Visual Acuity of the Midland Banded Water Snake Estimated from Evoked Telencephalic Potentials

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Running title: snake visual acuity

Abstract

The visual acuity of seven midland banded water snakes was measured by recording evoked responses from telencephalon to temporally modulated square wave grating patterns. Using conventional electrophysiological techniques and signal averaging, high contrast square wave gratings of different spatial frequencies were presented. Acuity was estimated by extrapolating relative response amplitude / $\log_{10}$ spatial frequency functions which yielded an average acuity of 4.25 cycles/deg. Refractive state was also estimated by recording evoked potentials to intermediate spatial frequencies with different lenses in front of the eye. Polynomial fits indicated that under the experimental conditions the snakes were around 6.4 diopters hyperopic suggesting a corrected acuity of 4.89 cycles/deg. Reduction of grating luminance resulted in a reduction in evoked potential acuity measurements. These results indicate that the spatial resolution of midland banded water snakes is the equal of cat; about 20/120 in human clinical terms.

Key words: snake, vision, acuity, evoked potential

Introduction

Snakes are commonly said to see poorly yet we know of few systematic studies of their vision (Jacobs, et al, 1992; but see Sillman, et al., 1997). Thought to have evolved from lizards under the selective pressures of a burrowing life style, snakes subsequently emerged from this fossorial existence to occupy virtually every major ecological niche (Bellairs and Underwood, 1951; Apesteguia and Zaher, 2006). Several differences between the visual systems of snakes and lizards are thought to reflect this period of subterranean living followed by a return to the surface and a renewed selective advantage for sight. Walls (1942), for example, notes that while lizard retinas are dominated by cone photoreceptors within snakes a wide range of rod/cone ratios can be found. Significant differences in the primary visual system have also been noted between snakes and lizards (Repérant, et al., 1992). The dorsal geniculate complex is more developed in snakes while the superficial layers of the optic tectum are regarded as impoverished.

Considering the unique evolutionary history of snakes, and its conjectured manifestations in visual system structure, interesting structure/function questions suggest themselves. For example, do similar rod/cone photoreceptor populations in species of snakes and lizards result in similar sensitivity under different levels of illumination? Do the impoverished superficial layers of optic tectum and hypertrophied dorsal geniculate complex in snakes produce any visual disabilities, or abilities, in comparison to lizard species with similar ecology?

We chose to begin with an investigation of spatial vision by recording visually evoked potentials from the telencephalon of banded water snakes. In humans, cats and dogs, acuity estimates based upon evoked potentials from primary visual cortex to grating

stimuli have been shown to coincide quite closely with behavior measures of acuity (Campbell and Maffei, 1970; Berkley and Watkins, 1973; Murphy, et al., 1997). In addition, visually evoked potentials have also been used to estimate the acuity of two turtle species. The freshwater red-eared slider (*Pseudemys scripta elegans)* had an average visual acuity of 6.1 cycles/deg (Northmore and Granda, 1991) and the loggerhead sea turtle (*Caretta caretta*) an average acuity of 5.6cycles/deg (Bartol, et al., 2001). Dudziak (1955, in Northmore and Granda) reported behavioral acuity as high as 10cycles/deg in the freshwater turtle *Emys orbicularis* which Northmore and Granda (1991) noted corresponded to the intercone spacing in red-eared slider and the upper range of their evoked potential acuity estimates.

We selected water snakes for study because they are widely distributed, use vision to both feed (Drummond, 1985) and flee, their refractive state has been studied (Schaeffel, 1991), they are active both day and night and are common in local streams.

Method

Subjects

Midland banded water snakes (*Nerodia sipedon pleuralis)*, collected in Birmingham, AL from Shades Creek between the Elder St. and Groover Dr. bridges (weights 23 to 212 grams), were studied. The snakes were maintained around 22° C on a 12hr light cycle in 38 liter aquaria with free access to drinking water and monthly feedings of fish. No experiments were conducted on snakes during their shed cycle.

Visual stimulation and signal processing

The visual stimuli were black/white vertical square wave gratings presented on a 43 by 30 cm KDS video monitor located 40 cm from the snake so that the stimulus display occupied a visual angle of 56deg by 41deg. The mean luminance of the display was 34cd/m^2 and the Michelson contrast of the gratings was maintained at 0.96.

The spatial frequency of the gratings was computer controlled and ranged from 0.038 to 5.09 cycles / deg. When no grating was present the screen was at 0.0 contrast and 34 cd/m^2. A stimulus epoch lasted 3sec and consisted of the grating appearing (time 0) followed by instantaneous one-half cycle displacement at 1sec and 2sec. Thus the first contrast modulation was off-on and the next two counter-phased. A stimulus epoch repeated every 0.5sec. Evoked potentials were generally recorded from over the snake's telencephalon and referenced to a subdermal site in the upper neck using Ag-AgCl electrodes (E215P probe & E255-A wire, In Vivo Metric, Healdsburg, CA). Neural signals were amplified, low/high pass filtered at 0.3-300hz, displayed on a digital oscilloscope and averaged by a laboratory computer for subsequent analysis. Responses to around 20-30 stimulus epochs were averaged for each stimulus condition. Evoked potential amplitudes were measured from baseline to the peak of the response.

Procedures

Snakes were initially anesthetized in a 1.89 liter clear plastic container with 0.5cc of isoflurane. Once the righting reflex disappeared (around 12-15min) the snake was quickly

intubated with a 2in 18 gage IV catheter which was connected by a 1in length of tubing to a Versaflex breathing device (EZ -104, Euthanex Corp, Palmer, PA). Once stabilized with 5% isoflurane in 0.5 liter/min of O_2, isoflurane was reduced to 3% for evoked potential recording. Isoflurane has been used in conjunction with sensory stimulation for both mammals and reptiles (Hartikainen and Rorarius, 1999; Moon and Terashima, 2002; Goris, et al., 2003; but see Rojas, et al., 2006) Expired gases were vented to a charcoal filter canister (EZ-250).

The snake was upon a wooden platform with its head positioned over a 3 x 3in hole up through which the anesthesia tube was double taped to its open lower jaw. A wooden craft stick, under its upper jaw, spanned the hole and a few pieces of medical tape positioned the snake on the platform. An electrode carrier from a rat stereotaxic was attached to the platform and located to the right of the snake's head. The platform itself was atop a 13in high plastic cylinder mounted upon a turntable such that the snake's orientation to the stimulus could be varied without changing the distance to the stimulus screen. A lens / filter holder was used to position a refraction lens, or neutral density filter, in front of the snake's left eye.

Initial experiments recorded from the dura surface through a small hole in the skull. Subsequently we found that very satisfactory potentials could be recorded from the surface of the skull overlying telencephalon after simply removing a small flap of skin.

During an experimental session an acuity experiment was conducted first by presenting stimulus epochs of different spatial frequencies ranging from 0.038 to 5.09 cycles / deg. The spatial frequency sequence of the stimuli was interleaved between epochs and average responses were based upon 20-30 epochs / spatial frequency. The

initial acuity experiment was often followed by a refraction experiment which entailed recording evoked potentials to an intermediate spatial frequency (0.66 or 1.27 cycles / deg) with different clinical trial lenses placed 1.0 cm in front of the snakes left eye and the snake oriented at 45 deg to the stimulus screen.

Most recording sessions involved several acuity experiments. The most frequent acuity experiments were conducted with the snake oriented at 45 deg to the stimulus (n=8) as this angle was estimated to put the plane of the pupil parallel to the stimulus display. Four acuity experiments were conducted with the snake oriented directly at the stimulus screen so as to allow binocular stimulation.

To investigate the effect of stimulus luminance upon acuity, three acuity experiments were conducted on one snake with neutral density filters (ND 1.8, 1.1, and 0.63) positioned in front of the snake's eye and the snake oriented at 45 deg to the stimulus screen. These luminance acuities were conducted following 10min of no stimulation with the ND 1.8 filter in front of the snake's eye. ND 1.8 testing was followed by testing with ND 1.1 and ND 0.63 filters.

The snake was either oriented at rightward 45 deg (favoring left eye stimulation) or 0 deg to the stimulus screen and we usually recorded from over the right telencephalon (n= 9 experiments) but occasionally over the left telencephalon (n= 4 experiments). One acuity experiment was conducted recording over the left optic tectum with the snake oriented at 0 deg. Recording sites were just lateral of the midline and located as multiples of the distance between the snake's snot and middle of the pupil (range 8.5mm to 12mm); telencephalon: 1.5, optic tectum: 1.75. These locations agree well with Figure 1 of Northcutt and Butler (1974).

Results

Acuity

Figure 1 presents the averaged potentials comprising an acuity series

 recorded from the right telencephalon with the snake oriented at 45 deg to the stimulus

screen and a 2.0 diopter lens in front of the snake's eye. Typical of the potentials we

recorded are a) decreasing amplitude with increasing spatial frequency, b) the first

potential (a response to the gratings presentation from 0.0 contrast) is smaller than the

next two (both responses to half cycle displacements of the grating) and c) there are

generally two peaks in each response.

All analysis was based upon the average of the second and third responses

because both were to grating counter-phase and were always larger than the first

response. Relative amplitude was derived by taking the largest potential in any

experiment as 1.0. Figure 2 illustrates the relative evoked potential amplitude as a

function of

grating spatial frequency for both the potentials illustrated in Figure 1 (using a 2.0 diopter

lens - squares) and using a plano (0.0 diopter - diamonds) lens. The regression lines were

extrapolated to zero on the spatial frequency axis and correspond to acuity estimates of

7.91 cycles/deg with the 2.0 diopter lens and 4.77 cycles/deg with the plano lens; the correlation coefficients (r^2) were 0.92 and 0.96 respectively.

Most acuity experiments were conducted with no refractive correction because data were analyzed later, there was a relatively small effect of focus upon evoked potential amplitude and an appropriate refractive correction (see below) could be applied post hoc. The average of these acuity experiments, with a stimulus orientation of 45 deg, was 4.25±1.7sd cycles/deg with an average $r^2 = 0.95±0.04$sd (n=8). When a snake was oriented at 0 deg to the stimulus the average acuity was 7.89±7.8 cycles/deg with an average $r^2 =0.94±0.05$sd (n=4). The difference in snake orientation to the stimulus screen was not statistically significant (one-tailed, t = -1.23, df =10, p = 0.12). Acuities obtained from the left telencephalon (n=2) fell within the range of those obtained from the right telencephalon (n=9) as did the acuity obtained from optic tectum (n=1).

Refraction

Figure 3 presents the results of one refraction experiment and illustrates relative evoked

Insert Figure 3 about here

 potential amplitude, for a 1.27 cycle/deg grating, as a function of lens focus. The data points are fit to a polynomial equation (y = -0.0037x^2 + 0.0788x + 0.5464; $r^2 = 0.90$) and indicate that a maximum amplitude evoked potential would be obtained with a 10.5 diopter lens in front of the snake's eye, i.e. the snake is 10.5 diopters hyperopic in the experimental situation. Four refraction experiments were conducted and the average 'best' refraction was 5.75 diopters (average $r^2 = 0.81±0.12$sd) based upon the average of

the individual polynomial fits. Another approach was based upon a polynomial computed

by averaging the terms of the individual polynomials (y = -0.0028x^2 + 0.0354x + 0.731)

and indicated 6.5 diopters as the 'best' lens.

Effect of luminance upon acuity

Figure 4 presents the effect of luminance upon acuity with the snake oriented at 45

Insert Figure 4 about here

deg to the stimulus screen. The three lowest luminance (0.54, 2.7 and 8 cd/m^2) acuities

were derived from individual relative evoked potential amplitude/spatial frequency

functions (average r^2 = 0.97±0.03sd) conducted with one snake while the value for 34

cd/m^2 was taken from the average of all other acuities functions under these conditions (n

= 8). As can be seen acuity was highest at 34 cd/m^2 and declines as mean luminance was

reduced. The solid line is a least square regression while the dashed line simply connects

the individual data points.

Discussion

Acuity

Our most common acuity experiments were with the snake oriented at 45 deg to the

stimulus screen, with no corrective lens in place, and yielded a mean acuity of 4.25

cycles/deg. While the differences between orientations of 45 deg and 0 deg were not

statistically significant, the two highest acuities observed were with 0 deg orientation: 18.8 cycles/deg from left telencephalon and 14.2 cycles/deg from left optic tectum. We are at a loss to explain the high variability in the 0 deg orientation acuity measurements. While it might be thought that a pronounced retinal specialization could produce this variability, because the visual display subtended 56deg by 41 deg, we think this unlikely.

The mean acuity of 4.25 cycles/deg is without refraction. However the average polynomial fit of the four refraction experiments indicated a largest evoked potential response using a 6.4 diopter lens and a 15% smaller response without refraction. These estimates suggest a correction of 1.15 should be applied when no refraction was used bringing the mean acuity to 4.89 cycles/deg. In the one acuity experiment we used a corrective lens (2.0 diopters) and the acuity was improved from 4.77 to 7.91 cycles/deg (i.e. Figure 2). The refraction experiment from this session (i.e. Figure 3) suggested that a 2.0 diopter lens would increase uncorrected acuity from 4.77 to 6.07 cycles/deg.

A water snake visual acuity of approximately 5 cycles/degree can be compared with the turtle acuities estimated from evoked potentials. Recording from optic tectum Northmore and Granda (1991) and Bartol et al (2001) reported average acuities of 6.12 and 5.6 cycles/deg respectively. While these reptile acuities do not match Old World primates (around 30 cycles/deg) they are, as Northmore and Granda noted "..good acuity for a small eye.." and compare favorably to some well studied non-primate mammals such as rat at 1.0 cycles/deg (Prusky, et al., 2000) and cat at 6.5 to2.5 cycles/deg (Berkley and Watkins, 1973; Pasternak and Merigan, 1981). In human clinical terms the acuity of the midland banded water snake is approximately 20/120.

The acuity of the midland banded water snake is good for a small eye, and better than some mammals with larger eyes. However we suspect that some other more visual snakes with larger eyes may have even better acuities. In this regard racers (*Coluber*) and coachwhips *(Masticophis*) come to mind.

Refraction

Relevant to the study at hand are the refractive measurements of Sivak (1977). Measurements were made with a retinoscope, under both unanaesthetized and ether anaesthetized conditions, from black racer (*Coluber constrictor)*, yellow rat snake *(Elaphe quadravittata)* and red rat snake (*Elaphe guttata)*. Sivak's (1977) mean unanaesthetized refraction was 0.44 diopters while the mean anaesthetized refraction was 9.6 diopters. In the present study with anaesthetized (isoflurane) water snakes, the mean refraction was 5.75 to 6.5 diopters and was obtained from evoked potential measurements. The fair agreement between Sivak (1977) and our anaesthetized refractive state estimates is complicated by the fact that retinoscopy systematically yields excess hyperopic values for small eyes. This small eye effect in retinoscopy, with an eye diameter of about 4mm, should produce an error of about 10 diopters (Glickstein and Millodot, 1970; Schaeffel and De Queiroz, 1990). However Glickstein and Millodot (1970) also found no effect of anesthesia upon retinoscopy measurements so the small-eye retinoscopy effect should have also affected Sivak's unanaesthetized measurements. A somewhat clearer picture emerges from Northmore and Granda (1991) who directly compared evoked potential and retinoscopically determined refractions in turtle.

Retinoscopy indicated about 4.8 diopters of hyperopia, in keeping with the small-eye effect. The evoked potential estimates suggesting the turtles were, on average, free of refractive error. Using infrared retinoscopy and three species of European water snakes, Schaeffel (1991) found about 10.0 diopters of hyperopia and concluded, considering the effect of eye size, these snakes likely properly focused in air. The results of the study at hand indicate that, under isoflurane anesthesia with a viewing distance of 40cm, the snakes were around 6 diopters hyperopic. However, because the equivalent power of snake eyes is high, well over 150 diopters based upon the relevant measurements of Sivak (1977), small refractive errors should produce little effect upon spatial resolution.

Effect of luminance upon acuity

Figure 4 illustrates the effect of mean luminance upon acuity. This result is preliminary as the data for the three lowest luminance levels are based upon one snake while the data for $34 cd/m^2$ are based upon all other snakes (n=6). The solid line is a linear regression fit to the data points and indicates a decline in spatial resolution as luminance is reduced. The dashed line, on the other hand, simply connects the data points but may suggest a branched effect of luminance upon acuity which is generally attributed to rod and cone function in many species including humans (Shaer, 1937) and cats (Pasternak and Merigan, 1981). If this branched interpretation is correct, our acuity estimate of around 5 cycles/degree at $34 cd/m^2$ is based upon solid cone function as the level section of such curves is typically taken as mesopic illumination of mixed rod and cone function.

Acknowledgements

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Figure captions

Figure 1: Evoked potentials recorded from the telencephalon in response to square-wave gratings of different spatial frequencies with a 2.0 diopter lens. X-axis is time (msec) from start of stimulus epoch; y-axis is computer referenced voltage. (prep. WS 7-25-06).

Figure 2: Relative evoked potential amplitude as a function of grating spatial frequency with a plano lens (diamonds) and a 2.0 diopter lens (squares). Snake was oriented at 45 deg to the stimulus screen. Extrapolation of the least squares lines to the x – axis indicated acuities of 4.7 cycles/deg (plano lens) and 7.9 cycles/deg (2.0 diopter lens) (prep. WS 7-25-06).

Figure 3: Relative evoked potential amplitude as a function of refraction lens power for a spatial frequency of 1.2 cycles/deg. Function peak corresponds to a 'best' lens of 10.5 diopters (prep. WS 7-25-06).

Figure 4: Evoked potential estimated acuity as a function of stimulus luminance. The solid straight line is a least squares regression while the dashed line directly connects the data points (prep WS 7-28-06).
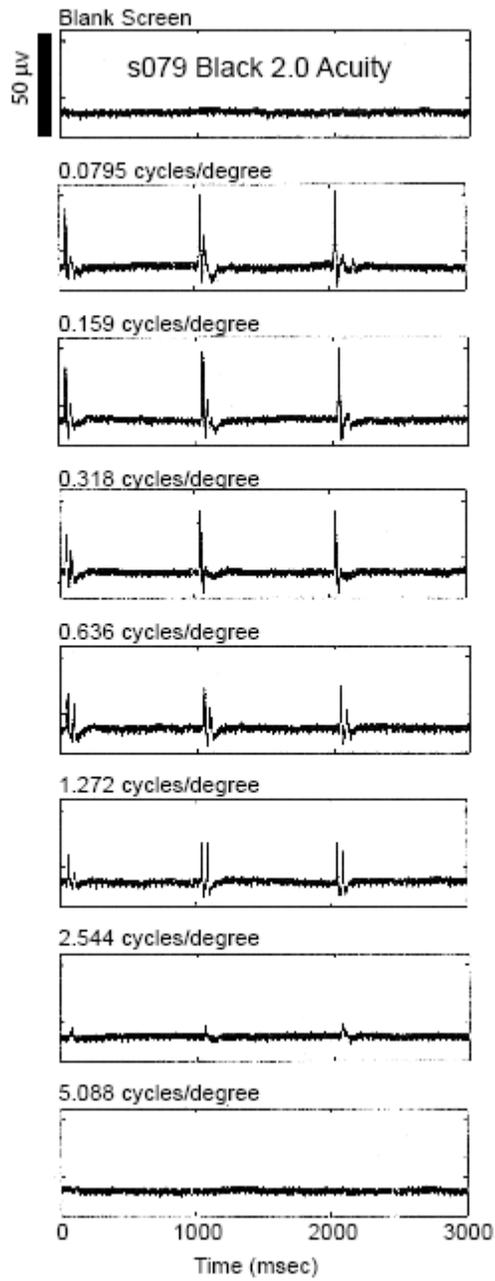
FIGURE 1- Baker, Gawne, Loop and Pullman
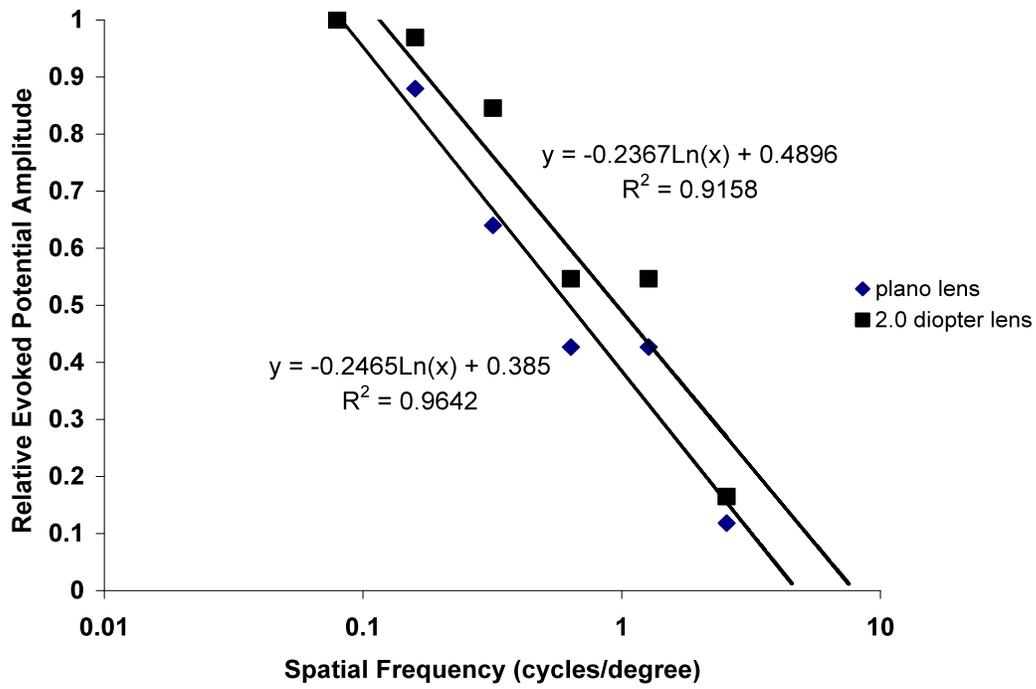
Figure 2 - Baker, Gawne, Loop and Pullman

$y = -0.2367Ln(x) + 0.4896$
$R^2 = 0.9158$

$y = -0.2465Ln(x) + 0.385$
$R^2 = 0.9642$

◆ plano lens
■ 2.0 diopter lens

Relative Evoked Potential Amplitude

Spatial Frequency (cycles/degree)

FIGURE 3 - Baker, Gawne, Loop and Pullman

$y = -0.0037x^2 + 0.0788x + 0.5464$
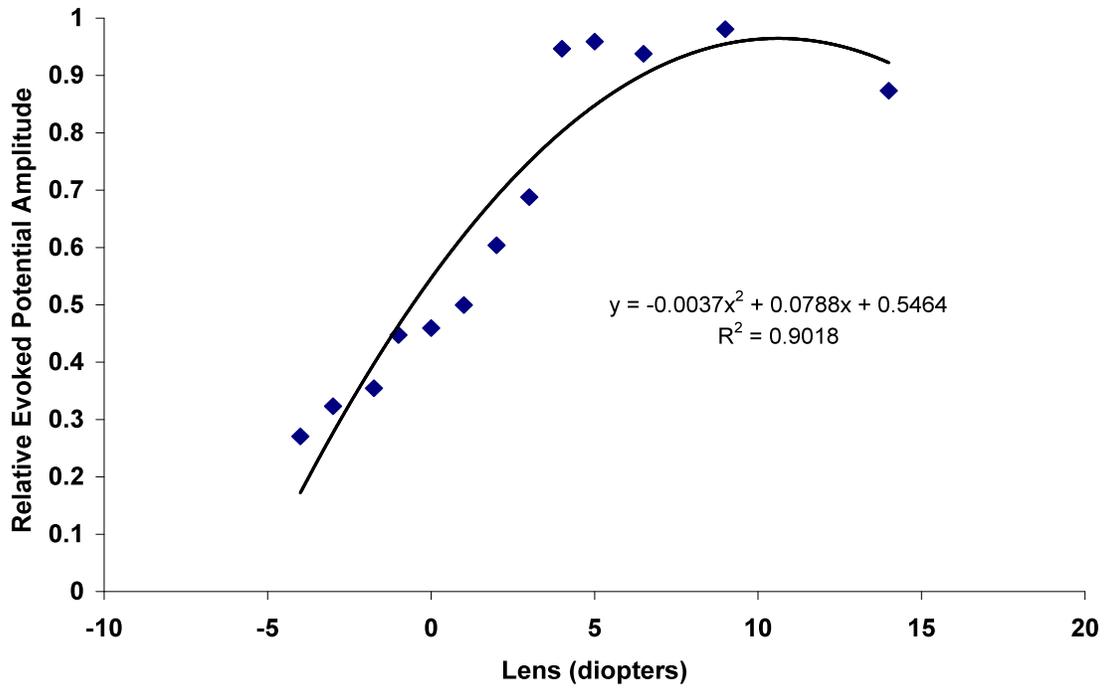$R^2 = 0.9018$

X-axis: Lens (diopters)
Y-axis: Relative Evoked Potential Amplitude

FIGURE 4 - Baker, Gawne, Loop and Pullman

$y = 0.5519 \text{Ln}(x) + 1.8721$

Acuity (cycles/degree)

Grating Luminance (cd/m^2)