Published in final edited form as:
Vision Res. 2008 March ; 48(6): 788–798. doi:10.1016/j.visres.2007.12.011.

Crowding Between First- and Second-Order Letters in Amblyopia

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Abstract

To test whether first- and second-order stimuli are processed independently in amblyopic vision, we measured thresholds for identifying a target letter flanked by two letters for all combinations of first- and second-order targets and flankers. We found that (1) the magnitude of crowding is greater for second- than for first-order letters for target and flankers of the same order type; (2) substantial but asymmetric cross-over crowding occurs such that stronger crowding is found for a second-order letter flanked by first-order letters than for the converse; (3) the spatial extent of crowding is independent of the order type of the letters. Our findings are consistent with the hypothesis that crowding results from an abnormal integration of target and flankers beyond the stage of feature detection, which takes place over a large distance in amblyopic vision.

Keywords

amblyopia; crowding; first order; second order; letter identification

INTRODUCTION

Objects in everyday life are rarely seen in complete isolation. When objects are in close proximity with one another, details of the target of interest may be difficult to discern. This effect, known as *crowding*, is ubiquitous in spatial vision, and represents a form of suppressive spatial interaction between visual objects.

Crowding affects task performance such as letter and face identification in people with normal vision (e.g. Bouma, 1970; Chung, Levi & Legge, 2001; Chung, Li & Levi, 2007; Martelli, Majaj & Pelli, 2005; Pelli, Palomares & Majaj, 2004), but its effect is often more pronounced in individuals with amblyopia (e. g. Flom, Weymouth & Kahneman, 1963; Hariharan, Levi & Klein, 2005; Hess, Dakin, Tewfik, & Brown, 2001; Hess & Jacobs, 1979; Levi, Hariharan & Klein, 2002c; Levi & Klein, 1985; Simmers, Gray, McGraw & Winn, 1999). Amblyopia is a developmental disorder of spatial vision almost always accompanied by the presence of strabismus, anisometropia or form deprivation early in life (Ciuffreda, Levi & Selenow, 1991). The signature of amblyopia is the presence of visual deficits in one eye that cannot be attributed to an identifiable ocular pathology. With respect to crowding, previous studies

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showed that the spatial extent of crowding (defined as the distance between a target and its surrounding objects) is much greater for individuals with amblyopia (especially those with strabismus) than for people with normal vision, even when the poor resolution in the amblyopic eye is taken into account (Hariharan et al, 2005; Hess et al, 2001; Levi et al, 2002c). Three main hypotheses have been suggested to account for the extensive crowding in amblyopia: (1) enlarged cortical receptive fields (Flom et al., 1963); (2) abnormal long-range inhibitory interactions (Levi & Klein, 1985; Bonneh, Sagi & Polat, 2004; Ellemberg, Hess & Arsenault, 2002; Wong, Levi & McGraw, 2005), and (3) abnormal integration of target and flankers beyond the stage of feature detection (Levi et al., 2002c; Pelli et al., 2004; Hariharan et al., 2005).

Given that crowding is a form of spatial interaction between visual objects, it can be utilized as a tool to study how the visual system processes and integrates information from different stimuli. Previously, we used crowding as a tool to examine whether or not first- and second-order visual stimuli are processed independently in normal foveal and peripheral vision. *First-order* targets refer to targets that differ from their background by a change in luminance, hence they are often referred to as luminance-defined targets. In the absence of a change in luminance, targets can still be distinguished from their background by variations in other stimulus attributes such as contrast, texture or motion. These stimuli are often referred to as *second-order* stimuli. There are conflicting reports as to whether or not first- and second-order information is processed via independent visual pathways (for a review, please refer to Chung et al, 2007). Our principal finding that there exists substantial cross-over crowding between first- and second-order letter stimuli in normal foveal and periheral vision, combined with a survey of the literature, offers a parsimonious explanation for the conflicting results — that first- and second-order processing remains separate at the initial stage of detection and feature extraction, but the signals are combined at a later integration stage (see also Rivest & Cavanagh, 1996).

Much of what we have learned about the visual deficits in individuals with amblyopia is based upon studies that used first-order stimuli. This knowledge is essential as it provides us with a better understanding of the visual deficits that occur primarily in visual cortex V1. Over the past several years, it has become evident that amblyopic deficits are found not only in V1, but also in the extrastriate cortex, as uncovered by several studies that examined second-order processing in amblyopia (Wong, Levi & McGraw, 2001;2005;Wong & Levi, 2005;Mansouri, Allen & Hess, 2005;Simmers, Ledgeway, Hess & McGraw, 2003;Simmers, Ledgeway & Hess, 2005). Results from these studies are often interpreted as an amplified amblyopic deficit in the extrastriate cortex that cannot be attributable to the first-order deficit. Indeed, Wong et al. (2005) found that spatial interactions in second-order target detection were abnormal in both eyes of amblyopic observers, and suggested that amblyopia results in predominantly inhibitory interactions between second-order neurons.

Considering that amblyopic deficits are found for both first- and second-order stimuli, it is of interest to examine the interaction between the processing of these two types of stimuli. In this study, we used crowding as a tool to probe into the properties of spatial interaction between first- and second-order signals in amblyopic vision. We were especially interested in the cross-over conditions (first-order target with second-order flankers, and *vice versa*). If abnormal crowding in amblyopia reflects abnormal integration of target and flankers beyond the stage of feature detection, we would expect strong cross-over crowding between first- and second-order stimuli. Hence, the primary question we asked in this study was whether or not there is cross-over crowding in the amblyopic visual system, as occurs in normal fovea and periphery. We shall quantify the effect of crowding by its magnitude (intensity) and its spatial extent. Previously, we proposed a framework to explain how first- and second-order letters interact in normal fovea and periphery, therefore, an auxiliary question of this study was whether or not this framework can be used to explain the combination rules of first- and second-order stimuli in amblyopic vision.

METHODS

Observers

Seven observers with amblyopia (five with strabismus, one with anisometropia and one with both strabismus and anisometropia), aged between 21 and 41 years, participated in this study. Table 1 summarizes the visual characteristics of these observers. All observers except JD had previously participated in a perceptual learning study to track the performance for identifying contrast-defined letter with practice (Chung, Li & Levi, 2006b). JD, however, had participated in an earlier study that involved detection of contrast-modulated static noise patches (Wong & Levi, 2005). Consequently, all observers were familiar with second-order stimuli. They all had best-corrected visual acuity of 20/20 or better in the non-amblyopic eye, and a difference in logMAR acuities between the two eyes that ranged between 0.14 to 0.90 log units. All observers wore their best optical corrections during the experiment. Written informed consent was obtained from each observer after the procedures of the experiment were explained and before the commencement of data collection.

Stimuli

To induce crowding, we presented a target letter in the presence of two flanking letters (we shall refer to these sequences of three random letters as “trigrams”) at small letter separations. Letters of each trigram were randomly picked, with equal probability, from the 26 lowercase letters of the Times-Roman alphabet. The task was to identify the middle, flanked target letter. The magnitude of crowding was defined as the contrast threshold elevation of identifying the target letters (middle letters of trigrams) in the presence of flanking letters when compared with the threshold for identifying unflanked (single) letters. We examined all four combinations of the order type of target and flanking letters (see Figure 1): a first-order letter flanked by first-order letters (referred to as the ‘111’ condition), a second-order target letter flanked by second-order letters (‘222’), a first-order letter flanked by second-order letters (‘212’) and a second-order letter flanked by first-order letters (‘121’). We refer to the latter two as the crossover conditions.

Methods of generating the first- and second-order stimuli were identical to those described in our previous paper (Chung et al, 2006a). In brief, our first-order (luminance-defined) letters were generated by assigning a different luminance value to the letter, compared with its mid-gray background. An array of white noise that followed a rectangular distribution and with a maximum luminance contrast of 0.25 (corresponding to a rms contrast of 0.07; unless otherwise stated, we used maximum luminance contrast to specify our contrast values throughout this manuscript) covered both the letter and its background. Our primary measurement for first-order letters was the contrast threshold for identifying these letters, where contrast was defined as the Weber contrast between the letter and its background, (letter luminance − background luminance)/background luminance. Second-order (contrast-defined) letters were generated by first constructing an array of white noise, and then assigning a different contrast to the subset of white noise that defined the letter, with respect to the contrast of the background (maximum luminance contrast = 0.25). The mean luminance of the letter and its background were the same. Thus, threshold for identifying second-order letters was defined as the absolute just-noticeable differential contrast (ΔC) that defined the letter from its background. For simplicity, we shall refer to this as the “contrast threshold” for second-order letters.

Psychophysical procedures

The psychophysical procedures used to determine thresholds for identifying the target letters were identical to those described in our previous paper (Chung et al, 2007). Each condition was tested in a separate block of trials. In each block of trials, we used the method of constant stimuli to present the stimulus letter at five stimulus levels (five letter sizes for size threshold measurement, five Weber contrast levels for contrast threshold measurement for first-order letters or five differential contrast, ΔC, threshold measurement for second-order letters), with each stimulus level presented 20 times within the block. Stimulus duration was 150 ms. A small fixation target was presented at the center of the display, which also corresponded to the center of the middle letter of each trigram to guide the observer's fixation. It disappeared 100 ms before the onset of the stimulus and reappeared immediately after the offset of the stimulus. Following the presentation of the stimulus in each trial, observers indicated their responses as to the identity of the letters using a keyboard. Audio feedback was provided to indicate whether or not the responses were correct. We used probit to fit a cumulative-Gaussian function to each block of data, where threshold was defined as the stimulus level that yielded 50% correct on the psychometric function, after correction for guessing (guessing rate = 1/26). Threshold reported in this paper represents the threshold estimate averaged across 4–6 blocks of the same condition.

Experimental design

The experimental design followed that of our previous paper (Chung et al, 2007). Observers were tested monocularly with the non-tested eye covered using a standard black eye patch. The two eyes of each observer were tested separately. For each eye, we first measured the size threshold for identifying single first-order letters of a fixed and high contrast of 0.7 and single second-order letters of a fixed ΔC of 0.7[1]. Because acuities differed between the non-amblyopic and amblyopic eyes, and between first-and second-order letters, viewing distance varied depending on the eye (non-amblyopic or amblyopic) and the order type of the letter being tested, and ranged from 400 cm (non-amblyopic eyes, first-order letters) to 42 cm (amblyopic eyes, second-order letters). Letter size used in subsequent testing (for both first- and second-order letters) was then set at 1.3× the size threshold[2] obtained for the second-order letters, and ranged between 0.83 to 1.3° for the non-amblyopic eyes, and 1 to 2° for the amblyopic eyes. Across all observers, the size threshold for second-order letters was 5.78 ± 2.59 (95% confidence intervals) times larger than that for first-order letters for the non-amblyopic eyes, and 6.04 ± 2.39 times for the amblyopic eyes. Note that these values were similar to those obtained before amblyopic or normal observers were trained on identifying second-order letters (Chung et al, 2006b). We then determined the contrast thresholds for identifying the first- and second-order flanking letters so that we could equate the visibility of the flankers in the main experiment (see below), regardless of whether they were first- or second-order letters. For these trials, we presented only the two flanking letters, i.e., trigrams without the middle stimulus letters and we measured the threshold for identification separately for the right and left flanking letters. Observers were asked to fixate the fixation target presented before and after each stimulus (see "Psychophysical procedures"). Viewing distance for these and subsequent measurements (main experiment, see below) was 42 cm. Observers were informed before each block of trials as to whether they should respond to the right or the left letters. As in the normal fovea and periphery, thresholds for identifying the left and right flanking letters were very similar for all observers, thus, we used the value averaged across 8–12 blocks of trials (two

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[1] The use of first-order letters of a fixed contrast of 0.7 and second-order of a ΔC of 0.7 for size threshold measurement was arbitrary, but a ΔC of 0.7 was close to maximum value that we could use to display second-order letters so as to avoid clipping of luminance values in the stimuli.

[2] The choice of using letter sizes equivalent to 1.3× the size threshold was also arbitrary. Our goal was to use a letter size that yielded a performance greater than 50%, but below 100%.

letter positions with 4–6 blocks for each letter position) to represent the identification threshold of the flanking letters.

In the main experiment in which we measured the crowding effect using trigrams, the contrast (for first-order letters) or the differential contrast (for second-order letters) of the flanking letters were set at 1.6× the respective threshold, as determined previously. We chose this value to equate for the flanker visibility in part because this was the value used in our previous study (Chung et al, 2007) and also this was likely to be the highest multiple of threshold that we could use to display second-order letters. Three letter separations (defined as center-to-center separations) were tested: 1×, 1.2× and 2× the height of the letter “x”. At each letter separation, thresholds were determined for each of the four trigram conditions (111, 222, 212 and 121). Thresholds for identifying single first- and second-order letters were also measured so that threshold elevation due to the presence of flanking letters could be determined.

Apparatus

Stimuli were generated on a Macintosh G4 computer with software written in Matlab (The MathWorks, MA) using the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997) and were displayed on a Sony 17” monitor (model number G400) at a mean luminance of 23 cd/m^2 (Berkeley) or a Mitsubishi Diamond Plus 15” monitor (model number N0701) at a mean luminance of 26 cd/m^2 (Houston). The luminance of the display was measured using a Minolta photometer. By combining the red and blue output of the display with the use of a video attenuator (Pelli & Zhang, 1991) and custom-written software (Tjan, personal communication), we obtained an effective 10-bit resolution on luminance after gamma-correction of the display. At a viewing distance of 42 cm (used for all conditions except when size thresholds were being determined), each pixel subtends 2.5 arc min.

RESULTS

Contrast thresholds for single (unflanked) letters were measured so that we could determine the magnitude of crowding (ratio of flanked to unflanked thresholds) for different letter separations. Because contrast threshold depends, to a certain extent, on the stimulus size, and we used different letter sizes for testing the non-amblyopic and amblyopic eyes, the comparison of contrast thresholds between the two eyes is not meaningful. However, given that we used the same letter size for presenting first- and second-order letters in each eye, a comparison of the contrast threshold ratio between first- and second-order letters across eyes would provide further evidence as to whether or not there is an additional second-order deficit in the amblyopic eye. Averaged across observers, the contrast threshold ratio of second- to first-order letters was 3.55 ± 0.83 for the non-amblyopic eyes and 4.88 ± 1.40 for the amblyopic eyes, consistent with previous observations (Wong et al, 2001) of an additional second-order deficit in the amblyopic eye.

The effect of crowding can be described by two parameters — the peak magnitude and the spatial extent of the effect. By plotting threshold elevation as a function of letter separation, we can visualize these two parameters easily. Figure 2A shows samples of these plots, for the four trigram conditions and for two observers, AP and RH. We define the peak magnitude of crowding as the magnitude of crowding at the smallest letter separation (in this case, 1× nominal separation), and the spatial extent of crowding as the separation at which the magnitude of the effect drops to a pre-determined criterion. To quantify these two parameters, we fit each set of threshold elevation vs. letter separation data with the following equation that describes a Gaussian function

$$y=1+a\exp\left(1-(x/b)^2\right)$$

where a represents the peak amplitude of the function and b represents the x value at which peak amplitude occurs (Chung et al, 2007). Here, we used a to represent the peak magnitude of the crowding effect and we defined the "critical distance", the letter separation that corresponds to 2 standard deviations from the peak of the Gaussian function, to represent the spatial extent of crowding. The critical distance was estimated based on the fitted parameters of the best fit curve, using the bootstrap resampling technique with 1000 resamplings (Chung et al, 2007). Figure 3 summarizes the critical distance (top panels) and the peak magnitude of the crowding effect (bottom panels) for the four trigram conditions. In each panel, colored symbols represent the values estimated for each observer, while the black filled circles represent the group-averaged value (± 95% confidence intervals). For comparison, we also include the normal foveal (unfilled gray symbols) and 10° eccentricity data (filled gray symbols), replotted from Chung et al (2007). Unless stated otherwise, in this paper, the error estimates associated with a group-averaged value represents the 95% confidence intervals, which in turn represent the upper and lower limits that are 2 standard deviations from the mean. A value that falls outside the 95% confidence limits implies a 5% probability ($p = 0.05$) that the value belongs to the distribution. We will make our inference as to whether or not data from two conditions are statistically different by comparing their respective ranges (mean ± 95% confidence intervals).

Critical distance for crowding

An interesting observation from Figure 3 is that the critical distance is virtually constant across the four trigram conditions, and averages 2.35 deg and 3.14 deg for the non-amblyopic and amblyopic eyes, respectively. The fact that the critical distance does not depend on whether the target or flanking letters are first- or second-order is consistent with our data obtained from the normal fovea and periphery (Chung et al, 2007), and with the notion that crowding involves a two-stage process where features are detected and extracted at the first-stage, after which information from the target and flankers is combined at the second-stage that is insensitive to the order type of individual letters.

As expected for first-order targets, the spatial extent of crowding is greater for the amblyopic (111: 2.91 ± 0.56 deg) than the non-amblyopic eye (111: 2.13 ± 0.12 deg). Consistent with Wong et al. (2005), we show that the result also holds for second-order targets, in our case, letter stimuli (222: 2.91 ± 0.26 for amblyopic eyes vs. 2.36 ± 0.19 for non-amblyopic eyes). Previously, Hariharan et al (2005), Hess et al (2001) and Levi et al (2002c) showed that for some amblyopic observers, the spatial extent of crowding is greater than predicted based on acuity alone. In Figure 2B we replotted the data shown in Figure 2A, with the letter separation normalized to letter size, thus yielding letter separation expressed as multiples of letter size. Once the acuity is taken into account, the plots of threshold elevation vs. letter separation become very similar between the non-amblyopic and amblyopic eyes, suggesting that the spatial extent of crowding is *not* greater in amblyopic than non-amblyopic eyes. This result does not contradict the previous finding because as shown by Hariharan et al (2005) and Levi et al (2002c), the difference in the spatial extent of crowding between the non-amblyopic and amblyopic eye is most obvious for letter sizes close to the acuity limit. For larger letters, the spatial extent of crowding becomes similar between the non-amblyopic and amblyopic eyes, as shown in this study. Here, we used a letter size equivalent to 1.3× the size-threshold for *second-order* letters, which averaged approximately 8× larger than the size-threshold for first-order letters. Therefore, it is not surprising that we did not find a difference in the scaled spatial extent of crowding between the non-amblyopic and amblyopic eyes.

Peak magnitude of crowding

As in normal foveal and peripheral vision (Chung et al, 2007), crowding is more substantial for second- than for first-order letter stimuli, for both the non-amblyopic and amblyopic eyes. Averaged across observers, the threshold elevation is 1.45 ± 0.21 for the 222 condition and 1.03 ± 0.03 for the 111 condition in the non-amblyopic eyes, and 1.44 ± 0.15 for the 222 condition and 1.11 ± 0.03 for the 111 condition in the amblyopic eyes (Figure 3). In our earlier paper, we put forward a framework to explain the crowding effect for first- and second-order letters in normal foveal and peripheral vision. One of the assumptions associated with the framework is that the visual system places more weighting on first- than second-order signals, therefore, the target (middle) letter for the 111 condition would be considered as more reliable than the target letter for the 222 condition. Another assumption states that target letters are weighted more (treated as more reliable by the visual system) than flankers, therefore when combined with the first assumption, the effects of the flankers for the 111 and 222 conditions are not the same, and could explain why the magnitude of crowding is more substantial for second- than for first-order letters. Here we show that data obtained in both the non-amblyopic and the amblyopic eyes of our observers are consistent with this assumption, and are qualitatively similar to what we observed in the normal fovea and periphery (Chung et al, 2007).

As noted earlier, previous studies on crowding in amblyopia showed that the spatial extent of crowding for small letters is much greater in the amblyopic eye than in the normal fovea, even when the poorer resolution in the amblyopic eye is taken into account (Hariharan et al, 2005; Hess et al, 2001; Levi et al, 2002c). For large letters, we show that the spatial extent of crowding is similar between the non-amblyopic and amblyopic eyes. Here, we show that the *magnitude* of the crowding effect is also similar between the non-amblyopic and the amblyopic eyes. Figure 4 plots the peak magnitude (threshold elevation at the nominal letter separation of 1×) as a function of the spatial extent of crowding (equivalent to the critical distance derived from our curve-fit) for the four trigram conditions and for the non-amblyopic and the amblyopic eyes separately. There are two interesting observations from this figure. First, there is no correlation between the peak magnitude and the spatial extent of crowding. A linear regression fit to the data (not shown in Figure 4) for the same-order crowding conditions (111 and 222: panel A) yielded a correlation coefficient of 0.19 ($t_{(df = 26)} = 0.995$, $p = 0.32$). For the cross-over crowding conditions (212 and 121: panel B), the correlation coefficient was 0.20 ($t_{(df} = 26) = 1.05$, $p = 0.30$). Second, the spread of the magnitude of crowding is similar between the non-amblyopic eyes (unfilled symbols) and the amblyopic eyes (filled symbols). We note that this result is clearly different from the effects of second-order flankers on second-order target detection, where not only the magnitude, but also the sign of interaction may be different in amblyopic and normal eyes (Wong et al., 2005).

The results described so far refer to the conditions in which the target and the flanking letters were of the same order type. What about cross-over crowding? In our previous paper, we found that the magnitude of the cross-over crowding conditions (121 and 212) is similar to that obtained for the 111 condition, in both the normal fovea and periphery (see Figure 3). For our amblyopic observers, the magnitude of crowding for the 212 condition (non-amblyopic eyes: 1.05 ± 0.07; amblyopic eyes: 1.14 ± 0.04) is indeed very similar to that for the 111 condition (non-amblyopic eyes: 1.03 ± 0.03; amblyopic eyes: 1.11 ± 0.03); however, the magnitude of crowding for the 121 condition (non-amblyopic eyes: 1.22 ± 0.08; amblyopic eyes: 1.34 ± 0.22) is apparently higher than those for the 212 or 111 conditions. This result, found in both the non-amblyopic and amblyopic eyes, suggests an asymmetric cross-over crowding that depends on whether the target letter is first- or second order. We shall return to the significance of this result in the Discussion.

Another interesting observation is that the magnitudes of crowding obtained for the non-amblyopic eyes are remarkably similar to those obtained in the normal fovea (gray unfilled symbols in Figure 3) for all but the 121 condition. For the amblyopic eyes, the magnitudes of crowding are slightly higher than those for the normal fovea by about 0.05 to 0.1 units, again, for all but the 121 condition. For both the non-amblyopic and amblyopic eyes, the magnitudes of crowding for the 121 condition are clearly higher (by about 0.2 to 0.3 units) than those obtained in the normal fovea.

DISCUSSION

The primary question we asked in this study was whether or not there is cross-over crowding in the amblyopic visual system, as occurs in normal fovea and periphery. We also asked the question of whether the framework that we proposed to explain the combination rules of first- and second-order stimuli in normal vision can be used to explain how first- and second-order stimuli are combined, and thus interact with one another, in amblyopic vision. To a first approximation, the findings from the amblyopic observers, for both the non-amblyopic and amblyopic eyes, show the same general findings as in normal fovea and periphery, namely, (1) for target and flankers of the same order type, the magnitude of crowding is larger for second- than for first-order letters; (2) there is substantial cross-over crowding suggesting interaction between first-and second-order information at the site where crowding occurs; (3) there is an asymmetry in the magnitude of the cross-over crowding such that crowding is stronger for a second-order target letter flanked by first-order letters (121) than for a first-order target letter flanked by second-order letters (212); (4) the spatial extent of crowding does not depend on the order type of the target or flanking letters. In the following subsections, we will first briefly describe our framework, then compare our findings with the predictions of the framework, as well as with the findings in normal fovea and periphery.

Framework

The framework we proposed earlier to explain how first- and second-order stimuli are combined in normal foveal and peripheral vision (Chung et al, 2007) is based upon several assumptions: (1) the visual system generally places more weighting on first-than second-order signals; (2) signals from targets are weighted more than signals from flankers and (3) flankers of the same order type as the target are given more weighting than flankers that differ from the order type of the target. Can we account for the findings obtained from the amblyopic observers based on this framework, taking into consideration the associated assumptions? In the following paragraph, we will present the various predictions based on the assumptions of our framework and how our data compare with these predictions. We will also compare the data obtained from the amblyopic observers with those obtained in the normal fovea and periphery.

The first prediction, based upon the first assumption, is that first-order target letters suffer from less crowding than second-order target letters, because first-order signals are given more weight than second-order signals and are thus considered as more reliable. As in the normal fovea and periphery, data obtained from the non-amblyopic and amblyopic eyes of our observers are consistent with this prediction. The magnitude of crowding is clearly smaller for the 111 condition than for the 222 condition, for the non-amblyopic (111: 1.027 ± 0.031; 222: 1.446 ± 0.210) and amblyopic eyes (111: 1.114 ± 0.025; 222: 1.438 ± 0.148) alike (Figure 3).

The second prediction is that for first-order targets, the flankers would be more effective for first- (same order) than second-order (different) letters, thus crowding should be stronger for the 111 than the 212 condition. Similarly, we also expect that for second-order targets, the 222 condition should yield more crowding than the 121 condition. These are true in the normal periphery. However, in the normal fovea, crowding virtually does not exist for the 111 condition, thereby leaving no room for the 212 condition to show an even smaller crowding effect (Chung et al, 2007; see also Figure 3). In this study, we found that both the non-amblyopic and amblyopic eyes conform to our prediction only for the second-order target conditions (crowding is stronger for the 222 than the 121 condition) but not for the first-order target conditions, akin to what we observed in the normal fovea. With first-order targets, because the 95% confidence intervals of the magnitude of crowding include the value of 1.0 (no crowding) for both the 111 and 212 conditions in the non-amblyopic eyes, we conclude that there is no significant crowding for these two conditions. For the amblyopic eyes, the 95% confidence intervals of the magnitude of crowding do not include the value of 1.0; however, these magnitudes are very small (111: 1.114 ± 0.025; 212: 1.143 ± 0.043). Therefore, we speculate that one reason why the amblyopic visual system does not conform to our prediction that the 111 condition should yield stronger crowding than the 212 condition is because of the small magnitude of crowding found in this study, i.e. a potential floor effect. However, it remains possible that our assumption is not entirely correct. In the normal periphery (Chung et al, 2007; see also Figure 3), crowding is clearly weaker for the 212 than the 111 condition. Here, in the amblyopic eyes, the magnitude of crowding is practically identical for the 111 and 212 conditions. In other words, the magnitude of crowding seems to be stronger for the 212 condition in amblyopic eyes than in the normal periphery. This difference in result between the normal periphery and the amblyopic eyes suggests that the amblyopic eyes may be more susceptible to the suppressive spatial interaction due to flankers, regardless of the specific properties of the flankers. Consistent with this notion, Wong et al (2005) showed that threshold for detecting a second-order sinusoidal patch increases in the presence of flanking patches, but the threshold elevation is independent of the orientation (collinear or orthogonal) of the flankers with respect to the target. It is also consistent with the finding that in the normal periphery no crowding occurs when the first-order target and flankers have orthogonal carrier orientations (Levi et al., 2002b), whereas in amblyopic vision crowding does occur with orthogonal target and flanker carrier orientations (Levi et al., 2002c).

When compared with the normal fovea and periphery, the non-amblyopic and amblyopic eyes exhibit at least one other qualitative difference in the pattern of results — the magnitude of crowding is virtually identical for the 121 and the 111 condition in the normal fovea and periphery; whereas the magnitude of crowding is larger for the 121 than the 111 condition in both the non-amblyopic and amblyopic eyes (Figure 3). According to our prediction, on one hand, because the flanker and the target letters are of the same order type, we would predict that the 111 condition should yield *more* crowding than the 121 condition. On the other hand, because first-order targets are usually given more weight than second-order targets, it follows that the signal (target letter) in the 111 condition is considered as more reliable, and thus the 111 condition should yield *less* crowding when compared with the 121 condition. Although these two predictions, both based upon our assumptions, seem contradictory, there is no reason why they cannot co-exist, and thus co-determine the final outcome of the magnitude of crowding. In fact, perhaps this is exactly why the magnitude of crowding for the 111 and 121 conditions are so similar in the normal fovea and periphery. In the amblyopic visual system (both the non-amblyopic and amblyopic eyes), however, there may exist additional factors that cause stronger crowding for the 121 condition. One candidate explanation is the documented extra loss in sensitivity in processing second-order information in the amblyopic visual system.

Amplified loss in sensitivity to second-order targets in amblyopia?

Several studies of second-order visual processing in amblyopia have shown that the amblyopic deficit is larger for second- than first-order stimuli, for stationary (Mansouri et al, 2005; Simmers et al , 2005; Wong et al, 2001) as well as motion stimuli (Simmers et al, 2003; 2005). For example, when comparing the detection thresholds for localized patches of first- or second-order sinusoids, Wong et al (2001) found an additional loss in sensitivity for detecting the second-order stimuli, even after equating for the first-order spatial input. In a later study, the same authors assessed the effect of second-order flankers on the detection threshold of a second-order target, and found that even though the flankers facilitate the detection of the target in people with normal vision, the effect is suppressive instead of facilitatory in amblyopic observers, in both the non-amblyopic and amblyopic eyes (Wong et al, 2005). Here, we found that the contrast threshold ratio for identifying single first- and second-order letters (of the same size) is larger for the amblyopic than the non-amblyopic eyes. All these findings provide evidence that amblyopic observers experience more difficulties processing second-order than first-order information, which may explain the larger magnitude of crowding shown for the 121 than the 111 condition, even though the flankers were of the same order type in both conditions.

Critical distance for crowding

As in the normal fovea and periphery, the critical distance for crowding does not seem to depend on the order type of the target and flanking letters (Figure 3). Averaged across the four trigram conditions, the critical distances for crowding are 2.35 and 3.14 deg, for the non-amblyopic and amblyopic eyes, respectively. The value obtained for the non-amblyopic eye is highly comparable to that obtained in the normal fovea (2.29 deg: Chung et al, 2007, see also Figure 3). Previously, Levi et al (2002c) examined the properties of crowding using E patterns that were made up of Gaussian or Gabor patches in amblyopic observers and compared the results with those obtained in the normal fovea (Levi et al, 2002a). They found that the properties of crowding obtained in the non-amblyopic eyes resemble those of the normal fovea — crowding is scale invariant and is attributable to simple contrast masking. Here, we showed that using our paradigm, the critical distance for crowding is also comparable between the non-amblyopic eyes and the normal fovea.

For traditional small first-order targets, the spatial extent of crowding has been reported to be larger in amblyopic eye than in the normal fovea (e. g. Flom et al, 1963; Hariharan et al, 2005; Hess et al, 2001; Hess & Jacobs, 1979; Levi et al, 2002c; Levi & Klein, 1985; Simmers et al, 1999). Hariharan et al (2005), Hess et al (2001) and Levi et al (2002c) further showed that for small letters, the larger extent of crowding in the amblyopic eyes is not simply due to a scale shift, i.e. the use of larger receptive field because of the poorer acuity. In this study, we showed that the larger crowding zone reported for the amblyopic eyes, when compared with normal fovea, also applies to second-order stimuli. This result is not surprising given the critical distances for crowding determined in this study do not depend on the order type of the letters.

Spatial scales for first- and second-order letters

The magnitude and the spatial extent of crowding have been shown to depend on the similarity between the target and its flankers, with respect to stimulus attributes such as spatial-frequency content (Chung et al, 2001), shape (Kooi, Toet, Tripathy & Levi, 1994; Nazir, 1992) color (Kooi et al, 1994), contrast polarity (Kooi et al, 1994), orientation (Andriessen & Bouma, 1976; Levi et al, 2002b; Hariharan et al, 2005) etc. In this study, the use of the same letter size for first- and second-order letters to construct our trigrams has at least two consequences in relation to the similarity or differences between the target and flankers, besides the obvious difference of the order type of the letters. First, our first- and second-order letters have different spectral composition. Specifically, the amplitude of the power spectrum of the first-order letters shows a clear peak around 2 c/letter, corresponding to the band of spatial frequencies most informative for letter identification (Chung, Legge & Tjan, 2002; Legge, Pelli, Rubin & Schleske, 1985; Majaj, Pelli, Kurshan & Palomares, 2002; Solomon & Pelli, 1994); while the power spectrum of the second-order letters is flat across a range of spatial frequencies, given that the second-order letters are composed of arrays of white noise. Previously, we found that perceptual learning of identification of second-order letters did not transfer to the task of identifying first-order letters of the same letter size, a finding that could be attributed to the use of different spatial scales in analyzing these letters (Chung et al, 2006a). However, the use of different spatial scales in analyzing first- and second-order letters would have predicted that crowding does not occur for the cross-over conditions.

Second, as we reported earlier, all observers had substantially (≈ 6 times) for first- than for second-order letters. When constructing the trigrams for each observer, we used one fixed letter size that corresponded to approximately 1.3× the size-threshold of identifying second-order letters in the amblyopic eyes, which also corresponded to approximately 8× the size-threshold of identifying first-order letters. In other words, the letter size used was near threshold size for second-order letters, but was very suprathreshold for first-order letters. This may in part explain why all four conditions (111, 222, 212 and 121) show similar extents of crowding. Unlike crowding in the normal fovea, the extent of crowding in amblyopic vision does not depend strongly on letter size when the stimuli are small (Levi et al, 2002c). However, when the stimuli are large, the extent of crowding increases, becoming similar to the normal fovea. Thus, the fact that the extent of crowding was similar across conditions maybe, at least in the normal fovea and that of the amblyopes, be a consequence of using the same letter size. Indeed, four of the observers in the current study participated in a separate study in which we determined the critical distance for crowding with near acuity size letters (Levi, Song & Pelli, 2007). Here, we show their critical distances, along with the mean of three normal observers at the fovea and 10° eccentricity in Figure 4 (small symbols). The critical distances obtained for stimuli near size-threshold in the normal fovea and both eyes of the amblyopic observers are much smaller than those obtained with the suprathreshold letters. This helps to explain why first- and second-order crowding in the fovea (normal and amblyopic) is similar in size. Interestingly, this is not the case for the normal periphery. The critical distance for near threshold size letters is quite similar to those obtained here with suprathreshold letters. However, the main point of this study was to examine cross-over crowding. The fact that we found substantial cross-over crowding despite a difference in the spectral composition between first- and second-order letters, and that first-order letters were much larger than the size thresholds when compared with second-order letters, imply that spatial scale, undoubtedly important at the early stage of analysis of letters, does not play an important role at the (later) stage of analysis where crowding occurs.

Neural basis of crowding in amblyopia

As noted in the Introduction, there are three main hypotheses to account for the extensive crowding in amblyopia: (1) enlarged cortical receptive fields (Flom et al., 1963); (2) abnormal long-range inhibitory interactions (Levi & Klein, 1985; Bonneh et al, 2004; Ellemberg et al, 2002; Wong et al, 2005), and (3) abnormal integration of target and flankers beyond the stage of feature detection (Levi et al., 2002; Pelli et al., 2004; Hariharan et al., 2005).

Our cross-over crowding results cannot be simply explained by enlarged receptive fields in early visual areas, since first- and second-order stimuli are processed separately and independently in early vision. It is less clear whether abnormal long-range inhibitory interactions occur between first-and second-order stimuli in early vision. Ellenberg et al (2002) suggest that an abnormal topographic representation of the stimulus (with normal contrast-coding) may account for the abnormal perceived contrast in strabismic (but not anisometropic) amblyopia. Specifically they state that “If there is a positional abnormality in amblyopia that precedes or occurs at the same point as the contrast/gain computation that underlies this task, then such an abnormality in contrast-coding would be expected.” It is not clear how such an early positional abnormality would explain cross-over crowding. An alternative idea is that crowding results from a combination or pooling of signals from the target and its flankers after the stage of feature extraction (Chung et al, 2001; Levi et al, 2002b; Parkes, Lund, Angelucci, Solomon & Morgan, 2001; Pelli et al, 2004). Indeed, Bonneh et al (2004) suggest that amblyopic crowding may reflect an inaccurate and scattered top-down attentional selection mechanism. Our result of a similar critical distance for crowding for the different trigram conditions is consistent with the view that the receptive field properties at the combination site are indifferent to the order type of letters. Our current finding provides additional evidence that the pooling of signals takes place over a large spatial distance in amblyopic vision.

Physiological studies have implicated the cortical area V4 as the plausible site for the combination of signals from different stimulus types (Ferrera, Nealey & Maunsell, 1992; 1994;Logothetis & Charles, 1990). In addition, V4 has also been implicated as the site for crowding in macaque monkeys (Motter, 2002). Previously, we compared the critical distances for crowding obtained from the normal fovea and periphery with the receptive field size in V4 as reported by Smith et al (2001) using the fMRI technique. We found that the total extent of crowding (2× critical distance for crowding) matches the receptive field size in V4 in the normal periphery (10° eccentricity) rather well. At the fovea, the total extent of crowding is much larger than the receptive field size in V4 as reported by Smith et al (2001). We attributed this difference to the different mechanisms underlying foveal and peripheral crowding. As suggested by Levi et al (2002a,b), foveal crowding is simply contrast masking whereas peripheral crowding represents the genuine crowding effect and is limited by the pooling of signals at the second (combination) stage of processing. Considering that the properties of crowding in the non-amblyopic eye resemble those in the normal fovea, while the properties of crowding in the amblyopic eye resemble those of genuine crowding (Levi et al, 2002c), our current findings suggest that the difference in the critical distances for crowding between the non-amblyopic and amblyopic eye can also be attributed to the different resolution limit of the two-stage process of crowding. In other words, the critical distances for crowding in the non-amblyopic and amblyopic eyes are limited by different mechanisms that may have different neural origins.

ACKNOWLEDGMENTS

This study was supported by research grants R01-EY12810 (STLC) and R01-EY01728 (DML) from the National Eye Institute, National Institutes of Health.

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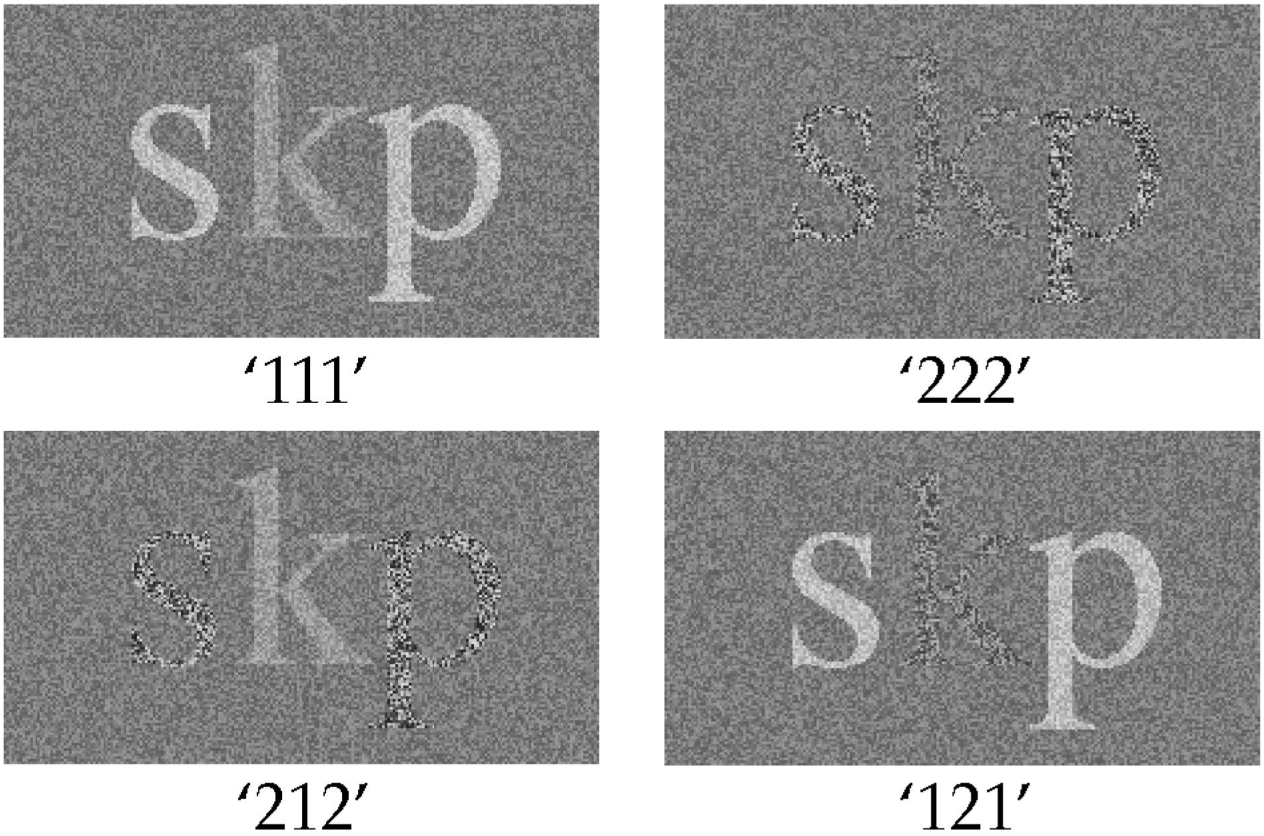

'111' '222'

'212' '121'

Figure 1.

The trigram "skp" illustrated for the four testing conditions: (top-left) a first-order target (middle) letter flanked by two first-order letters (the "111" condition); (top-right) a second-order target letter flanked by second-order letters ("222"); (bottom-left) a first-order target letter flanked by second-order letters ("212") and (bottom-right) a second-order target letter flanked by first-order letters ("121").

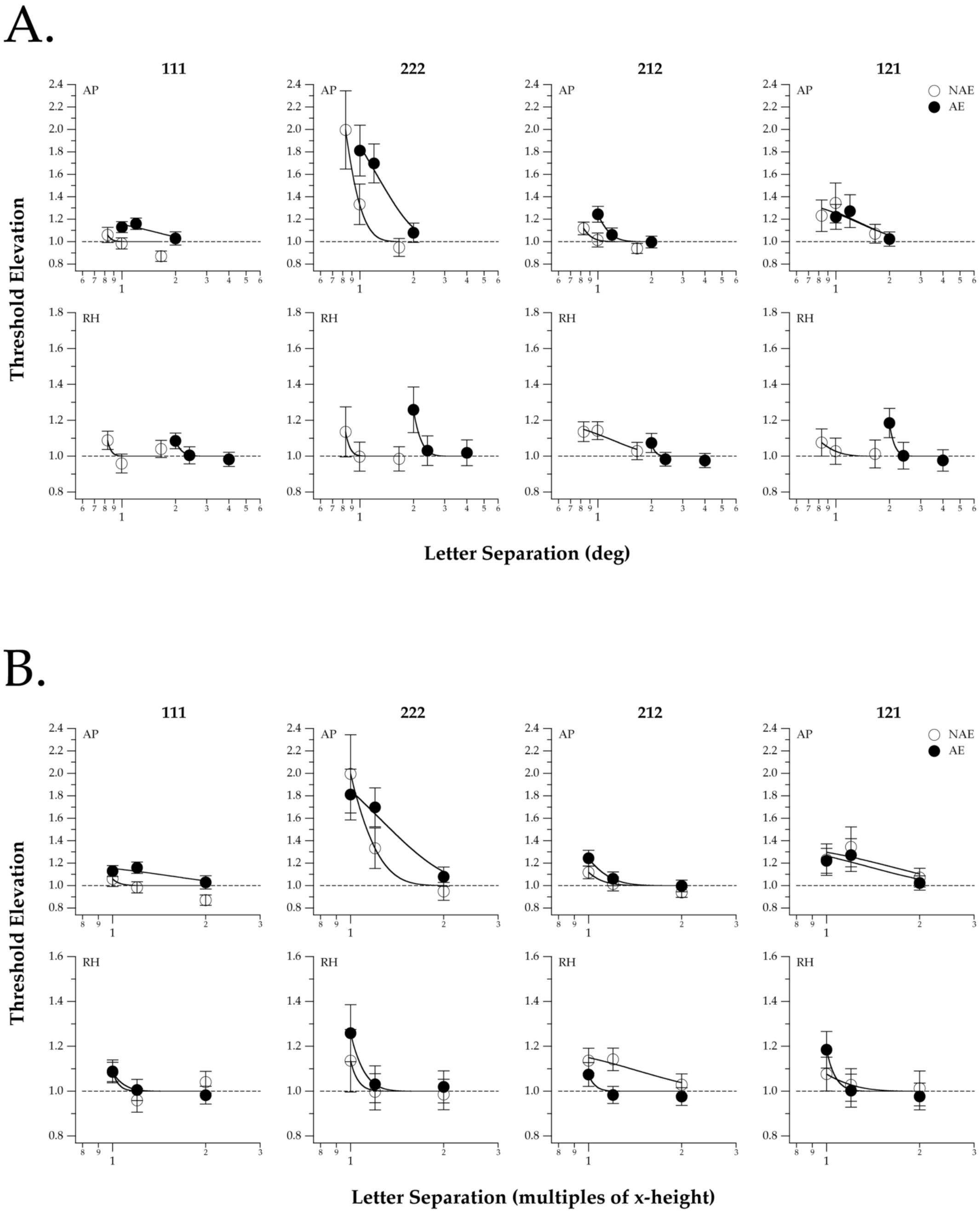

Figure 2.

Threshold elevation, ratio of contrast threshold for identifying flanked and unflanked (single) letters, is plotted as a function of letter separation in (A) degrees or (B) multiples of letter size, for the four trigram conditions. Data shown are obtained from observers AP and RH, but are representative of other five observers. Solid lines represent the best-fit curves (see text for details) through each set of data. Dashed lines represent the null effect (absence of threshold elevation). Error bars represent ± 1 S.E.M.

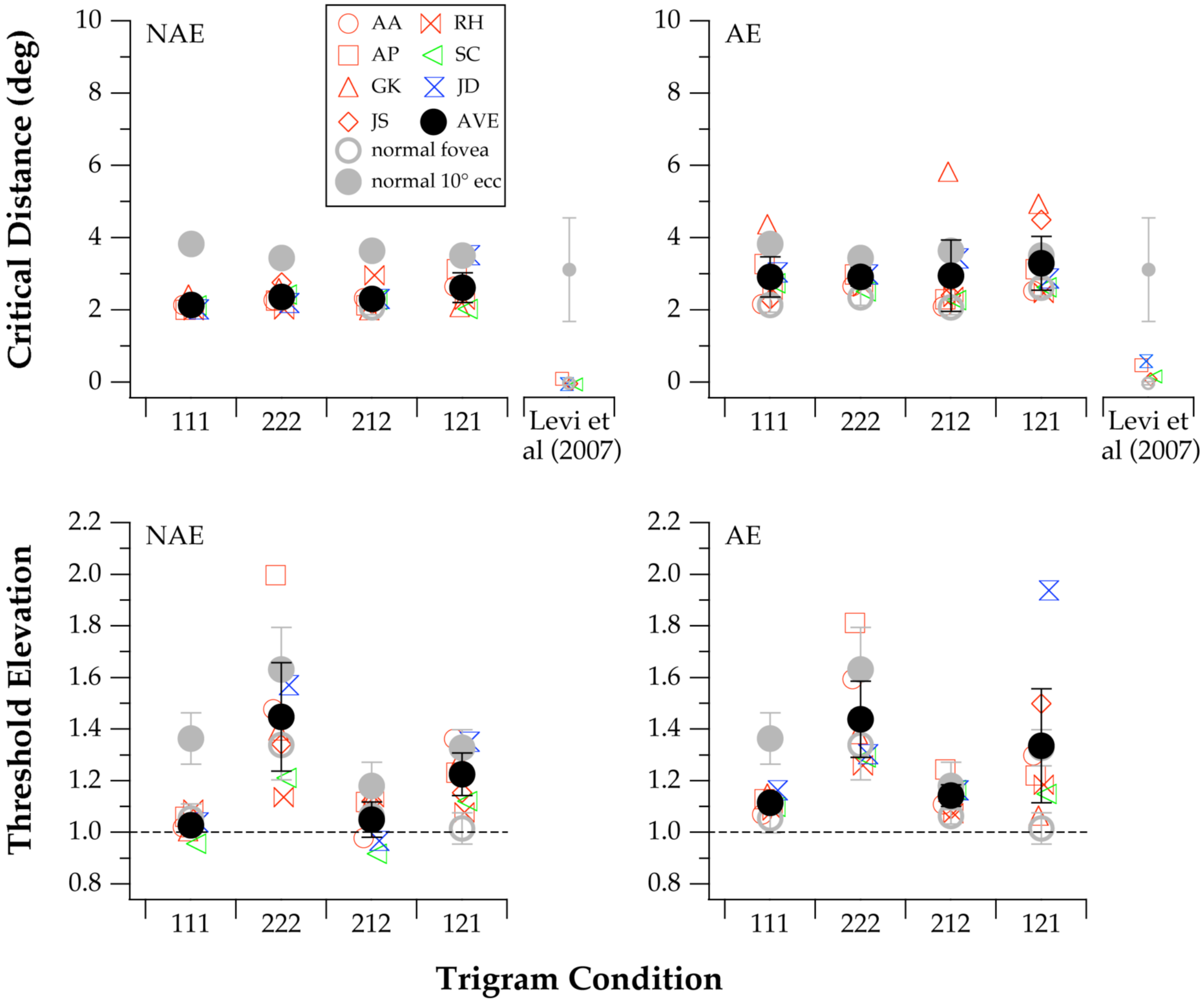

Figure 3.

Critical distance in degrees (upper panels), representing the spatial extent of crowding and threshold elevation (lower panels) representing the peak magnitude of crowding are plotted for the four trigram conditions, for the non-amblyopic (left) and amblyopic eyes (right). Data for each observer are plotted as different colored symbols (red: strabismic observers; green: anisometropic observer; blue: strabismicanisometropic observer) and the group-averaged values (± 95% confidence intervals) are plotted as filled black circles. For comparison, data from the normal fovea (unfilled gray symbols) and periphery (10° eccentricity: filled gray symbols) are replotted from Chung et al (2007) as gray symbols, and the extent of crowding measured using stimuli close to size thresholds from Levi et al (2007) are replotted as small symbols. Dashed lines in the lower panels represent the null effect (no crowding).

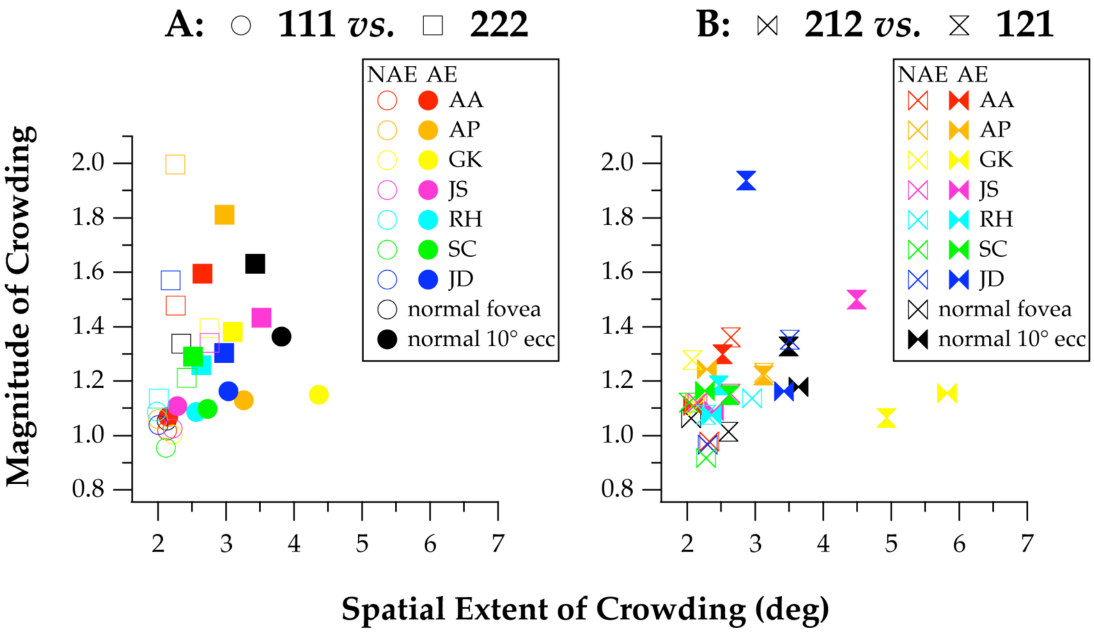

Figure 4.

Scatter plots showing the magnitude of crowding as a function of the spatial extent of crowding (deg; values replotted from the "critical distance" in Figure 3) for the non-amblyopic (NAE: unfilled symbols) and amblyopic (AE: filled symbols) eyes of each observer (different color). Panel A presents data for the same-order crowding (111 and 222 conditions) while panel B presents data for the cross-over conditions (212 and 121). Although the spatial extent of crowding seems to be larger for the amblyopic than the non-amblyopic eyes (note the horizontal displacement between filled and unfilled symbols), the ranges of the magnitude of crowding are similar between the two eyes.

Table 1

Visual characteristics of the amblyopic observers.

Observer	Gender	Age (years)	Type	Eye	Visual Acuity (logMAR)	Refractive Errors	Eye Alignment	Stereoacuity (if any)	Letter Size Used (deg)
AA	F	29	Strab	OD	0.14	-2.00/-2.25×180	>30Δ Alt ET		1.17
				OS	-0.04	-3.75/-2.00×005	10Δ RHyperT		1.17
AP	F	21	Strab	OD	-0.16	-1.50/-0.50×180	3-4Δ LET		0.83
				OS	0.40	-0.75/-0.25×180	2Δ LHyperT		1
GK	M	25	Strab	OD	0.04	+0.50/-2.25×010	12Δ RET		1.33
				OS	-0.10	+0.50/-2.25×170	10Δ RHyperT		0.83
JS	F	21	Strab	OD	-0.10	+1.25	6-8Δ LET		1.3
				OS	0.30	+1.00	4-6Δ LHyperT		1.5
RH	M	41	Strab	OD	-0.10	-1.00/-0.50×170	Microtropia 2Δ	200″	0.83
				OS	0.50	-1.50/-1.50×010	LET		2
SC	M	30	Aniso	OD	-0.14	+0.50		70″	1
				OS	0.32	+3.25/-0.50×155			1.67
JD	M	21	Strab +	OD	-0.10	+2.50	3Δ LET		1
			Aniso	OS	0.80	+5.00			2