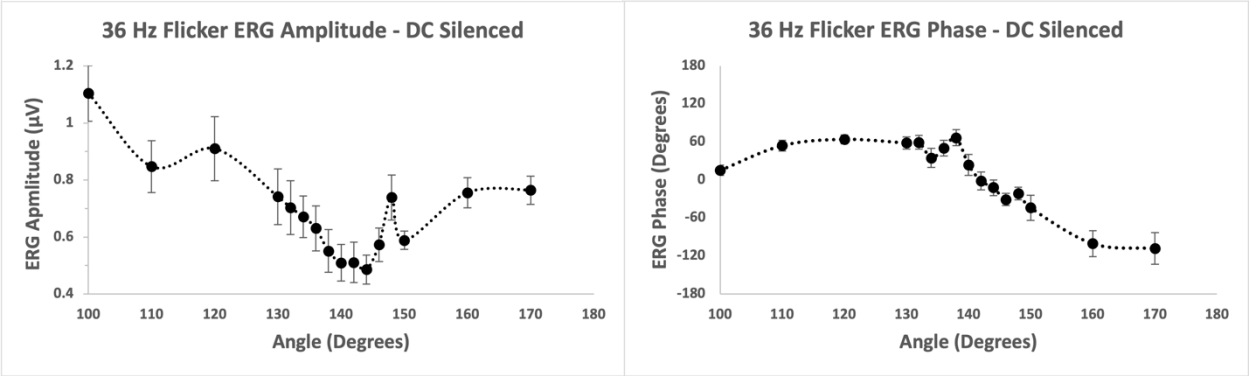


Figure 9: Average amplitude (left) and phase (right) of the first harmonic of the Fourier transform of the ERG response to sinusoidal 36 Hz stimulus with the DC silenced. The error bars show ±1 SE.

Experiment 4: Flicker ERGs of the chick chromatic system with varying temporal frequency:

The minimum luminance ERG response determined in Experiment 2, with the DC active, is at R/(R+G) ratio of 0.04, while for experiment 3, the minimum luminance response was recorded at 142 degrees. To test whether these two conditions are probing the same mechanism we tested the temporal response function as measured using ERGs, with and without DC stimulation.

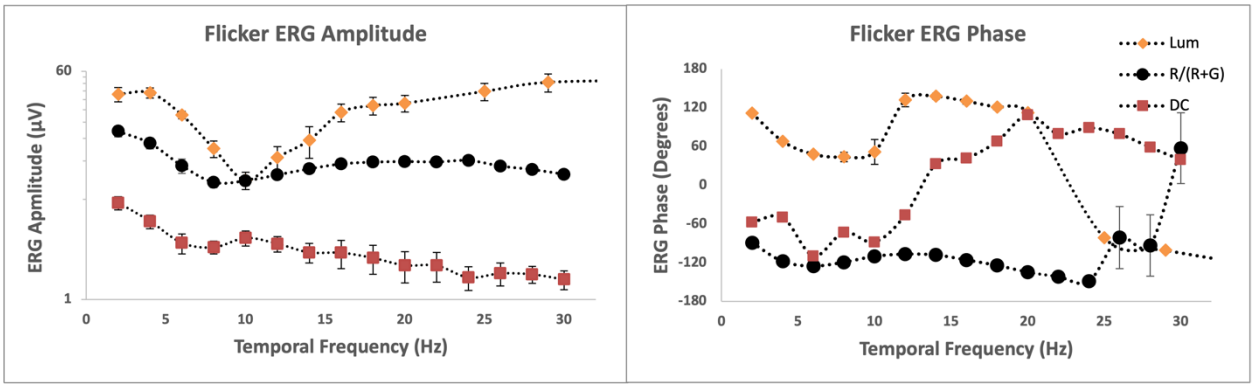


Figure 10: Average amplitude (left) and phase (right) of the first harmonic of the Fourier transform of the ERG response as a function of temporal frequencies measured for R/(R+G) = 0.04 (filled circles) and DC angle of 142 degrees (filled squares). For comparison we also include the data from experiment 1 (filled diamonds). The error bars show ±1 SE.

Figure 10 shows the ERG amplitude (left) and phase (right) for the two conditions that minimize the luminance response of the chick's retina as a function of temporal frequency. It can be seen that the temporal response function for the two conditions (R/(R+G) and DC silenced) is qualitatively the same, albeit the absolute amplitude values differ. We can see that there is an increase in the ERG amplitude for frequencies below approximately 10 Hz for both conditions, Beyond 10 Hz, the ERG amplitude for the R/(R+G) condition remains relatively stable while it decreases for the DC-silenced condition. The decrease in the DC condition amplitude is manifested with an increase in phase, while the phase for the R/(R+G) condition remains relatively stable (with the exception of the three last data points). Due to the low amplitude of the DC condition, the signal to noise ratio was calculated for each temporal frequency. Despite the low amplitude, the ratio is greater than 1 for the majority. There are also error bars depicting ±1 SE for both the amplitude and phase although these error bars are generally too small to see.

Frequency (Hz)	Signal to Noise Ratio
2	0.704333173
4	1.131040107
6	0.773293798
8	2.327565451
10	2.048564699
12	3.208465959
14	1.809312381
16	2.229135698
18	2.225624547
20	2.062489322
22	2.464364546
24	2.908466313
26	3.697253679
28	3.419356071
30	3.91491204

Table 2: Signal to noise ratio for each temporal frequency for the DC condition.

DISCUSSION

In this study, we used flicker ERGs to measure the chick's luminance and chromatic ERG response as a function of temporal frequencies using stimuli of varying chromatic content with and without DC activation via silent substitution. The results indicate a minimal role for the DC in chromatic processing of red-green flicker in conditions that limit luminance input, supporting the predominant theory for the function of the DC and suggesting similarities between human and chick postreceptoral pathways.

Luminance Flicker ERGs in Chicks:

We recorded the chick ERG response to luminance flicker at a range of temporal frequencies. Figure 5 shows the flicker ERG response amplitude (left) reached a maximum at a temporal frequency of approximately 36 Hz with the ERG amplitude decreasing on either side of the peak sensitivity and forming a second maximum between 2-4Hz. A minimum trough formed between the two peaks at approximately 10-12 Hz and a second minimum formed in the higher frequency range above 80Hz. The peak amplitude at 36 Hz indicates the temporal modulation which primes the chick visual system to maximally respond to luminance stimulation. Temporal frequencies on either side of this peak resulted in lower response amplitudes and we have chosen to use 12 Hz as the low temporal frequency in subsequent experiments for achieving a minimum luminance response. Figure 5 shows the phase of the luminance ERG (right) forming a polyphasic function with a steep drop off at 20Hz and a steep increase at 60Hz. The initial phase shift of roughly 180 degrees corresponds with a slight decrease in the slope of the response amplitude. The second 180-degree shift in phase corresponds with another slight decrease in the response amplitude slope. This may indicate points where the different cone types are responding differently to the

stimuli. Further study would be needed to see the response to luminance flicker of each cone type.

These results are in agreement with previous testing in humans and primates where flicker ERG resulted in minimums between temporal frequencies of 10-20Hz (Odom et al., 1992; Viswanathan et al., 2002). The chick ERG response to luminance flicker as a function of temporal frequency shows similar bandpass characteristics to human flicker ERG responses. A study by Jarvis et. al., (2000) measured the photopic flicker sensitivity of the chick. Their study used psychophysical methods and found the peak chick sensitivity to photopic flicker was around 15Hz. Measuring over a range of luminance levels, the chick flicker sensitivity was considerably lower than humans except at high temporal frequencies. Though the psychophysical peak was shifted to around 15 Hz in chicks, and the amplitude reduced, the shape of the chick temporal frequency sensitivity to luminance flicker function was found to be similar to humans. This study design relies on the chick's perception of the stimuli before making a forced choice between two options and this limits the sensitivity. While the psychophysical task is not directly comparable to the ERG results we found, it does reinforce the underlying similarities between chick and human luminance processing. The shape of the luminance flicker sensitivity curve is dictated by a low pass and high pass filter and is the result of photoreceptor processing and inhibition from horizontal and amacrine cells (Kelly, 1971a; Rovamo, Raninen, Lukkarinen, & Donner, 1996; Rovamo, Raninen, & Donner, 1999). Similar photoreceptors and lateral inhibitory feedback networks exist in the chick retina (Walls, 1942) and both species share similar bandpass temporal response characteristics (Jarvis et al., 2002).

Previous studies have also analyzed the phase change resulting from flicker ERG. This could provide further information about the underlying mechanism responsible for processing luminance flicker. We would expect a change in the timing characteristics of the ERG response between high and low temporal frequencies probably indicating different processing pathways.

Flicker ERGs with varying R/(R+G) ratio:

We created a sinusoidal stimulus with varying ratios of red and green light and recorded chick ERG responses at temporal frequencies of 12 and 36 Hz. This was modeled after the technique used by Kremers et. al., (2010). The 36 Hz results in Figure 7 (top) showed a minimum ERG amplitude (i.e., no or minimal luminance modulation) accompanied by a corresponding phase shift at a R/(R+G) ratio of 0.04. The 12 Hz ERG in Figure 7 (bottom) showed relatively constant amplitude and phase across the range of R/(R+G) ratios. Figure 6 shows the raw data recorded from a single chick. The 36 Hz data (left) visually shows the change in amplitude across three R/(R+G) ratios but also the change in phase between the 0, 0.04 and 1. The 12 Hz (right) shows stable amplitude and phase throughout.

When compared to the human results, the minimum amplitude in the 36 Hz testing formed at a lower R/(R+G) ratio, shifted to the left, indicating that increased green contrast/higher M- cone stimulation was required to achieve a minimum luminance response. There is also a steady increase in amplitude with increasing R/(R+G) indicating a small increase in the red content of the stimulus causes large increases in the ERG amplitude. Comparing the response amplitude at a R/(R+G) ratio of 0 where the stimulus is comprised of only green light and a R/(R+G) ratio of 1 where the stimulus is only red, the response to red light is ~10x greater than it is to the green. In contrast, Figure 2 shows the human ERG amplitudes at R/(R+G) ratios

of 0 and 1 are relatively equal reflecting a balanced input from the L and M- cones to postreceptoral mechanisms (Kremers et al., 2010). The minimum found at 0.04 R/(R+G) can be expressed in terms of the L- and M-cone contrast as 7% and 93%, respectively, while the DC-contrast is ~40%. We could speculate, therefore, that the chick retina has a strong long-wavelength bias that might be due to the presence of the DCs (with a sensitivity skewed towards longer wavelengths) in addition to the L-cones.

There are several theories for the function of the DC but previous studies suggest the DC plays a role in achromatic vision with input to luminance pathways and contributing to motion detection (Jones and Osorio, 2004; Sun and Frost, 1997; Campenhausen and Kirschfeld, 1998; Olsson et al. 2015). It has also been shown that chicks are able to discriminate visual textures based on luminance contrast but not when these patterns are isoluminant to the DCs (Jones & Osorio., 2004). This implies that only the single cones are responsible for chromatic vision (Osorio, Vorobyev, & Jones, 1999; Vorobyev & Osorio, 1998). While this is widely believed, the DC is not ubiquitous for all avian species and raptors do not have DCs which suggests they use single cones for luminance vision (Potier et al., 2018). In a study of spatial contrast sensitivity in budgerigars (Melopsittacus undulatus), gratings with both luminance and chromatic contrast are detected with a higher frequency compared to stimuli with either luminance or chromatic contrast alone. This implies that the single and DC pathways are not completely separated (Lind et al., 2012).

The shift to a lower R/(R+G) ratio could also be due to an increased number of L- cones, or increased L-cone sensitivity. It is also possible that chicks use single L- and M-cone inputs for their chromatic vision but when it comes to luminance changes they only rely on the DC excitation. In the Kremers et. al., (2010) study, ERG testing was performed on trichromatic and

deuteranopic subjects. The disparity in ERG responses between the trichromatic and dichromatic subjects served as a control to prove ERG responses were from L- and M-cone driven chromatic and luminance channel activity, with minimal input from S-cones and rods. The deuteranopic 36 Hz ERG amplitude shares a similar V-shape function with the minimal shifted to lower R/(R+G) ratio as we saw in the chick's 36 Hz ERG responses. This indicates that increased green LED contrast was needed to compensate for the lack of M-cones (in deuteranopes) or for the long-wavelength sensitivity bias (in chicks) in order to achieve a balanced response from both cone types.

Another possible explanation for the differences seen between humans and chicks could be the luminance levels measured in units of cd/m^2 based on the human luminosity function. When measuring across 14 varying lighting conditions, the average chicken lumens were 37% higher than human lumens (Saunders et al., 2008). This may explain the significant bias towards longer wavelength light seen in the chick. Previous studies have also shown that retinal illuminance in chicks can be 10x greater than that of humans (Jarvis et al., 2003). These differences are evident in the spectral sensitivity curve for the chick which has much higher and broader peaks when compared to human. The S- and UV- cones of chick contribute to a more robust sensitivity to shorter wavelength light compared to humans. While our results show an overwhelming bias towards longer wavelength, it would be more thorough to include experiments to control for the input from S- and UV- cones as well.

It would also have been beneficial to observe the 2nd order harmonic amplitude and phase as it is likely there are higher order processing affecting the ERG response as indicated by the double peaks seen in the 12Hz raw data in figure 4c. A higher harmonic nonlinear component is a well-documented part of the human flicker ERG. (Odom et al 1992). The graph of the 2^{nd}

order harmonic amplitude and phase of the 12Hz data are shown below (Figure 11). This shows a minimum at a R/(R+G) ratio of .7 with a corresponding shift in phase. This supports the theory that there is higher order processing of another visual mechanism at the lower temporal frequency.

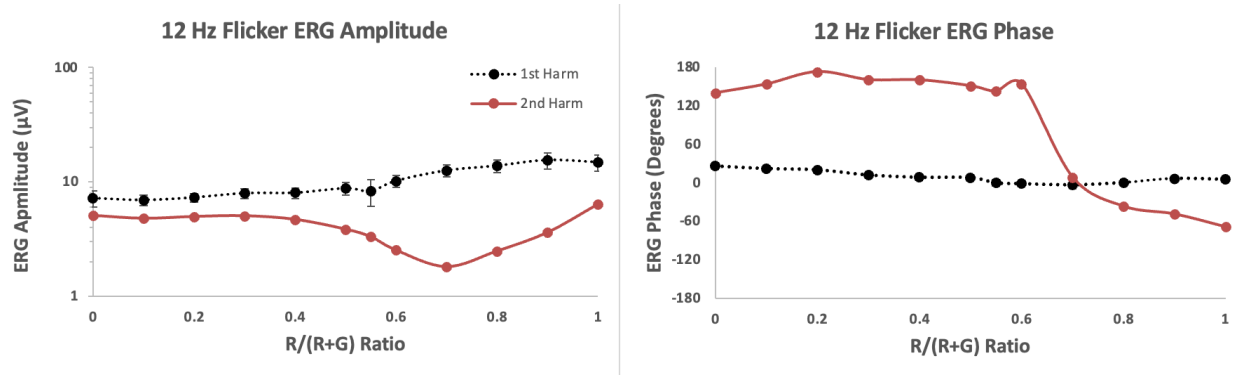


Figure 11. Average amplitude (left) and phase (right) of the first and second harmonics of the Fourier transform of the ERG response to sinusoidal modulation of stimuli with R/(R+G) ranging from 0 to 1 measured at 12 Hz respectively. The error bars show ±1 SE.

<u>36 Hz flicker ERG without DC modulation:</u>

The chicks have an extra DC that should contribute to a certain extent to the ERG responses we described above. In order to investigate the DC's contribution to the flicker ERG response, we created a new stimulus using the silent substitution method, so as to record ERG responses without (or minimal) input from the DCs.

The chick ERG response to the stimulus that elicits no DC modulation is seen in Figure 9. The response amplitude (left) forms a distinct V-shape with the highest maxima occurring initially at an angle of 100 degrees before decreasing to a minimum at an angle of 142 degrees with a corresponding 180-degree shift in the phase of the first harmonic (right). The shift in

phase is also visible in Figure 8 where the dashed line shows the change in phase after the minimum amplitude is reached at 142 degrees.

This result is most easily compared to the previous Flicker ERG testing in terms of cone contrast. With the DC silenced, the minimum response amplitude is reached when the L-cone contrast is 24%, and the M-cone contrast is 18%. With the DC active, the data indicates that chicks have minimum response at an R/(R+G) ratio of 0.04 which corresponds to 7% L-cone contrast, 93% M-cone contrast, and 40% DC contrast. From this result, we can infer that without input from the DC, the chick's L- and M-cone input to the ERG responses is more balanced, even though slightly biased towards the M-cones since a slightly higher L-cone contrast is required in this case. This is a stark difference compared to the previous testing which required 93% M-cone contrast to balance the L and DC input.

As the amount of red in the stimulus increased at a higher angle, the response amplitudes did not increase as dramatically as was seen with the R/(R+G) experiment. Overall, the DC-silenced ERG amplitudes were significantly lower than the ERG amplitudes recorded with the DC active. This difference in the amplitude, most likely is due to the contribution of the DC (stimulated at 40% contrast) in the second experiment, while its contrast is 0% in the third experiment. The DC is the most populous photoreceptor in the chick retina and new studies have shown that of the 15 types of bipolar cells in the chick retina, 13 receive input from DCs with all bipolar cells bistratified or multistratified. This indicates the outer plexiform layer of the chick forms a more multifaceted network than previously thought (Gunther et al., 2022). The DC dominance in both density and connectivity may explain the significant changes in amplitude seen between the two testing conditions both with and without input from the DCs. Further

studies should be conducted that silence L/M- cone activity and isolate the activity of the DC, to further characterize the DC's contribution in the ERG signal.

Flicker ERGs of the chick chromatic system with varying temporal frequency:

The two minimum response amplitudes from experiment 2 and 3 were used to measure ERG responses along a range of temporal frequencies to compare the temporal frequency response function when the DC is active or silenced, respectively (Figure 10 left). Both results show maximum response amplitude at lower temporal frequencies while the amplitude decreases with increasing temporal frequency. We again see an amplitude difference between the two functions, most likely due to the excitation or not of the DC. Overall, both temporal response functions appear to be low-pass, consistent with the properties of a chromatic system, suggesting that both conditions tapped the same visual processing pathway. Figure 10 also shows the luminance flicker ERG response amplitude and phase from experiment 1. Comparing the three data sets next to each other gives a sense of the function of both the luminance and chromatic systems. There is a change in the slope of the amplitude of all three conditions around 8-10Hz from negative to positive, if only briefly for the R/(R+G) and DC conditions. The R/(R+G) condition does show a slightly prolonged increase in slope with increasing temporal frequency in a similar fashion to the luminance response which may indicate some activity of the double cone creating a luminance response in our R/(R+G) condition. The change in slope at 10Hz is also reflected in the phase data of the luminance and R/(R+G) conditions with a phase shift occurring for both. A second larger phase shift occurs for the luminance condition at 20Hz.

Parry et al. (2012) used the isoluminant point to record human ERG responses across a range of temporal frequencies. They showed a separation of magno- and parvocellular ERG

responses with a single red-green isoluminant stimulus of which the first harmonic component reflected a low pass temporal frequency response function consistent with the expected response of midget bipolar cells of the parvocellular pathway. Our results are very similar in appearance and we could infer that the underlying mechanism is the chick's equivalent to the human parvocellular pathway. This is surprising given the anatomical differences between the human and chick retina where chicks have significantly fewer "midget-like" bipolar cells with single cone connectivity and no anatomical equivalent for the midget RGC, which comprises 80–90% of all RGCs in humans (Seifert et al., 2020). The anatomical basis for the chick magnocellular equivalent is more robust with extensive multi-stratified/diffuse RGCs with dendritic branches throughout the IPL (Seifert et al., 2020).

Based on the similarity of the two temporal response functions, we could infer that the excitation, or not, of the DC, does not alter significantly the temporal characteristics of the chromatic system, as long as the L- and M-cones are balanced so as to minimize a luminance contribution.

Very little is known about the temporal characteristics of the chick luminance or chromatic system. The vast majority of studies using the chick have focused on eye growth and emmetropization using a cause and effect model, treating the visual processing of the chick as a black box where a visual stimulus is used and the effect on eye growth is measured with no speculation as to the underlying process causing the effect. It has been shown the mechanism underlying emmetropization is more sensitive to achromatic stimulation than chromatic at high temporal frequencies (Cottaris and De Valois, 1998; Petrova et al., 2013a), and high temporal frequency stimulation has been shown to decrease eye growth (Rohrer et al., 1995; Gawne et al., 2014). There is significant evidence in the chick and other similar animal models that the inverse

is true with lower temporal frequency flicker acting as a signal to increase eye growth leading to more myopic refractions (Crewther et al., 2016). These characteristics could indicate an underlying neural mechanism where the chromatic system is dictating eye growth at lower temporal frequencies and the luminance system is dominant at higher temporal frequencies. This is supported by our study where the response amplitude was highest at low temporal frequency without input from the luminance pathway and gradually decreased with increasing temporal frequency as the chromatic input weakened.

CONCLUSIONS

Using flicker ERGs we have demonstrated a luminance and a chromatic pathway in chicks. The ERG response to red and green flicker in chicks was complicated by the presence of a DC with a broad spectral sensitivity. With an active DC, the chick ERG response is strongly influenced by the long-wavelength sensitive photopigment present in both double- and L-cones. When the DC is silenced, the L- and M-cone contribution to the ERG response is more balanced and similar to that found in humans. Therefore, it appears that the DC adds additional long-wavelength bias in the chick's visual system that detects luminance contrast. The minimum response amplitude to red green flicker, where the luminance response is minimized, can be used in studies of eye growth to determine the effects of chromatic contrast on eye growth.

REFERENCES

- Bach, M., Brigell, M. G., Hawlina, M., Holder, G. E., Johnson, M. A., McCulloch, D. L., Meigen, T., & Viswanathan, S. (2013). ISCEV standard for clinical pattern electroretinography (PERG): 2012 update. Documenta Ophthalmologica. Advances in Ophthalmology, 126(1), 1–7. https://doi.org/10.1007/s10633-012-9353-y
- Barbur, J. L., Prescott, N. B., Douglas, R. H., Jarvis, J. R., & Wathes, C. M. (2002). A comparative study of stimulus-specific pupil responses in the domestic fowl (Gallus gallus domesticus) and the human. Vision Research, 42(2), 249–255. https://doi.org/10.1016/s0042-6989(01)00279-6
- Berardi, N., Domenici, L., Gravina, A., & Maffei, L. (1990). Pattern ERG in rats following section of the optic nerve. Experimental Brain Research, 79(3), 539–546. https://doi.org/10.1007/BF00229323
- Bowles, K. E., & Kraft, T. W. (2012). ERG Critical Flicker Frequency Assessment in Humans. In M. M. LaVail, J. D. Ash, R. E. Anderson, J. G. Hollyfield, & C. Grimm (Eds.), Retinal Degenerative Diseases (pp. 503–509). Springer US. https://doi.org/10.1007/978-1-4614-0631-0_63
- Bowmaker, J. K. (2008). Evolution of vertebrate visual pigments. Vision Research, 48(20), 2022–2041. https://doi.org/10.1016/j.visres.2008.03.025
- Brainard, D. H., Roorda, A., Yamauchi, Y., Calderone, J. B., Metha, A., Neitz, M., Neitz, J., Williams, D. R., & Jacobs, G. H. (2000). Functional consequences of the relative numbers of L and M cones. Journal of the Optical Society of America. A, Optics, Image Science, and Vision, 17(3), 607–614. https://doi.org/10.1364/josaa.17.000607

- Bruhn, S. L., & Cepko, C. L. (1996). Development of the pattern of photoreceptors in the chick retina. Journal of Neuroscience, 16(4), 1430–1439. https://doi.org/10.1523/JNEUROSCI.16-04-01430.1996
- Bueno, J. M., Giakoumaki, A., Gualda, E. J., Schaeffel, F., & Artal, P. (2011). Analysis of the chicken retina with an adaptive optics multiphoton microscope. Biomedical Optics Express, 2(6), 1637–1648. https://doi.org/10.1364/BOE.2.001637
- Bush, R. A., & Sieving, P. A. (1996). Inner retinal contributions to the primate photopic fast flicker electroretinogram. JOSA A, 13(3), 557–565. https://doi.org/10.1364/JOSAA.13.000557
- Chui, T. Y. P., Song, H., & Burns, S. A. (2008). Individual variations in human cone photoreceptor packing density: Variations with refractive error. Investigative Ophthalmology & Visual Science, 49(10), 4679–4687. https://doi.org/10.1167/iovs.08-2135
- Cottaris, N. P., & De Valois, R. L. (1998). Temporal dynamics of chromatic tuning in macaque primary visual cortex. Nature, 395(6705), 896–900. https://doi.org/10.1038/27666
- Crewther, S. G., Barutchu, A., Murphy, M. J., & Crewther, D. P. (2006). Low frequency temporal modulation of light promotes a myopic shift in refractive compensation to all spectacle lenses. Experimental Eye Research, 83(2), 322–328. https://doi.org/10.1016/j.exer.2005.12.016
- da Silva, S., & Cepko, C. L. (2017). Fgf8 Expression and Degradation of Retinoic Acid Are Required for Patterning a High-Acuity Area in the Retina. Developmental Cell, 42(1), 68-81.e6. https://doi.org/10.1016/j.devcel.2017.05.024

- De Monasterio, F. M., Gouras, P., & Tolhurst, D. J. (1975). Concealed colour opponency in ganglion cells of the rhesus monkey retina. The Journal of Physiology, 251(1), 217–229. https://doi.org/10.1113/jphysiol.1975.sp011088
- Derrington, A. M., Krauskopf, J., & Lennie, P. (1984). Chromatic mechanisms in lateral geniculate nucleus of macaque. The Journal of Physiology, 357, 241–265.
- Derrington, A. M., & Lennie, P. (1984). Spatial and temporal contrast sensitivities of neurones in lateral geniculate nucleus of macaque. The Journal of Physiology, 357, 219–240. https://doi.org/10.1113/jphysiol.1984.sp015498
- Dirani, M., Zhou, B., Hornbeak, D., Chang, B. C., Gazzard, G., Chia, A., Ling, Y., Selvaraj, P., Young, T. L., Varma, R., Wong, T. Y., & Saw, S. M. (2010). Prevalence and causes of decreased visual acuity in Singaporean Chinese preschoolers. The British Journal of Ophthalmology, 94(12), 1561–1565. https://doi.org/10.1136/bjo.2009.173104
- Dreher, B., Fukada, Y., & Rodieck, R. W. (1976). Identification, classification and anatomical segregation of cells with X-like and Y-like properties in the lateral geniculate nucleus of old-world primates. The Journal of Physiology, 258(2), 433–452. https://doi.org/10.1113/jphysiol.1976.sp011429
- Estévez, O., & Spekreijse, H. (1982). The "silent substitution" method in visual research. Vision Research, 22(6), 681–691. https://doi.org/10.1016/0042-6989(82)90104-3
- Estévez, O., & Spekreuse, H. (1974). A spectral compensation method for determining the flicker characteristics of the human colour mechanisms. Vision Research, 14(9), 823–830. https://doi.org/10.1016/0042-6989(74)90147-3
- Evans, C. S., & Evans, L. (1999). Chicken food calls are functionally referential. Animal Behaviour, 58(2), 307–319. https://doi.org/10.1006/anbe.1999.1143

- Fan, D. S. P., Cheung, E. Y. Y., Lai, R. Y. K., Kwok, A. K. H., & Lam, D. S. C. (2004). Myopia progression among preschool Chinese children in Hong Kong. Annals of the Academy of Medicine, Singapore, 33(1), 39–43.
- Fischer, A. J., Stanke, J. J., Aloisio, G., Hoy, H., & Stell, W. K. (2007). Heterogeneity of horizontal cells in the chicken retina. Journal of Comparative Neurology, 500(6), 1154–1171. https://doi.org/10.1002/cne.21236
- Giordano, L., Friedman, D. S., Repka, M. X., Katz, J., Ibironke, J., Hawes, P., & Tielsch, J. M. (2009). Prevalence of refractive error among preschool children in an urban population: The Baltimore Pediatric Eye Disease Study. Ophthalmology, 116(4), 739–746, 746.e1-4. https://doi.org/10.1016/j.ophtha.2008.12.030
- Günther, A., Dedek, K., Haverkamp, S., Irsen, S., Briggman, K. L., & Mouritsen, H. (2021). Double Cones and the Diverse Connectivity of Photoreceptors and Bipolar Cells in an Avian Retina. Journal of Neuroscience, 41(23), 5015–5028. https://doi.org/10.1523/JNEUROSCI.2495-20.2021
- Guo, K., Yang, D. Y., Wang, Y., Yang, X. R., Jing, X. X., Guo, Y. Y., Zhu, D., You, Q. S., Tao, Y., & Jonas, J. B. (2015). Prevalence of Myopia in Schoolchildren in Ejina: The Gobi Desert Children Eye Study. Investigative Ophthalmology & Visual Science, 56(3), 1769–1774. https://doi.org/10.1167/iovs.14-15737
- Guo, X., Fu, M., Ding, X., Morgan, I. G., Zeng, Y., & He, M. (2017). Significant Axial Elongation with Minimal Change in Refraction in 3- to 6-Year-Old Chinese Preschoolers: The Shenzhen Kindergarten Eye Study. Ophthalmology, 124(12), 1826–1838. https://doi.org/10.1016/j.ophtha.2017.05.030

- Harrison, J. M., O'Connor, P. S., Young, R. S., Kincaid, M., & Bentley, R. (1987). The pattern ERG in man following surgical resection of the optic nerve. Investigative Ophthalmology & Visual Science, 28(3), 492–499.
- Hart, N. S. (2001). The Visual Ecology of Avian Photoreceptors. Progress in Retinal and Eye Research, 20(5), 675–703. https://doi.org/10.1016/S1350-9462(01)00009-X
- He, M., Zeng, J., Liu, Y., Xu, J., Pokharel, G. P., & Ellwein, L. B. (2004). Refractive error and visual impairment in urban children in southern china. Investigative Ophthalmology & Visual Science, 45(3), 793–799. https://doi.org/10.1167/iovs.03-1051
- Hicks, T. P., Lee, B. B., & Vidyasagar, T. R. (1983). The responses of cells in macaque lateral geniculate nucleus to sinusoidal gratings. The Journal of Physiology, 337, 183–200.
- Hodos, W., & Kuenzel, W. J. (1984). Retinal-image degradation produces ocular enlargement in chicks. Investigative Ophthalmology & Visual Science, 25(6), 652–659.
- Holden, B. A., Fricke, T. R., Wilson, D. A., Jong, M., Naidoo, K. S., Sankaridurg, P., Wong, T. Y., Naduvilath, T. J., & Resnikoff, S. (2016). Global Prevalence of Myopia and High Myopia and Temporal Trends from 2000 through 2050. Ophthalmology, 123(5), 1036–1042. https://doi.org/10.1016/j.ophtha.2016.01.006
- Holder, G. E. (2001). Pattern electroretinography (PERG) and an integrated approach to visual pathway diagnosis. Progress in Retinal and Eye Research, 20(4), 531–561. https://doi.org/10.1016/s1350-9462(00)00030-6
- Hurvich, L. M., & Jameson, D. (1955). Some quantitative aspects of an opponent-colors theory. II. Brightness, saturation, and hue in normal and dichromatic vision. Journal of the Optical Society of America, 45(8), 602–616. https://doi.org/10.1364/josa.45.000602

- Jacobs, G. H., & Deegan, J. F. (1994). Spectral sensitivity, photopigments, and color vision in the guinea pig (Cavia porcellus). Behavioral Neuroscience, 108(5), 993–1004. https://doi.org/10.1037//0735-7044.108.5.993
- Jacobs, G. H., & Neitz, J. (1993). Electrophysiological estimates of individual variation in the L/M cone ratio. In B. Drum (Ed.), Colour Vision Deficiencies XI: Proceedings of the eleventh Symposium of the International Research Group on Colour Vision Deficiencies, held in Sydney, Australia 21–23 June 1991 including the joint IRGCVD-AIC Meeting on Mechanisms of Colour Vision 24 June 1991 (pp. 107–112). Springer Netherlands. https://doi.org/10.1007/978-94-011-1856-9_11
- Jarvis, E. (2008). Neural systems for vocal learning in birds and humans: A synopsis. Journal of Ornithology / DO-G, 148, 35–44. https://doi.org/10.1007/s10336-007-0243-0
- Jarvis, J. R., Prescott, N. B., & Wathes, C. M. (2003). A mechanistic inter-species comparison of flicker sensitivity. Vision Research, 43(16), 1723–1734. https://doi.org/10.1016/s0042-6989(03)00253-0
- Jones, C. D., & Osorio, D. (2004). Discrimination of oriented visual textures by poultry chicks. Vision Research, 44(1), 83–89. https://doi.org/10.1016/j.visres.2003.08.014
- Jones, C. D., Osorio, D., & Baddeley, R. J. (2001). Colour categorization by domestic chicks. Proceedings of the Royal Society of London. Series B: Biological Sciences, 268(1481), 2077–2084. https://doi.org/10.1098/rspb.2001.1734
- Kaplan, E., & Shapley, R. M. (1986). The primate retina contains two types of ganglion cells, with high and low contrast sensitivity. Proceedings of the National Academy of Sciences, 83(8), 2755–2757. https://doi.org/10.1073/pnas.83.8.2755

- Kelber, A. (2019). Bird colour vision – from cones to perception. Current Opinion in Behavioral Sciences, 30, 34–40. https://doi.org/10.1016/j.cobeha.2019.05.003
- Kelber, A., & Osorio, D. (2010). From spectral information to animal colour vision: Experiments and concepts. Proceedings. Biological Sciences, 277(1688), 1617–1625. https://doi.org/10.1098/rspb.2009.2118
- Kelly, D. H. (1971). Theory of Flicker and Transient Responses, I. Uniform Fields*. JOSA, 61(4), 537–546. https://doi.org/10.1364/JOSA.61.000537
- Kram, Y. A., Mantey, S., & Corbo, J. C. (2010). Avian Cone Photoreceptors Tile the Retina as Five Independent, Self-Organizing Mosaics. PLoS ONE, 5(2), e8992. https://doi.org/10.1371/journal.pone.0008992
- Kremers, J., & Link, B. (2008). Electroretinographic responses that may reflect activity of parvo- and magnocellular postreceptoral visual pathways. Journal of Vision, 8(15), 11. https://doi.org/10.1167/8.15.11
- Kremers, J., Rodriguez, A. R., de Lima Silveira, L. C., & da Silva Filho, M. (2010). Flicker ERGs Representing Chromaticity and Luminance Signals. Invest Ophthalmol Vis Sci., 51(1), 577-587. https://doi.org/10.1167/8.15.11
- Kremers, J., & Meierkord, S. (1999). Rod–cone-interactions in deuteranopic observers: Models and dynamics. Vision Research. https://doi.org/10.1016/S0042-6989(99)00027-9
- Kremers, J., Scholl, H. P. N., Knau, H., Berendschot, T. T. J. M., Usui, T., & Sharpe, L. T. (2000). L/M cone ratios in human trichromats assessed by psychophysics, electroretinography, and retinal densitometry. JOSA A, 17(3), 517–526. https://doi.org/10.1364/JOSAA.17.000517

- Lan, W., Zhao, F., Lin, L., Li, Z., Zeng, J., Yang, Z., & Morgan, I. G. (2013). Refractive errors in 3-6 year-old Chinese children: A very low prevalence of myopia? PloS One, 8(10), e78003. https://doi.org/10.1371/journal.pone.0078003
- Lee, B. B., Pokorny, J., Smith, V. C., Martin, P. R., & Valbergt, A. (1990). Luminance and chromatic modulation sensitivity of macaque ganglion cells and human observers. JOSA A, 7(12), 2223–2236. https://doi.org/10.1364/JOSAA.7.002223
- Lee, B. B., Valberg, A., Tigwell, D. A., & Tryti, J. (1987). An account of responses of spectrally opponent neurons in macaque lateral geniculate nucleus to successive contrast. Proceedings of the Royal Society of London. Series B, Biological Sciences, 230(1260), 293–314. https://doi.org/10.1098/rspb.1987.0021
- Lennie, P., & D'Zmura, M. (1988). Mechanisms of color vision. Critical Reviews in Neurobiology, 3(4), 333–400.
- Li, Z., Xu, K., Wu, S., Lv, J., Jin, D., Song, Z., Wang, Z., & Liu, P. (2014). Population-based survey of refractive error among school-aged children in rural northern China: The Heilongjiang eye study. Clinical & Experimental Ophthalmology, 42(4), 379–384. https://doi.org/10.1111/ceo.12198
- Lind, O., Sunesson, T., Mitkus, M., & Kelber, A. (2012). Luminance-dependence of spatial vision in budgerigars (Melopsittacus undulatus) and Bourke's parrots (Neopsephotus bourkii). Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology, 198(1), 69–77. https://doi.org/10.1007/s00359-011-0689-7
- Lisney, T. J., Ekesten, B., Tauson, R., Håstad, O., & Ödeen, A. (2012). Using electroretinograms to assess flicker fusion frequency in domestic hens Gallus gallus domesticus. Vision Research, 62, 125–133. https://doi.org/10.1016/j.visres.2012.04.002

- Lisney, T. J., Rubene, D., Rózsa, J., Løvlie, H., Håstad, O., & Ödeen, A. (2011). Behavioural assessment of flicker fusion frequency in chicken Gallus gallus domesticus. Vision Research, 51(12), 1324–1332. https://doi.org/10.1016/j.visres.2011.04.009
- Livingstone, M., & Hubel, D. (1988). Segregation of form, color, movement, and depth: Anatomy, physiology, and perception. Science (New York, N.Y.), 240(4853), 740–749. https://doi.org/10.1126/science.3283936
- Ma, Y., Qu, X., Zhu, X., Xu, X., Zhu, J., Sankaridurg, P., Lin, S., Lu, L., Zhao, R., Wang, L., Shi, H., Tan, H., You, X., Yuan, H., Sun, S., Wang, M., He, X., Zou, H., & Congdon, N. (2016). Age-Specific Prevalence of Visual Impairment and Refractive Error in Children Aged 3-10 Years in Shanghai, China. Investigative Ophthalmology & Visual Science, 57(14), 6188–6196. https://doi.org/10.1167/iovs.16-20243
- Mandelman, T., & Sivak, J. G. (1983). Longitudinal chromatic aberration of the vertebrate eye. Vision Research, 23(12), 1555–1559. https://doi.org/10.1016/0042-6989(83)90169-4
- Masland, R. H. (2001). The fundamental plan of the retina. Nature Neuroscience, 4(9), 877–886. https://doi.org/10.1038/nn0901-877
- McCulloch, D. L., Marmor, M. F., Brigell, M. G., Hamilton, R., Holder, G. E., Tzekov, R., & Bach, M. (2015). ISCEV Standard for full-field clinical electroretinography (2015 update). Documenta Ophthalmologica. Advances in Ophthalmology, 130(1), 1–12. https://doi.org/10.1007/s10633-014-9473-7
- Merigan, W. H., & Maunsell, J. H. (1993). How parallel are the primate visual pathways? Annual Review of Neuroscience, 16, 369–402. https://doi.org/10.1146/annurev.ne.16.030193.002101

- Miller, R. F., & Dowling, J. E. (1970). Intracellular responses of the Müller (glial) cells of mudpuppy retina: Their relation to b-wave of the electroretinogram. Journal of Neurophysiology, 33(3), 323–341. https://doi.org/10.1152/jn.1970.33.3.323
- Miura, G., Wang, M. H., Ivers, K. M., & Frishman, L. J. (2009). Retinal pathway origins of the pattern ERG of the mouse. Experimental Eye Research, 89(1), 49–62. https://doi.org/10.1016/j.exer.2009.02.009
- Mollon, J. D. (1989). "Tho' she kneel'd in that place where they grew..." The uses and origins of primate colour vision. The Journal of Experimental Biology, 146, 21–38.
- Morgan, I. G., & Rose, K. A. (2013). Myopia and international educational performance. Ophthalmic & Physiological Optics: The Journal of the British College of Ophthalmic Opticians (Optometrists), 33(3), 329–338. https://doi.org/10.1111/opo.12040
- Morris, V. B. (1970). Symmetry in a receptor mosaic demonstrated in the chick from the frequencies, spacing and arrangement of the types of retinal receptor. Journal of Comparative Neurology, 140(3), 359–397. https://doi.org/10.1002/cne.901400308
- Murray, I. J., Parry, N. R. A., Kremers, J., Stepien, M., & Schild, A. (2004). Photoreceptor topography and cone-specific electroretinograms. Visual Neuroscience, 21(3), 231–235. https://doi.org/10.1017/s0952523804213268
- Mutti, D. O., Mitchell, G. L., Jones, L. A., Friedman, N. E., Frane, S. L., Lin, W. K., Moeschberger, M. L., & Zadnik, K. (2009). Accommodation, Acuity, and their Relationship to Emmetropization in Infants. Optometry and Vision Science : Official Publication of the American Academy of Optometry, 86(6), 666–676. https://doi.org/10.1097/OPX.0b013e3181a6174f

- Naito, J., & Chen, Y. (2004). Morphological features of chick retinal ganglion cells. Anatomical Science International, 79(4), 213. https://doi.org/10.1111/j.1447-073x.2004.00084.x
- Nassi, J. J., & Callaway, E. M. (2009). Parallel processing strategies of the primate visual system. Nature Reviews. Neuroscience, 10(5), 360–372. https://doi.org/10.1038/nrn2619
- Odom, J., REITS, D., BURGERS, N., & Riemslag, F. (1992). Flicker Electroretinograms: A Systems Analytic Approach. Optometry and Vision Science : Official Publication of the American Academy of Optometry, 69, 106–116. https://doi.org/10.1097/00006324-199202000-00003
- Okano, T., Kojima, D., Fukada, Y., Shichida, Y., & Yoshizawa, T. (1992). Primary structures of chicken cone visual pigments: Vertebrate rhodopsins have evolved out of cone visual pigments. Proceedings of the National Academy of Sciences of the United States of America, 89(13), 5932–5936.
- Olsson, P., Lind, O., & Kelber, A. (2015). Bird colour vision: Behavioural thresholds reveal receptor noise. Journal of Experimental Biology, 218(2), 184–193. https://doi.org/10.1242/jeb.111187
- Osorio, D., Jones, C. D., & Vorobyev, M. (1999). Accurate memory for colour but not pattern contrast in chicks. Current Biology, 9(4), 199–202. https://doi.org/10.1016/S0960-9822(99)80089-X
- Osorio, D., Miklósi, A., & Gonda, Zs. (1999). Visual Ecology and Perception of Coloration Patterns by Domestic Chicks. Evolutionary Ecology, 13(7), 673–689. https://doi.org/10.1023/A:1011059715610

- Osorio, D., Vorobyev, M., & Jones, C. D. (1999). Colour vision of domestic chicks. The Journal of Experimental Biology, 202(Pt 21), 2951–2959.
- Ostrin, L. A., Choh, V., & Wildsoet, C. F. (2016). The pattern ERG in chicks – Stimulus dependence and optic nerve section. Vision Research, 128, 45–52. https://doi.org/10.1016/j.visres.2016.09.009
- Parry, N. R. A., Murray, I. J., Panorgias, A., McKeefry, D. J., Lee, B. B., & Kremers, J. (2012). Simultaneous chromatic and luminance human electroretinogram responses. The Journal of Physiology, 590(13), 3141–3154. https://doi.org/10.1113/jphysiol.2011.226951
- Penn, R. D., & Hagins, W. A. (1969). Signal transmission along retinal rods and the origin of the electroretinographic a-wave. Nature, 223(5202), 201–204. https://doi.org/10.1038/223201a0
- Petrova, D., Henning, G. B., & Stockman, A. (2013). The temporal characteristics of the early and late stages of L- and M-cone pathways that signal brightness. Journal of Vision, 13(7), 15. https://doi.org/10.1167/13.7.15
- Potier, S., Mitkus, M., & Kelber, A. (n.d.). High resolution of colour vision, but low contrast sensitivity in a diurnal raptor. Proceedings of the Royal Society B: Biological Sciences, 285(1885), 20181036. https://doi.org/10.1098/rspb.2018.1036
- Prescott, N. B., Wathes, C. M., & Jarvis, J. R. (2003). Light, vision and the welfare of poultry. Animal Welfare, 12(2), 269–288.
- Quesada, A., Prada, F. A., & Genis-Galvez, J. M. (1988). Bipolar cells in the chicken retina. Journal of Morphology, 197(3), 337–351. https://doi.org/10.1002/jmor.1051970308

- Railton, R. C. R., Mary Foster, T., & Temple, W. (2009). A comparison of two methods for assessing critical flicker fusion frequency in hens. Behavioural Processes, 80(2), 196–200. https://doi.org/10.1016/j.beproc.2008.11.016
- Rovamo, J., Raninen, A., & Donner, K. (1999). The effects of temporal noise and retinal illuminance on foveal flicker sensitivity. Vision Research, 39(3), 533–550. https://doi.org/10.1016/S0042-6989(98)00120-5
- Rovamo, J., Raninen, A., Lukkarinen, S., & Donner, K. (1996). Flicker Sensitivity as a Function of Spectral Density of External White Temporal Noise. Vision Research, 36(23), 3767–3774. https://doi.org/10.1016/0042-6989(96)00108-3
- Rubene, D., Håstad, O., Tauson, R., Wall, H., & Ödeen, A. (2010). The presence of UV wavelengths improves the temporal resolution of the avian visual system. Journal of Experimental Biology, 213(19), 3357–3363. https://doi.org/10.1242/jeb.042424
- Rucker and Kruger—2004—Accommodation responses to stimuli in cone contras.pdf. (n.d.). Retrieved December 5, 2021, from https://www.sunyopt.edu/includes/kruger/pdf/8.pdf
- Rucker, F. (2019). Monochromatic and white light and the regulation of eye growth. Experimental Eye Research, 184, 172–182. https://doi.org/10.1016/j.exer.2019.04.020
- Rucker, F., Britton, S., Spatcher, M., & Hanowsky, S. (2015). Blue Light Protects Against Temporal Frequency Sensitive Refractive Changes. Investigative Ophthalmology & Visual Science, 56(10), 6121–6131. https://doi.org/10.1167/iovs.15-17238
- Rucker, F. J., & Kruger, P. B. (2004). Accommodation responses to stimuli in cone contrast space. Vision Research, 44(25), 2931–2944. https://doi.org/10.1016/j.visres.2004.07.005

- Rucker, F. J., & Wallman, J. (2012). Chicks use changes in luminance and chromatic contrast as indicators of the sign of defocus. Journal of Vision, 12(6), 23. https://doi.org/10.1167/12.6.23
- Sawusch, M., Pokorny, J., & Smith, V. C. (1987). Clinical electroretinography for short wavelength sensitive cones. Investigative Ophthalmology & Visual Science, 28(6), 966–974.
- Schaeffel, F., Glasser, A., & Howland, H. C. (1988). Accommodation, refractive error and eye growth in chickens. Vision Research, 28(5), 639–657. https://doi.org/10.1016/0042-6989(88)90113-7
- Schaeffel, F., & Howland, H. C. (1991). Properties of the feedback loops controlling eye growth and refractive state in the chicken. Vision Research, 31(4), 717–734. https://doi.org/10.1016/0042-6989(91)90011-s
- Schaeffel, F., Rohrer, B., Zrenner, E., & Lemmer, T. (1991). Diurnal control of rod function in the chicken. Visual Neuroscience, 6(6), 641–653. https://doi.org/10.1017/S0952523800002637
- Schiller, P. H., Logothetis, N. K., & Charles, E. R. (1990). Functions of the colour-opponent and broad-band channels of the visual system. Nature, 343(6253), 68–70. https://doi.org/10.1038/343068a0
- Schmid, K. L. (1994). Visual and retinal control of eye growth and refraction [Phd, Queensland University of Technology]. https://eprints.qut.edu.au/36710/
- Schmid, K. L., & Wildsoet, C. F. (1998). Assessment of visual acuity and contrast sensitivity in the chick using an optokinetic nystagmus paradigm. Vision Research, 38(17), 2629–2634. https://doi.org/10.1016/S0042-6989(97)00446-X

- Schwahn, H. N., & Schaeffel, F. (1997). Flicker parameters are different for suppression of myopia and hyperopia. Vision Research, 37(19), 2661–2673. https://doi.org/10.1016/S0042-6989(97)00114-4
- Seidemann, A., & Schaeffel, F. (2002). Effects of longitudinal chromatic aberration on accommodation and emmetropization. Vision Research, 42(21), 2409–2417. https://doi.org/10.1016/s0042-6989(02)00262-6
- Seifert, M., Baden, T., & Osorio, D. (2020). The retinal basis of vision in chicken. Seminars in Cell & Developmental Biology, 106, 106–115. https://doi.org/10.1016/j.semcdb.2020.03.011
- Shapley, R. (1990). Visual Sensitivity And Parallel Retinocortical Channels. Annual Review of Psychology, 41, 635–658. https://doi.org/10.1146/annurev.ps.41.020190.003223
- Shapley, R., & Hugh Perry, V. (1986). Cat and monkey retinal ganglion cells and their visual functional roles. Trends in Neurosciences, 9, 229–235. https://doi.org/10.1016/0166-2236(86)90064-0
- Smith, R. L., Nishimura, Y., & Raviola, G. (1985). Interreceptor junction in the double cone of the chicken retina. Journal of Submicroscopic Cytology, 17(2), 183–186.
- Stavenga, D. G., & Wilts, B. D. (2014). Oil droplets of bird eyes: Microlenses acting as spectral filters. Philosophical Transactions of the Royal Society B: Biological Sciences, 369(1636), 20130041. https://doi.org/10.1098/rstb.2013.0041
- Stockton, R. A., & Slaughter, M. M. (1989). B-wave of the electroretinogram. A reflection of ON bipolar cell activity. Journal of General Physiology, 93(1), 101–122. https://doi.org/10.1085/jgp.93.1.101

- Sun, H., & Crossland, W. j. (2000). Quantitative assessment of localization and colocalization of glutamate, aspartate, glycine, and GABA immunoreactivity in the chick retina. The Anatomical Record, 260(2), 158–179. https://doi.org/10.1002/1097-0185(20001001)260:2<158::AID-AR60>3.0.CO;2-V
- Sun, H.-J., & Frost, B. J. (1997). Motion processing in pigeon tectum: Equiluminant chromatic mechanisms. Experimental Brain Research, 116(3), 434–444. https://doi.org/10.1007/PL00005771
- Tran, N., Chiu, S., Tian, Y., & Wildsoet, C. F. (2008). The significance of retinal image contrast and spatial frequency composition for eye growth modulation in young chicks. Vision Research, 48(15), 1655–1662. https://doi.org/10.1016/j.visres.2008.03.022
- Usui, T., Kremers, J., Sharpe, L. T., & Zrenner, E. (1998). Flicker cone electroretinogram in dichromats and trichromats. Vision Research, 38(21), 3391–3396. https://doi.org/10.1016/s0042-6989(97)00466-5
- v. Campenhausen, M., & Kirschfeld, K. (1998). Spectral sensitivity of the accessory optic system of the pigeon. Journal of Comparative Physiology A, 183(1), 1–6. https://doi.org/10.1007/s003590050229
- Valberg, A., Lee, B. B., Kaiser, P. K., & Kremers, J. (1992). Responses of macaque ganglion cells to movement of chromatic borders. The Journal of Physiology, 458(1), 579–602. https://doi.org/10.1113/jphysiol.1992.sp019435
- Viswanathan, S., Frishman, L. J., & Robson, J. G. (2002). Inner-retinal contributions to the photopic sinusoidal flicker electroretinogram of macaques. Documenta Ophthalmologica, 105(2), 223–242. https://doi.org/10.1023/A:1020505104334

- Viswanathan, S., Frishman, L. J., Robson, J. G., & Walters, J. W. (2001). The photopic negative response of the flash electroretinogram in primary open angle glaucoma. Investigative Ophthalmology & Visual Science, 42(2), 514–522.
- Vorobyev, M. (2003). Coloured oil droplets enhance colour discrimination. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1521), 1255–1261. https://doi.org/10.1098/rspb.2003.2381
- Vorobyev, M., & Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. Proceedings of the Royal Society of London. Series B: Biological Sciences, 265(1394), 351–358. https://doi.org/10.1098/rspb.1998.0302
- Wahlin, K. J., Hackler, L., Adler, R., & Zack, D. J. (2010). Alternative Splicing of Neuroligin and Its Protein Distribution in the Outer Plexiform Layer of the Chicken Retina. The Journal of Comparative Neurology, 518(24), 4938–4962. https://doi.org/10.1002/cne.22499
- Wallman, J. (1993). Chapter 6 Retinal control of eye growth and refraction. Progress in Retinal Research, 12, 133–153. https://doi.org/10.1016/0278-4327(93)90007-G
- Wallman, J., Turkel, J., & Trachtman, J. (1978). Extreme myopia produced by modest change in early visual experience. Science (New York, N.Y.), 201(4362), 1249–1251. https://doi.org/10.1126/science.694514
- Walls, G. L. (1944). THE VERTEBRATE EYE AND ITS ADAPTIVE RADIATION. The Journal of Nervous and Mental Disease, 100(3), 332.
- Wang, Y., & Frost, B. J. (1992). Time to collision is signalled by neurons in the nucleus rotundus of pigeons. Nature, 356(6366), 236–238. https://doi.org/10.1038/356236a0
- Wässle, H. (2004). Parallel processing in the mammalian retina. Nature Reviews Neuroscience, 5(10), 747–757. https://doi.org/10.1038/nrn1497

- Weller, C., Lindstrom, S. H., De Grip, W. J., & Wilson, M. (2009). The area centralis in the chicken retina contains efferent target amacrine cells. Visual Neuroscience, 26(2), 249–254. https://doi.org/10.1017/S0952523808080917
- Wen, G., Tarczy-Hornoch, K., McKean-Cowdin, R., Cotter, S. A., Borchert, M., Lin, J., Kim, J., Varma, R., & Multi-Ethnic Pediatric Eye Disease Study Group. (2013). Prevalence of myopia, hyperopia, and astigmatism in non-Hispanic white and Asian children: Multi-ethnic pediatric eye disease study. Ophthalmology, 120(10), 2109–2116. https://doi.org/10.1016/j.ophtha.2013.06.039
- Wilby, D., & Roberts, N. W. (2017). Optical influence of oil droplets on cone photoreceptor sensitivity. The Journal of Experimental Biology, 220(Pt 11), 1997–2004. https://doi.org/10.1242/jeb.152918
- Wilby, D., Toomey, M. B., Olsson, P., Frederiksen, R., Cornwall, M. C., Oulton, R., Kelber, A., Corbo, J. C., & Roberts, N. W. (2015). Optics of cone photoreceptors in the chicken (Gallus gallus domesticus). Journal of the Royal Society, Interface, 12(111), 20150591. https://doi.org/10.1098/rsif.2015.0591
- Wildsoet, C. F., Howland, H. C., Falconer, S., & Dick, K. (1993a). Chromatic aberration and accommodation: Their role in emmetropization in the chick. Vision Research, 33(12), 1593–1603. https://doi.org/10.1016/0042-6989(93)90026-s
- Wildsoet, C. F., Howland, H. C., Falconer, S., & Dick, K. (1993b). Chromatic aberration and accommodation: Their role in emmetropization in the chick. Vision Research, 33(12), 1593–1603. https://doi.org/10.1016/0042-6989(93)90026-S
- Wildsoet, C., & Pettigrew, J. (1988). Experimental myopia and anamalous eye growth patterns unaffected by optic nerve section in chickens: Evidence for local control of eye growth.

Undefined. https://www.semanticscholar.org/paper/Experimental-myopia-and-anamalous-eye-growth-by-in-Wildsoet-Pettigrew/75314e8ff01d8ff98bf01cd04c236da584aa2876

- Wu, J. F., Bi, H. S., Wang, S. M., Hu, Y. Y., Wu, H., Sun, W., Lu, T. L., Wang, X. R., & Jonas, J. B. (2013). Refractive error, visual acuity and causes of vision loss in children in Shandong, China. The Shandong Children Eye Study. PloS One, 8(12), e82763. https://doi.org/10.1371/journal.pone.0082763
- Wu, L. J., You, Q. S., Duan, J. L., Luo, Y. X., Liu, L. J., Li, X., Gao, Q., Zhu, H. P., He, Y., Xu, L., Jonas, J. B., Wang, W., & Guo, X. H. (2015). Prevalence and associated factors of myopia in high-school students in Beijing. PloS One, 10(3), e0120764. https://doi.org/10.1371/journal.pone.0120764
- Wyszecki, G., & Stiles, W. S. (n.d.). Concepts and Methods, Quantitative Data and Formulae,. 9.
- Yokoyama, S. (2002). Molecular evolution of color vision in vertebrates. Gene, 300(1), 69–78. https://doi.org/10.1016/S0378-1119(02)00845-4
- Yokoyama, S. (2008). Evolution of dim-light and color vision pigments. Annual Review of Genomics and Human Genetics, 9, 259–282. https://doi.org/10.1146/annurev.genom.9.081307.164228
- You, Q. S., Wu, L. J., Duan, J. L., Luo, Y. X., Liu, L. J., Li, X., Gao, Q., Wang, W., Xu, L., Jonas, J. B., & Guo, X. H. (2014). Prevalence of myopia in school children in greater Beijing: The Beijing Childhood Eye Study. Acta Ophthalmologica, 92(5), e398-406. https://doi.org/10.1111/aos.12299