UNIVERSITY of York

This is a repository copy of *Steady-State Contrast Response Functions Provide a Sensitive and Objective Index of Amblyopic Deficits*.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/103307/

Version: Published Version

---

**Article:**
Baker, Daniel H. orcid.org/0000-0002-0161-443X, Simard, Mathieu, Saint-Amour, Dave et al. (1 more author) (2015) Steady-State Contrast Response Functions Provide a Sensitive and Objective Index of Amblyopic Deficits. Investigative ophthalmology & visual science. pp. 1208-1216. ISSN 0146-0404

https://doi.org/10.1167/iovs.14-15611

---

**Reuse**
This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here: https://creativecommons.org/licenses/

**Takedown**
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

White Rose university consortium
Universities of Leeds, Sheffield & York

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

# Steady-State Contrast Response Functions Provide a Sensitive and Objective Index of Amblyopic Deficits

Daniel H. Baker,[1] Mathieu Simard,[2] Dave Saint-Amour,[2] and Robert F. Hess[3]

[1]Department of Psychology, University of York, Heslington, York, United Kingdom
[2]Department of Psychology, Université du Québec à Montréal, Montréal, Canada
[3]McGill Vision Research, Department of Ophthalmology, McGill University, Montréal, Canada

Correspondence: Daniel H. Baker, Department of Psychology, University of York, Heslington, York, YO10 5DD, UK;
daniel.baker@york.ac.uk.

Submitted: September 4, 2014
Accepted: January 23, 2015

Citation: Baker DH, Simard M, Saint-Amour D, Hess RF. Steady-state contrast response functions provide a sensitive and objective index of amblyopic deficits. *Invest Ophthalmol Vis Sci*. 2015;56:1208–1216.
DOI:10.1167/iovs.14-15611

**Purpose.** Visual deficits in amblyopia are neural in origin, yet are difficult to characterize with functional magnetic resonance imagery (fMRI). Our aim was to develop an objective electroencephalography (EEG) paradigm that can be used to provide a clinically useful index of amblyopic deficits.

**Methods.** We used steady-state visual evoked potentials (SSVEPs) to measure full contrast response functions in both amblyopic ($n = 10$, strabismic or mixed amblyopia, mean age: 44 years) and control ($n = 5$, mean age: 31 years) observers, both with and without a dichoptic mask.

**Results.** At the highest target contrast, the ratio of amplitudes across the weaker and stronger eyes was highly correlated ($r = 0.76$) with the acuity ratio between the eyes. We also found that the contrast response function in the amblyopic eye had both a greatly reduced amplitude and a shallower slope, but that surprisingly dichoptic masking was weaker than in controls. The results were compared with the predictions of a computational model of amblyopia and suggest a modification to the model whereby excitatory (but not suppressive) signals are attenuated in the amblyopic eye.

**Conclusions.** We suggest that SSVEPs offer a sensitive and objective measure of the ocular imbalance in amblyopia and could be used to assess the efficacy of amblyopia therapies currently under development.

Keywords: amblyopia, steady-state EEG, dichoptic masking, contrast

The defining characteristic of amblyopia is a loss of visual sensitivity in one eye that is neural, rather than optical, in nature (although in some cases optical anisometropia is the causal factor, deficits persist following correction). This manifests as a reduction of visual acuity and contrast sensitivity, as well as deficits in higher-level tasks such as motion perception. Because amblyopia is typically unilateral, stereopsis rarely develops normally, and most amblyopes lack functional binocular vision.

Recently, several methods have been proposed for treating amblyopia in adults, and children beyond the "critical period" (until approximately the age of 8 years) during which traditional patching therapy is effective. These methods include perceptual learning,[1] electrical[2] or magnetic[3,4] brain stimulation at the scalp, monocular game-based methods,[5,6] and dichoptic game-based approaches designed to provided a balanced binocular input.[7] Although several treatments have shown promise, their efficacy is typically assessed by using psychophysical (behavioral) tasks, which might improve with practice and are prone to motivational and response bias effects. A more objective index of visual improvement is clearly required to evaluate treatments in large-scale clinical trials.

One possible objective measure is the functional magnetic resonance imagery (fMRI) blood-oxygen-level dependent (BOLD) response. Previous studies have reported lower amplitudes and greater latencies for the BOLD response in the amblyopic eye, compared with the fellow eye.[8–13] Yet the amplitude reduction is surprisingly mild for even quite severe amblyopes.[10] Furthermore, MRI equipment is expensive and not generally available to clinical practitioners outside of major facilities.

Measuring neural activity at the scalp using electroencephalography (EEG) offers an alternative objective measure that is cheaper and more straightforward to implement. Several studies have reported abnormalities in the amplitude and latency of visual evoked potentials (VEPs) such as the P100 and N170 for stimuli shown in the amblyopic eye.[14–18] Other studies have used steady-state visual evoked potentials (SSVEPs) to compare the amplitude of normal and amblyopic responses to flickering stimuli.[19–21] However, such work has typically measured responses at only a single contrast level, which is insufficient to provide a complete picture of the amblyopic deficit for contrast processing. Ideally, full contrast response functions should be measured for each eye to obtain a better estimate of the amblyopic deficit. This could be repeated with a dichoptic mask in the untested eye, to permit measurement of suppression between the eyes.

## Testing a Model of Amblyopia

In particular, empirical contrast response functions can be compared with the predictions of computational models of amblyopia. Baker et al.[22] proposed a model of contrast vision in amblyopia in which the response to stimuli in the amblyopic eye is attenuated before binocular summation and inhibition. This

Copyright 2015 The Association for Research in Vision and Ophthalmology, Inc.

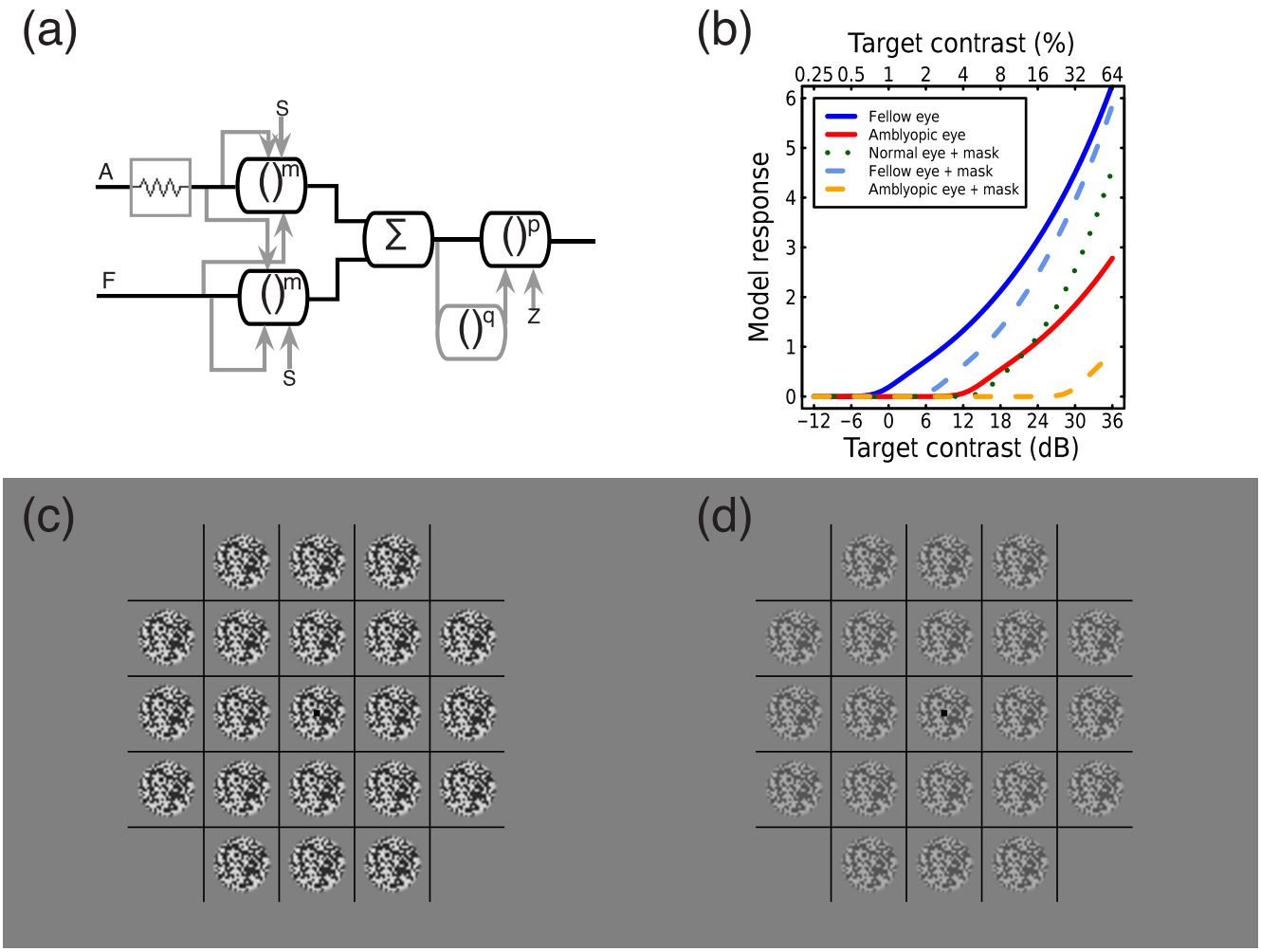


**Figure 1.** Model architecture, predictions, and example stimuli. The model diagram shown in (**a**) illustrates the model of Baker et al.[22] described in the text. (**b**) Model predictions for contrast response functions for several ocular combinations of mask and target. The orientation of the grids in (**c**, **d**) were varied randomly from trial to trial to minimize local adaptation.

architecture (Fig. 1a) is able to account for the pattern of contrast discrimination functions obtained from strabismic amblyopes, and related models have proved successful in other paradigms.[23]

The model makes predictions (see Methods section for details) about the pattern of contrast response functions that should be obtained when using steady-state EEG. As shown in Figure 1b, the model predicts a monocular response that increases monotonically with contrast (solid blue curve). The attenuator in the amblyopic eye reduces this response (solid red curve), effectively shifting the curve to the right. In normal observers (with no attenuation), a dichoptic mask shown to the nontarget eye also produces a rightward shift of the contrast response function (dotted green curve). This is a classic contrast gain control effect that resembles closely those measured with overlaid masks in both single- and multiunit electrophysiology,[24,25] and steady-state EEG[26,27] studies. In amblyopia, the attenuator reduces the suppressive effect from the amblyopic onto the fellow eye (dashed light blue curve is to the left of the green dotted curve) and increases the suppressive effect from the fellow onto the amblyopic eye (dashed orange curve), resulting in an imbalance of binocular suppression.

### Aims and Objectives

Here, we test the predictions of this computational model by measuring steady-state contrast response functions in normal observers and in amblyopes. Our aim was to quantify the amblyopic deficit by using this paradigm, to provide a benchmark that might be used in future evaluations of amblyopia therapies.

## Methods

### Apparatus and Stimuli

Stimuli were patches of static white noise windowed by a raised cosine envelope to subtend 3° of visual angle. The patches were tiled in a 5 × 5 grid, with the corner patches removed to avoid cropping at some grid orientations (see Figs. 1c, 1d). To promote binocular fusion, a series of vertical and horizontal lines crossed the display in between each patch. The orientation of the grid and placement of the patches was rotated by a random amount on each trial to minimize local contrast adaptation.

Target stimuli had maximum RMS contrasts ranging from 3% to 51% in logarithmic steps. Mask stimuli had a maximum root-mean-square (RMS) contrast of 26%. Target stimuli flickered at 10 Hz and mask stimuli flickered at 12 Hz. The flicker was sinusoidal on/off flicker (e.g., the contrast varied from 0% to 100% of the maximum, and did not reverse the phase polarity of the stimuli).

**Table.** Clinical Details of Amblyopic Observers

| Observer | Age/ Sex | Amblyopia | Optical Correction | Acuity | Stereoacuity | Age Detected | Patching | Surgery |
|---|---|---|---|---|---|---|---|---|
| EL | 42/M | Strabismic, left esotropia 11° | RE: +0.50/−1.00 × 0<br>LE: −0.25/−0.50 × 120 | RE: 20/16<br>LE: 20/50 | None | Unknown | Age 6–12 y | 19 and 25 y |
| EV | 23/F | Strabismic, right exotropia 15° | RE: none<br>LE: −0.75/0.50 × 60 | RE: 20/100<br>LE: 20/20 | None | <3 y | Until age 7 y | 5 y |
| ND | 59/F | Mixed, right esotropia 5° | RE: +6.00 (+2.50)<br>LE: +3.00 (+2.50)/−1.00 × 73 | RE: 20/200<br>LE: 20/20 | None | 12 y | 6 mo | None |
| AD | 29/F | Strabismic, right esotropia 15° | RE: none<br>LE: −0.5 | RE: 20/100<br>LE: 20/20 | None | 4 y | 4 y | 7 y |
| KM | 55/F | Strabismic, right esotropia 20° | RE: plano/−0.50 × 95<br>LE: −0.25/−0.50 × 90 | RE: 20/40<br>LE: 20/20 | None | 6 mo | None | 6 mo and 4 y |
| AA | 28/M | Strabismic, right esotropia 10° | RE: none<br>LE: none | RE: 20/80<br>LE: 20/32 | None | <2 y | 4 y | 2 and 6 y |
| GH | 51/M | Mixed, left esotropia 6° | RE: −1.25/−0.50 × 30<br>LE: +2.50/−1.50 × 75 | RE: 20/32<br>LE: 20/50 | 200 s | 11 y | None | None |
| LR | 43/M | Mixed, left exotropia 10° | RE: −1.00 D<br>LE: +1.00 D | RE: 20/12.5<br>LE: 20/32 | 70 s | 6 y | 6 mo | None |
| AR | 53/M | Strabismic, small left esotropia | RE: none<br>LE: none | RE: 20/12.5<br>LE: 20/63 | None | 20 y | None | None |
| CD | 59/F | Strabismic, right esotropia 5° | RE: none<br>LE: none | RE: 20/50<br>LE: 20/16 | 200 s | 2 y | 3 y | 2 y |

LE, left eye; RE, right eye.

All stimuli were presented by using virtual reality goggles (Z800 3DVisor; eMagin Corp., Bellevue, WA, USA) driven by a DualHead2Go display adaptor (Matrox Electronic Systems Ltd., Montreal, QC, Canada). The goggles used organic light-emitting diode (OLED) displays which have a linear response and do not require gamma correction. The resolution of each display was 800 × 600 pixels with a simultaneous refresh rate of 60 Hz and a corresponding visual field of 32 × 23 degrees. One pixel on the screen subtended 2.4 minutes of arc at the eye. The mean luminance of the goggles was 60 cd/m$^2$.

We recorded EEG signals by using *Ag-AgCl* electrodes located around the occipital pole, namely, *Oz* and *POz*. The signals were amplified and digitized by using a BrainAmp (BrainProducts GmbH, Gilching, Germany) and saved for offline analysis in Matlab (MathWorks, Inc., Natick, MA, USA). The display system was synchronized with the recording computer by using an Arduino-based trigger device. We present data averaged across electrodes *Oz* and *POz* in all subsequent analyses.

## Procedure

Observers were seated in a shielded room, wearing the display goggles and EEG electrodes. Target stimuli were shown to either the left or right eye at a range of contrasts, for trials of 11 seconds with intertrial intervals of 3 seconds. On some trials, the nontarget eye viewed a blank screen showing only the fusion grid and a central fixation point. On the remainder of trials, a dichoptic mask was shown to the nontarget eye. This was spatially identical to the target but flickered at a different temporal frequency. There was no task during trials—observers were instructed to attend to the central fixation point and asked to avoid blinking during stimulus presentation. Each observer completed five blocks of the experiment (lasting approximately 10 minutes each), with opportunities to rest in between. This yielded 10 repetitions of each condition per observer.

Because we were interested in the relative orderings of the contrast response functions, and not the absolute amplitudes (which are determined in part by extraneous variables such as skull and scalp thickness, cortical folding, and electrode impedance) we normalized the data for each observer before averaging. This was done by subtracting the mean amplitude across all functions at a given temporal frequency (e.g., the target frequency of 10 Hz) and dividing by the maximum unsigned amplitude. These normalized data were used in all statistical tests. We then rescaled by the averaged normalization factors for display purposes. The phase variance data were computed by taking the angular component from the Fourier transform at the target frequency (10 Hz) and calculating its variance across trials, using circular statistics.

## Observers

Ten adult amblyopes and five adult control observers completed the experiment (a power analysis based on previous work[21] indicated that we should recruit a minimum of eight amblyopes). Clinical details of the amblyopes are given in the Table; their mean age was 44 years. The control observers had no known abnormalities of binocular vision; their mean age was 31 years. Four controls were emmetropic, the fifth had a binocular spherical correction of –0.75 diopters. Observers wore their prescribed optical correction if required, and we measured visual acuity by using a logMAR chart (at 4 meters) and converted to equivalent Snellen acuity. The stronger eye (dominant or fellow) was tested first, and we defined acuity as the lowest line where all letters were reported correctly. We assessed stereovision by using the Randot stereotest and checked binocular single vision with the Worth four dot test. All observers gave informed consent, and the research protocols were consistent with the 2008 Declaration of Helsinki.

## Details of Model Simulations

We predicted the pattern of contrast response functions in normal observers and in amblyopes by using the attenuator model of Baker et al.[22] Although the model includes a binocular summation process, when the stimuli in the two eyes flicker at different frequencies we do not expect their EEG responses to sum across the two fundamental frequencies

Investigative Ophthalmology & Visual Science

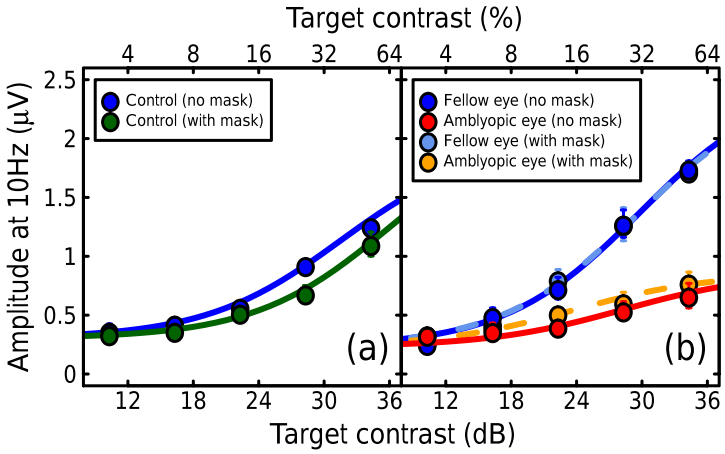


**Figure 2.** Contrast response functions for control observers (**a**) and amblyopes (**b**) averaged across electrodes Oz and POz. *Error bars* give ±1 standard error across observers (controls $n = 5$; amblyopes $n = 10$) after normalization (see Procedure section for details). *Curves* are the fits of a descriptive model detailed in the text.

(though there may be intermodulation responses[26]). Therefore, the response of the model to stimuli in the nonamblyopic eye was given by

$$monresp_F = \frac{C_F^m}{S + C_F + aC_A} \quad (1)$$

and

$$output_F = \frac{monresp_F^p}{Z + monresp_F^q}, \quad (2)$$

where $C$ is contrast, the subscripts $A$ and $F$ denote the amblyopic and fellow eyes, respectively, $a$ is an attenuation factor with a value of 0.2, and other parameters had values derived from previous work ($m = 1.3$, $S = 1$, $p = 7.995$, $q = 6.5$, $Z = 0.01$; for interpretation of the model parameters see Baker et al.[22] and Meese et al.[28]). The response for the amblyopic eye was similar, but with the attenuation applied to the amblyopic input

$$monresp_A = \frac{(aC_A)^m}{S + aC_A + C_F}, \quad (3)$$

with a corresponding version of Equation 2 for $monresp_A$. The curves in Figure 1b were produced by calculating the model response (Equation 2) for a range of inputs to the amblyopic and fellow eyes. Predictions for control observers were the same but for $a = 1$.

## Results

We averaged contrast response functions across the left and right eyes of our control participants. The blue data in Figure 2a show how the amplitude at the target frequency (10 Hz) increased as a function of target contrast. This is consistent with previous work.[26,27] When a 12-Hz mask was shown to the other eye at high contrast, this slightly reduced the response to the target (Fig. 2a, green data), particularly at 26% target contrast. This gain control effect is similar to those reported previously,[26,27] though somewhat weaker.

A two-way repeated measures ANOVA on the normalized amplitudes (which satisfied the assumption of sphericity) revealed a significant effect of target contrast ($F_{4,16} = 52.3$, $P < 0.001$, partial $\eta^2 = 0.93$), but no significant effect of mask contrast ($F_{1,16} = 6.94$, $P = 0.58$) and no interaction between the two variables ($F_{4,16} = 1.94$, $P = 0.15$). However, since we would expect to see no effect of mask contrast at low target contrasts (where the driven target response is negligible) or perhaps at high contrasts (because of saturation) it may be that inclusion of the full range of target contrasts explains this lack of a statistical effect of mask contrast. This was confirmed by running a paired samples $t$-test comparing the mask versus no-mask data at 26% target contrast, which did reveal a significant difference ($t = 3.63$, $df = 4$, $P = 0.022$).

Similar monotonic contrast response functions to the target stimuli were obtained in our amblyopic observers (Fig. 2b). However, the response in the amblyopic eye (red data) was much weaker than that in the fellow eye (dark blue), and appeared to be shallower in slope when plotted on a linear $y$-axis. There was no appreciable masking in either the fellow eye (light blue) or the amblyopic eye (orange). Indeed, there appeared to be a slight increase in response in the amblyopic eye when the mask was added (orange data). We consider possible explanations for this below.

We performed a three-way repeated measures ANOVA on the amblyope data, with eye (fellow or amblyopic), mask contrast (0% or 26%), and target contrast (five levels) as factors. Data showed significant deviations from sphericity for the contrast condition ($W = 0.056$, $P = 0.013$) and eye*contrast interaction ($W = 0.038$, $P = 0.005$), so we report Greenhouse-Geisser corrected values for these effects. There were highly significant effects of eye ($F_{1,9} = 28.3$, $P < 0.001$, partial $\eta^2 = 0.76$) and target contrast ($F_{2.05,18.4} = 59.1$, $P < 0.001$, partial $\eta^2 = 0.87$), and a significant interaction between the two ($F_{1.8,15.8} = 33.7$, $P < 0.001$, partial $\eta^2 = 0.79$). There was no significant effect of mask contrast ($F_{1,9} = 0.67$, $P = 0.43$), and no other interactions were significant (all $P > 0.05$).

As an index of the amount of binocular imbalance, we first calculated the ratio (nondominant/dominant eye) of amplitudes for responses at the highest target contrast for each observer. These are plotted in Figure 3a against the ratio of acuities (nondominant/dominant eye). A significant relationship is apparent ($r = 0.76$, $P < 0.001$, calculated using log acuity ratios), such that the greater the acuity difference between the eyes, the greater the reduction in neural response to stimuli shown in the weaker eye (this reduced to $r = 0.40$, $P = 0.25$ when the control data were omitted, largely due to the reduction in power).

We also fitted our data with a descriptive gain control equation[25] ($resp = R_{max}{}^*C^{1.4}/(Z^{1.4} + C^{1.4}) + B$) that had two free parameters ($R_{max}$ and $Z$) for each 5-point contrast

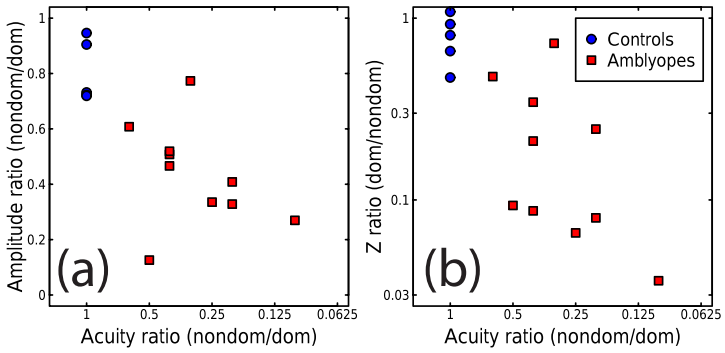


**Figure 3.** (**a**) Ratio of EEG amplitudes across weaker and stronger (nondominant and dominant) eyes for the highest contrast target stimulus, plotted against the ratio of visual acuities for all observers. (**b**) Ratio of fitted saturation constants (*Z*) against acuity ratio. Because low sensitivity is associated with a large *Z* value, the ratio was calculated as dominant/nondominant for comparison with (**a**).

response function (example fits are shown in Fig. 2), and an overall baseline parameter (*B*) common across all functions for a given observer. The $R_{max}$ parameter proved uninformative, remaining relatively constant across conditions (for the normalized data, even setting it to unity for all functions and refitting with only the *Z* and *B* parameters free made no difference to our findings). The saturation constant (*Z*) tracked the response reduction in the amblyopic eye. In Figure 3b we plot the ratio of fitted saturation constants across the eyes against the acuity ratio, in the same format as Figure 3a. This was highly correlated with the acuity ratio ($r = 0.79$, $P < 0.001$, calculated using log ratios), though again the correlation reduced when the control observers were omitted ($r = 0.56$, $P = 0.09$). Overall, the neural measurements were associated with real world visual ability and the magnitude of the amblyopic deficit.

The mask had a somewhat weaker effect than expected from the model predictions (Fig. 1b) and previous work.[26,27] To confirm that the mask was exciting neural responses, we measured the amplitude of the 12-Hz component of the EEG signal. These are presented in Figure 4 in the same format as Figure 2. There was a strong response to the mask when presented to the control observers (Fig. 4a, green data), which declined slightly as the target contrast increased (see Busse et al.[25] and Baker and Vilidaitė[27]). A strong response was also observed for the fellow eye of the amblyopes (Fig. 4b, orange data). There were significant effects of mask contrast for both control ($F_{1,4} = 14.0$, $P < 0.001$, partial $\eta^2 = 0.78$) and amblyopic ($F_{1,9} = 32.7$, $P < 0.001$, partial $\eta^2 = 0.78$) observers.

When the mask was shown to the amblyopic eye, it evoked a very weak response (Fig. 4b, light blue data), barely above the baseline levels of noise when it was absent (Fig. 4b, red and dark blue data). The weak response to the mask in the amblyopic eye explains the lack of a masking effect on the target shown by the light blue data in Figure 2b. However, it is not clear why a mask in the fellow eye did not suppress the target response in the amblyopic eye (Fig. 2b, compare red and orange data), as it was clearly exciting sufficient neurons to drive a strong response (Fig. 4b, orange data).

To further address this issue, we calculated the phase variance of the responses. This is a measure of (inverse) coherence, caused by stronger inputs leading to greater phase locking of the SSVEP signal, and can provide a more sensitive measure than raw amplitude.[27] The phase variance of responses at the target frequency is shown in Figure 5. These data replicate the main features of the raw amplitudes (Fig. 2) but inverted. The masking effect in the control observers was clearer (left panel), but was still not evident in the amblyopes (right panel).

Lastly, we examined the intertrial variance of the amplitude at the target frequency. Consistent with previous reports,[27] the variance increased with amplitude. We regressed variance against amplitude for control participants, and both the amblyopic and fellow eyes of the amblyopes. The regression

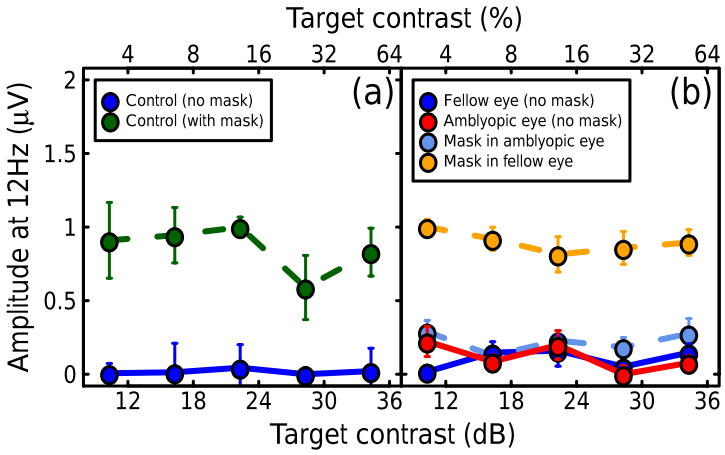


**Figure 4.** Steady-state visual evoked potential amplitudes at the mask frequency (12 Hz) as a function of target contrast for control observers (**a**) and amblyopes (**b**). In conditions when the mask was absent, the response was equivalent to the noise baseline of the system (equipment plus observer). *Error bars* give ±1 standard error across observers (control $n = 5$; amblyope $n = 10$) after normalization (see Procedure section for details).

Investigative Ophthalmology & Visual Science

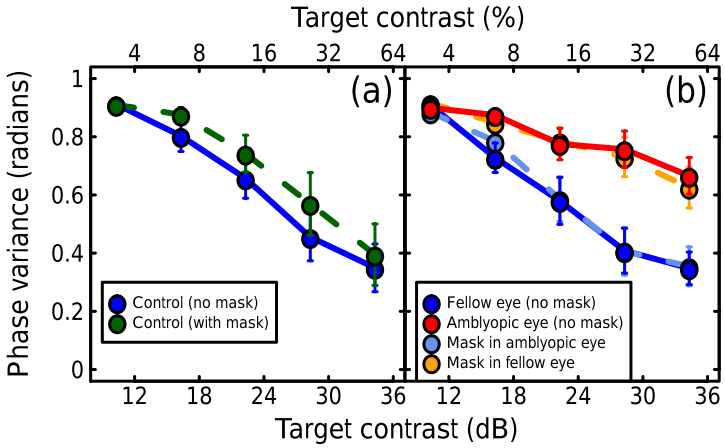


**Figure 5.** Trial-by-trial phase variance at 10 Hz as a function of target contrast for control observers (**a**) and amblyopes (**b**), calculated by using circular statistics. The functions show the same ordering as the amplitudes in Figure 2, but are inverted. *Error bars* give ±1 standard error across observers (control $n = 5$; amblyope $n = 10$).

intercept and slope were very similar in all data sets, suggesting that noise was not substantially increased in the responses from amblyopic eyes. To support this, we computed the Fano factor (variance/mean) for each participant at each target contrast level. An ANOVA comparing Fano factors across groups was not significant ($F_{2,299} = 0.11$, $P = 0.895$), and there was no clear effect of target contrast. In summary, we do not find evidence of increased noise in amblyopia when using this EEG technique.

## Discussion

We measured SSVEPs in 10 amblyopic and 5 control observers. In all observers, amplitude and signal coherence at the target frequency increased as a function of target contrast, though the responses were greatly attenuated in amblyopic eyes. Dichoptic masks had a suppressive effect in control observers, but no overall effect in amblyopes for either eye. We discuss this in relation to the predictions of a computational model of amblyopia, and the potential of SSVEPs to give an objective index of visual dysfunction in amblyopia.

### Model Predictions

We tested three predictions from the model of Baker et al.[22] The first prediction was that responses would be reduced in the amblyopic eye. This was clearly the case, as can be seen by comparing the dark blue and red functions in Figure 2b. A second prediction was that dichoptic masks shown to the amblyopic eye would produce less suppression than in control observers. This was also confirmed, as suppression was not observed in the fellow eye of amblyopes (Fig. 2b, light and dark blue functions overlap), whereas it was for control observers (blue and green functions in Fig. 2a).

The third prediction was that there would be stronger suppression from dichoptic masks shown to the fellow eye than in control observers. This was not the case, as responses were similar for targets shown to the amblyopic eye, regardless of the presence of a dichoptic mask in the fellow eye (Fig. 2b, red and orange functions). In fact, close inspection of the data of individual subjects revealed that some amblyopes showed a clear response increase when the dichoptic mask was added (not shown). This is especially surprising because of the widespread assumption that suppression from the fellow eye onto the amblyopic eye is particularly profound. Why might this occur? We suggest three possibilities that are not mutually exclusive: facilitation, release from inhibition, and synchronization.

Facilitation (an improvement in contrast detection thresholds) can be caused by overlaid pedestal stimuli,[29,30] adjacent stimuli,[31,32] stimuli remote in spatial frequency[33] or orientation,[34,35] and (controversially) low contrast noise stimuli.[36] Dichoptic facilitation has also been reported,[28,37] though this is typically very weak (threshold improvements of less than a factor of two), and occurs at pedestal contrasts much lower than the 26% contrast mask used here. Although it is conceivable that amblyopes might experience abnormal levels of dichoptic facilitation that could overcome interocular suppression, there is no evidence of this in studies measuring detection thresholds in the presence of dichoptic masks.[22,38]

A related concept is the release from a standing level of inhibition.[39] If amblyopes experience abnormal suppression from neurons responding to incidental stimuli in the fellow eye (e.g., the mean luminance[40]), this inhibition itself might be suppressed by a binocularly matched target stimulus. Related effects have been reported in normal observers, whereby the suppression from a dichoptic mask is released by binocular matching.[41,42] However, it is worth noting that recent studies have found no abnormalities in interocular suppression in amblyopes once sensitivity differences have been accounted for[43,44] (though see also Harrad and Hess[38]), and no evidence of abnormal masking from mean luminance.[44]

Some physiological studies have reported reduced synchronization of neural firing in amblyopic eye of strabismic animals.[45] If this lack of synchronization is part of the cause of the reduced SSVEP amplitude measured for the amblyopic eye (see below for further discussion of this idea), then perhaps presentation of a strong driving stimulus in the fellow eye can entrain binocular neurons responding to both eyes and increase the overall level of response. Because of the frequency selectivity of the SSVEP technique, this might manifest as an increase at the target frequency only, as seen in Figure 2b (compare red and orange functions).

In principle any of the explanations above, or some combination thereof, might be responsible for the slight increase in response we found to stimuli in the amblyopic eye when a mask was presented in the fellow eye. Future studies using more sensitive techniques within a similar paradigm

Investigative Ophthalmology & Visual Science

(i.e., magnetoencephalography [MEG][46]) may shed light on this issue.

### Causes of Amblyopic Signal Reduction

What is responsible for the reduction in SSVEP responses to stimuli presented in the amblyopic eye? One possibility is that the proportion of visual cortex that responds to input from this eye is reduced, perhaps with a corresponding increase in the number of neurons driven by the fellow eye (though we note that such imbalances are not striking in neurophysiological work[47]). An equally plausible explanation would be reduced responses from an otherwise normal neural population driven by the amblyopic eye.

An alternative possibility, as mentioned above, might be that many neurons still respond to inputs to the amblyopic eye, but there is a lack of synchronized firing.[45] Since the SSVEP is an aggregate measure over many millions of neurons, a lack of synchronization will cause signals to cancel, so the observed evoked response will be reduced. This is consistent with the increased phase variance for amblyopic stimulation at a given contrast level, relative to stimulation of the fellow eye (compare blue and red functions in Fig. 5b), though we note that masking also increases phase variance (compare blue and green functions in Fig. 5a; see also Baker and Vilidaitė[27]). This idea is also consistent with the much smaller proportional difference in amblyopic versus fellow eye responses measured with fMRI.[10] If neurons are still firing, albeit in a desynchronized fashion, they will consume oxygen and result in measurable BOLD signals, but may not relay useful information.

A generalized suppression is often considered to be a primary factor in amblyopia. This idea is not inconsistent with the reduction of SSVEP amplitudes. However, our failure to observe strong interocular suppression from the fellow eye onto the amblyopic eye is inconsistent with this imbalance being solely due to suppression from the fellow eye. In most clinical accounts, and in the predictions of the model of Baker et al.[22] (see Fig. 1b), such suppression should be stronger in amblyopes than in control subjects. An alternative interpretation of generalized suppression is that it corresponds to the attenuator in the model of Baker et al.[22] This is equivalent to a long-term sensitivity reduction, rather than a transient, active (stimulus-driven) process of suppression.

In the model of Baker et al,[22] signal attenuation takes place in the amblyopic channel before the first stage of contrast gain control (see Fig. 1a). This predicts an approximately lateral translation in the contrast response function (compare dark blue and red functions in Fig. 1b), resembling a contrast gain control effect.[24,48] However, empirically we found a reduction in slope of the contrast response function in the amblyopic eye (compare blue and red functions in Fig. 2b). We confirmed this by calculating the slope of the best fitting regression lines for the individual contrast functions of each observer. These were significantly shallower in the amblyopic eye than in the fellow eye (paired *t*-test, $t = 2.30$, $P < 0.05$, $df = 9$).

In the model, this behavior can be produced by applying attenuation to the numerator but not the denominator of the first stage. This involves changing Equation 3 to the following:

$$monresp_A = \frac{(aC_A)^m}{S + C_A + C_F}, \quad (4)$$

with all terms retaining their previous meanings. This implies attenuation of excitatory pathways[49] only, with suppressive pathways left unaffected. Such a modification makes little difference to the model's predictions for contrast discrimination behavior, so it remains capable of explaining the dipper functions of Baker et al.[22] (see also Ding et al.[23] for further variations of related models).

### Monitoring Changes Over Time

Regardless of the cause of the amblyopic deficit, our primary finding is that the maximum amplitude and slope of the contrast response function is greatly reduced in the amblyopic eye. Because steady-state EEG measurements have good signal-to-noise ratios, and require only a small number of electrodes, this is an ideal objective measurement for clinical monitoring. For clinical deployment, the dichoptic mask conditions could be omitted, and a more limited range of target contrasts tested than in the present study, with fewer repetitions. This would reduce the testing time from approximately 50 minutes in the experiments reported here to less than 15 minutes.

A number of amblyopia treatments have been proposed in recent years, with the aim of improving function in the amblyopic eye alone, and some of restoring binocularity (e.g., stereopsis). Steady-state visual evoked potential measurements can be obtained reasonably rapidly, perhaps even in the patient's own home, using modern consumer-grade EEG equipment intended for gaming.[50] Using this objective technique to monitor improvements in visual function avoids issues of bias and practice inherent in psychophysical measures of visual performance, and it is substantially cheaper than MRI and MEG techniques.

### Strengths and Limitations of the Current Study

The present study is the first to measure full steady-state contrast response functions in amblyopic observers and the first to directly test the predictions of a computational model of amblyopic vision by using electrophysiological measures. The dichoptic masking conditions produced weaker effects than anticipated, even in control participants. This could be a consequence of the broadband mask stimulus (though this was similar to previous work[26]), or the relatively high temporal frequencies we used. Future work will aim to extend the range of conditions to include binocular stimulation with matched temporal frequencies, to estimate binocular summation processes as well as the interocular suppression observed with mismatched temporal frequencies. Although the sample size used here was sufficient to show the very large deficits in the amblyopic eye, detecting subtler effects may require larger numbers of participants.

## Conclusions

We measured contrast response functions by using SSVEPs, for both eyes of amblyopic observers. Consistent with model predictions, we found a marked reduction in response amplitude for stimuli presented in the amblyopic eye. The ratio of responses between amblyopic and fellow eyes was highly correlated with the visual acuity of the amblyopic eye. We suggest that this method might provide a sensitive and objective measure of any improvements gained through amblyopia therapy, particularly those relating to signal synchrony.

### *Acknowledgments*

Supported in part by the Wellcome Trust (ref: 105624) through the Centre for Chronic Diseases and Disorders (C2D2) at the University of York. Also supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC), the FRSQ Visual Heath Research Network awarded to DSA and

RFH, and the Canadian Institutes of Health Research (CIHR No.108-18, No. 53346).

Disclosure: **D.H. Baker**, None; **M. Simard**, None; **D. Saint-Amour**, None; **R.F. Hess**, None

## *References*

1. Polat U, Ma-Naim T, Belkin M, Sagi D. Improving vision in adult amblyopia by perceptual learning. *Proc Natl Acad Sci U S A*. 2004;101:6692–6697.
2. Spiegel DP, Byblow WD, Hess RF, Thompson B. Anodal transcranial direct current stimulation transiently improves contrast sensitivity and normalizes visual cortex activation in individuals with amblyopia. *Neurorehabil Neural Repair*. 2013;27:760–769.
3. Clavagnier S, Thompson B, Hess RF. Long lasting effects of daily theta burst rTMS sessions in the human amblyopic cortex. *Brain Stimul*. 2013;6:860–867.
4. Thompson B, Mansouri B, Koski L, Hess RF. Brain plasticity in the adult: modulation of function in amblyopia with rTMS. *Curr Biol*. 2008;18:1067–1071.
5. To L, Thompson B, Blum JR, Maehara G, Hess RF, Cooperstock JR. A game platform for treatment of amblyopia. *IEEE Trans Neural Syst Rehabil Eng*. 2011;19:280–289.
6. Li RW, Ngo C, Nguyen J, Levi DM. Video-game play induces plasticity in the visual system of adults with amblyopia. *PLoS Biol*. 2011;9:e1001135.
7. Hess RF, Mansouri B, Thompson B. A binocular approach to treating amblyopia: antisuppression therapy. *Optom Vis Sci*. 2010;87:697–704.
8. Barnes GR, Hess RF, Dumoulin SO, Achtman RL, Pike GB. The cortical deficit in humans with strabismic amblyopia. *J Physiol*. 2001;533(pt 1):281–297.
9. Algaze A, Roberts C, Leguire L, Schmalbrock P, Rogers G. Functional magnetic resonance imaging as a tool for investigating amblyopia in the human visual cortex: a pilot study. *J AAPOS*. 2002;6:300–308.
10. Li X, Dumoulin SO, Mansouri B, Hess RF. Cortical deficits in human amblyopia: their regional distribution and their relationship to the contrast detection deficit. *Invest Ophthalmol Vis Sci*. 2007;48:1575–1591.
11. Conner IP, Odom JV, Schwartz TL, Mendola JD. Monocular activation of V1 and V2 in amblyopic adults measured with functional magnetic resonance imaging. *J AAPOS*. 2007;11:341–350.
12. Hess RF, Li X, Lu G, Thompson B, Hansen BC. The contrast dependence of the cortical fMRI deficit in amblyopia; a selective loss at higher contrasts. *Hum Brain Mapp*. 2010;31:1233–1248.
13. Farivar R, Thompson B, Mansouri B, Hess RF. Interocular suppression in strabismic amblyopia results in an attenuated and delayed hemodynamic response function in early visual cortex. *J Vis*. 2011;11(14):16, 1–12.
14. Bankó ÉM, Körtvélyes J, Németh J, Weiss B, Vidnyánszky Z. Amblyopic deficits in the timing and strength of visual cortical responses to faces. *Cortex*. 2013;49:1013–1024.
15. Bankó ÉM, Körtvélyes J, Weiss B, Vidnyánszky Z. How the visual cortex handles stimulus noise: insights from amblyopia. *PLoS One*. 2013;8:e66583.
16. Joosse MV, Esme DL, Schimsheimer RJ, Verspeek SAM, Vermeulen MHL, van Minderhout EM. Visual evoked potentials during suppression in exotropic and esotropic strabismics: strabismic suppression objectified. *Graefes Arch Clin Exp Ophthalmol*. 2005;243:142–150.
17. Oner A, Coskun M, Evereklioglu C, Dogan H. Pattern VEP is a useful technique in monitoring the effectiveness of occlusion therapy in amblyopic eyes under occlusion therapy. *Doc Ophthalmol*. 2004;109:223–227.
18. He H-Y, Ray B, Dennis K, Quinlan EM. Experience-dependent recovery of vision following chronic deprivation amblyopia. *Nat Neurosci*. 2007;10:1134–1136.
19. Bagolini B, Falsini B, Cermola S, Porciatti V. Binocular interactions and steady-state VEPs: a study in normal and defective binocular vision (part II). *Graefes Arch Clin Exp Ophthalmol*. 1994;232:737–744.
20. Johansson B, Jakobsson P. Fourier analysis of steady-state visual evoked potentials in subjects with normal and defective stereo vision. *Doc Ophthalmol*. 2000;101:233–246.
21. Johansson B, Jakobsson P. Fourier-analysed steady-state VEPs in pre-school children with and without normal binocularity. *Doc Ophthalmol*. 2006;112:13–22.
22. Baker DH, Meese TS, Hess RF. Contrast masking in strabismic amblyopia: attenuation, noise, interocular suppression and binocular summation. *Vis Res*. 2008;48:1625–1640.
23. Ding J, Klein SA, Levi DM. Binocular combination in abnormal binocular vision. *J Vis*. 2013;13(2):14.
24. Heeger DJ. Normalization of cell responses in cat striate cortex. *Vis Neurosci*. 1992;9:181–197.
25. Busse L, Wade AR, Carandini M. Representation of concurrent stimuli by population activity in visual cortex. *Neuron*. 2009;64:931–942. doi:10.1016/j.neuron.2009.11.004.
26. Tsai JJ, Wade AR, Norcia AM. Dynamics of normalization underlying masking in human visual cortex. *J Neurosci*. 2012;32:2783–2789. doi:10.1523/JNEUROSCI.4485-11.2012.
27. Baker DH, Vilidaitė G. Broadband noise masks suppress neural responses to narrowband stimuli. *Front Psychol*. 2014;5:736.
28. Meese TS, Georgeson MA, Baker DH. Binocular contrast vision at and above threshold. *J Vis*. 2006;6(11):1224–1243.
29. Nachmias J, Sansbury RV. Letter: grating contrast: discrimination may be better than detection. *Vis Res*. 1974;14:1039–1042.
30. Legge GE, Foley JM. Contrast masking in human vision. *J Opt Soc Am*. 1980;70:1458–1471.
31. Polat U, Sagi D. Lateral interactions between spatial channels: suppression and facilitation revealed by lateral masking experiments. *Vis Res*. 1993;33:993–999.
32. Meese TS, Summers RJ, Holmes DJ, Wallis SA. Contextual modulation involves suppression and facilitation from the centre and the surround. *J Vis*. 2007;7(4):7, 1–21.
33. Meese TS, Holmes DJ, Challinor KL. Remote facilitation in the Fourier domain. *Vis Res*. 2007;47:1112–1119.
34. Meese TS, Holmes DJ. Spatial and temporal dependencies of cross-orientation suppression in human vision. *Proc Biol Sci*. 2007;274:127–136.
35. Meese TS, Baker DH. Cross-orientation masking is speed invariant between ocular pathways but speed dependent within them. *J Vis*. 2009;9(5):2.1–15.
36. Goris RLT, Wagemans J, Wichmann FA. Modelling contrast discrimination data suggest both the pedestal effect and stochastic resonance to be caused by the same mechanism. *J Vis*. 2008;8(15):17.1–21.
37. Legge GE. Spatial frequency masking in human vision: binocular interactions. *J Opt Soc Am*. 1979;69:838–847.
38. Harrad RA, Hess RF. Binocular integration of contrast information in amblyopia. *Vis Res*. 1992;32:2135–2150.
39. Morrone MC, Burr DC. Evidence for the existence and development of visual inhibition in humans. *Nature*. 1986;321:235–237.
40. Yang J, Stevenson SB. Post-retinal processing of background luminance. *Vis Res*. 1999;39:4045–4051.
41. Meese TS, Hess RF. Interocular suppression is gated by interocular feature matching. *Vis Res*. 2005;45:9–15.

42. Baker DH, Meese TS, Summers RJ. Psychophysical evidence for two routes to suppression before binocular summation of signals in human vision. *Neuroscience*. 2007;146:435–448.
43. Huang P-C, Baker DH, Hess RF. Interocular suppression in normal and amblyopic vision: spatio-temporal properties. *J Vis*. 2012;12(11):29, 1–12.
44. Zhou J, McNeal S, Babu RJ, Baker DH, Bobier WR, Hess RF. Time course of dichoptic masking in normals and suppression in amblyopes. *Invest Ophthalmol Vis Sci*. 2014;55:4098–4104.
45. Roelfsema PR, König P, Engel AK, Sireteanu R, Singer W. Reduced synchronization in the visual cortex of cats with strabismic amblyopia. *Eur J Neurosci*. 1994;6:1645–1655.
46. Chadnova E, Reynaud A, Clavagnier S, Baker DH, Baillet S, Hess RF. Dynamics of dichoptic masking in the primary visual cortex. *BMC Neurosci*. 2014;15(suppl 1):P145.
47. Sengpiel F, Blakemore C. The neural basis of suppression and amblyopia in strabismus. *Eye*. 1996;10:250–258.
48. Carandini M, Heeger DJ. Normalization as a canonical neural computation. *Nat Rev Neurosci*. 2012;13:51–62.
49. Foley JM. Human luminance pattern-vision mechanisms: masking experiments require a new model. *J Opt Soc Am*. 1994;11:1710–1719.
50. Badcock NA, Mousikou P, Mahajan Y, de Lissa P, Thie J, McArthur G. Validation of the Emotiv EPOC(®) EEG gaming system for measuring research quality auditory ERPs. *PeerJ*. 2013;1:e38.

Investigative Ophthalmology & Visual Science