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The pattern ERG in chicks – Stimulus dependence and optic nerve section

Lisa A. Ostrin[a,*,1], Vivian Choh[b,1], Christine F. Wildsoet[c,2]

[a] University of Houston College of Optometry, 4901 Calhoun, Houston, TX 77004, USA
[b] University of Waterloo, 200 University West, Waterloo, ON N2L 3G1, Canada
[c] University of California Berkeley, School of Optometry, 588 Minor Hall, Berkeley, CA 94720, USA

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ABSTRACT

The chick is widely used in studies of eye growth regulation and myopia. The aim of this study was to explore the utility of pattern (p)ERG as a tool to assess retinal function in such studies. Effects of optical defocus and diffusing blur, manipulations used to alter eye growth experimentally, were evaluated. PERGs were recorded from White-Leghorn chickens, using a checkerboard pattern, including 8 spatial frequencies (0.05–2.2 c/d SF), 13 contrast levels (1–100%), and 8 temporal reversal frequencies (0.5–20 Hz). The acute effects of defocus and diffusing blur were examined. Flash- and pERGs were also recorded from chicks that underwent monocular optic nerve section (ONS), to explore the contribution of retinal ganglion cells (RGCs). Measurements were made up to 6 weeks post-ONS, complemented with SD-OCT imaging. In normal chicks, the response to 1 Hz, 100% contrast stimuli showed positive- and negative-going waveforms at 43 ms (P1) and 75 ms (N95), respectively, with 0.06–0.1 c/d SF eliciting the largest P1 amplitudes of 21.9 ± 2.5 μV. Contrast levels above 5% yielded measurable P1 responses. Responses were transient and monophasic for 0.5–5 Hz reversal rates, with higher temporal frequencies yielding steady state responses. Defocus and diffusing blur decreased pERG amplitude across all SFs. pERG responses remained normal after ONS, despite the loss of RGCs. In conclusion, chicks show robust pERG responses, which are attenuated by defocus and diffusing blur. The pERG response is not affected by ONS, suggesting that RGCs do not contribute to the chick pERG.

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1. Introduction

The chick is an important and widely used model for studies aimed at understanding human eye growth regulation and dysregulation, including myopia (Schaeffel, Glasser, & Howland, 1988; Wallman & Winawer, 2004; Wildsoet & Schmid, 2000). Given that experimental investigations into eye growth regulation commonly use visual manipulations that alter retinal image quality, such as spatial form deprivation or optical defocus, and related evidence implicating local retinal circuits, an *in vivo* measure of retinal function, such as the electroretinogram (ERG), is a useful research tool for such studies.

The ERG is recorded at the cornea as a voltage change originating in the retina in response to visual stimulation. The components of the resulting waveform can provide information about the integrity of various retinal cell types. The simplest recording paradigm makes use of flash stimuli to record full field ERGs, with four main components generally identifiable, traceable to photoreceptors (a-wave) (Penn & Hagins, 1969), ON bipolar cells (b-wave) (Robson & Frishman, 1995), the inner retina (photopic negative response, PhNR) (Viswanathan, Frishman, Robson, Harwerth, & Smith, 1999) and the retinal pigment epithelium (c-wave) (Gallemore & Steinberg, 1990). However the pattern (p)ERG, which makes use of more complex stimuli, either alternating checkerboards or grating stimuli, is potentially more informative for investigations involving visual manipulations, offering insight into their effects on retinal activity. The response in this case comprises only nonlinear signals, as the a- and b- waves cancel (Miura, Wang, Ivers, & Frishman, 2009). In humans, the pERG response is composed of two key components, an early positive component (P50) and a later negative-going component (N95) (Berninger & Arden, 1988; Riggs, Johnson, & Schick, 1964).

Comprehensive studies to determine the origins of the pERG component waveforms have been carried out in mice

* Corresponding author.
E-mail addresses: lostrin@central.uh.edu (L.A. Ostrin), vchoh@uwaterloo.ca (V. Choh), wildsoet@berkeley.edu (C.F. Wildsoet).
[1] Present address.
[2] Present address, all work performed here.

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(Miura et al., 2009) and monkeys (Luo & Frishman, 2011). In mice, the p1 (analogous to P50 in primates), was found to originate from cells in the ON pathway while the n2 (N95 in primates) originates from cells in the OFF pathway. The pERG response is abolished with the loss of retinal ganglion cells after optic nerve crush in mice, as in other mammals and primates. For example, in cats (Mafei & Fiorentini, 1981) and monkeys (Maffei, Fiorentini, Bisti, & Hollander, 1985), the pERG response is reduced to noise level following optic nerve section and subsequent retinal ganglion cell degeneration. In monkeys, non-spiking cells in the ON pathway appear to contribute to the P50 response, in addition to retinal ganglion cells (Luo & Frishman, 2011).

Despite the potential utility of the pERG for studies of eye growth regulation in chicks, a systematic study to determine the characteristics and origins of the pERG response in the chick is lacking. The pERG has been measured previously in a number of other avian species. In one study, investigators used pERGs as a tool to evaluate the contrast sensitivity function (CSF) of six species, including the starling, barn owl, wood-pecker, pigeon, quail and kestrel (Zhao, Wang, Chen, Xu, & Jonas, 2013). In relation to studies into the origin of the pERG components in birds, the pigeon has been most extensively studied (Bagnoli, Porciatti, Francesconi, & Barsellotti, 1984; Blondeau, Lamarche, Lafond, & Brunette, 1987; Blondeau, Olivier, Brunette, Zaharia, & Lafond, 1986; Porciatti, Francesconi, & Bagnoli, 1985; Vaegan & Arden, 1987). Results showed that, in contrast to findings in primates and other mammals, the pigeon pERG response is not dependent on the functional integrity of retinal ganglion cells, as evident by pERG amplitudes in optic nerve section eyes comparable to control eyes up to nine months following surgery.

The aim of the study described here was to characterize the chick pERG in terms of spatial and temporal frequency, as well as contrast-dependence, with optic nerve section (ONS) used as a lesioning tool to eliminate retinal ganglion cells to investigate their contribution to the pERG response. Additional testing specifically evaluated the acute effects of optical defocus and diffusing blur, which are the two most common types of visual stimulus manipulations used in myopia research.

2. Materials and methods

2.1. Animals

Forty-two White-Leghorn chickens were used in this study, with an age range spanning 2 days to 6 weeks. Chicks were housed in a 12-h light/dark cycle, with food and water available *ad libitum*. Methods were approved by the UC Berkeley Animal Care and Use Committee and conformed to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

For all ERG recordings, chicks were anesthetized with 60 mg/kg ketamine and 4 mg/kg xylazine and placed prone on a water-filled heat mat at 40° C. Eyelids were held open with a speculum. The depth of anesthesia was compatible with continued action of the nictitating membrane during the procedure, thereby preventing corneal dehydration. DTL electrodes were placed along the lower lid margin, in contact with the cornea. The reference and ground electrodes were platinum needles, placed subcutaneously in the dorsal neck and thigh skin, respectively, as described previously (Rymer et al., 2007). Stimuli were generated and recorded using an Espion system (Diagnosys, Ltd, Lowell MA, USA). Protocols were adapted from the guidelines of the International Society for Clinical Electrophysiology of Vision (Bach et al., 2013, McCulloch et al., 2015).

2.2. Flash ERGs

Photopic flash ERGs were recorded binocularly in 8 normal White-Leghorn chicks (aged 2–3 weeks), using two colorburst ganzfeld stimulators (Diagnosys Ltd, Lowell MA, USA) placed directly in front of the undilated eyes. Full field ERG parameters used a flash luminance of 3.0 cd.s.m-2 and 1 s inter-stimulus interval, against a background illuminance of 25 cd.m-2. The responses to twenty flashes were averaged and subsequently analyzed. Sampling frequency was 1000 Hz, with a pre-trigger time of 10 ms and post-trigger time of 500 ms. Data were exported to excel and peaks for each response were manually marked and averaged. Amplitudes and implicit times of the a- and b-waves are reported. The a-wave amplitude was measured from the origin to the trough of the initial negative-going wave. The b-wave amplitude was measured from the trough of the a-wave to the following positive peak. Oscillatory potentials were isolated with a band pass filter of 50–300 Hz, as used previously (Frishman et al., 1996, Miura et al., 2009). Scotopic recordings were not undertaken because rods are known to be nonfunctional in chickens during the day (Schaeffel, Rohrer, Lemmer, & Zrenner, 1991).

2.3. pERG in normal animals

Monocular pERGs were recorded from 18 normal White-Leghorn chicks (aged 2–3 weeks), using an alternating checkerboard pattern stimulus displayed on a 36 × 27 cm cathode ray tube (CRT) monitor, which subtended an angle of ~75° at the eye (Espion, Diagnosys Ltd, Lowell MA, USA). The mean luminance of the display was 30.1 cd/m2 for a uniform field (IL1700, International Light Technologies, MA, USA). The chicks were positioned to visually align the line of site from the area centralis of the eye being recorded with the center of the stimulus display. Eyes were not cyclopleged and pupils were not dilated.

The various stimulus conditions tested are summarized in Table 1. The spatial frequency (SF) threshold was determined using an alternating 4 Hz checkerboard pattern at 100% contrast. Recordings were obtained for eight SFs, starting with the lowest frequency, 0.05 c/d (largest pattern), and increasing to 2.2 c/d, with 100 reversals per SF. Contrast sensitivity was tested using a reversal rate of 4 Hz, for only one, mid-range SF, a 0.1 c/d pattern; recordings were obtained for 13 contrast levels, ranging from 1 and

Table 1
pERG stimulus parameters used for each condition tested.

Condition	Test	Lens	Contrast (%)	Temporal Frequency (Hz)	Spatial Frequency (c/d)
Normal	Spatial frequency threshold	None	100	4	0.05, 0.63, 0.10, 0.18, 0.54, 0.63, 1.1, 2.2
Normal	Contrast sensitivity	None	1, 2.5, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100	4	0.1
Normal	Temporal frequency threshold	None	100	0.5, 1, 2, 3, 4, 5, 10, 20	0.1
Normal	Effects of defocus	−10, −5, +5, +10 D	100	4	0.05, 0.63, 0.10, 0.18, 0.54, 0.63, 1.1, 2.2
Normal	Effects of diffusing blur	0.6, 0.2, <0.1, LP Bangerter filters	100	4	0.05, 0.63, 0.10, 0.18, 0.54, 0.63, 1.1, 2.2
ONS	Effects of ganglion cell loss	None	100	4	0.05, 0.63, 0.10, 0.18, 0.54, 0.63, 1.1, 2.2

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