# Reduced fixation stability induced by peripheral viewing does not contribute to crowding

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Attending to peripheral visual targets while maintaining central fixation, a process that involves covert attention, reduces fixation stability. Here, we tested the hypothesis that changes in fixation stability induced by peripheral viewing contribute to crowding in peripheral vision by increasing positional uncertainty. We first assessed whether fixation was less stable during peripheral versus central (foveal) viewing for both crowded and uncrowded stimuli. We then tested whether fixation stability during peripheral viewing was associated with the extent of crowding. Fourteen participants performed a tumbling E orientation discrimination task at three different eccentricities (0°, 5°, 10°). The target was presented with or without flankers. Fixational eye movements were measured using an infrared video-based eyetracker. A central fixation cross was provided for the two peripheral viewing conditions, and optotype size was scaled for each eccentricity. Discrimination of appropriately scaled uncrowded stimuli was unaffected by eccentricity, whereas discrimination of crowded stimuli deteriorated dramatically with eccentricity, despite scaling. Both crowded and uncrowded peripheral stimuli were associated with reduced fixation stability, increased microsaccadic amplitude, and a greater proportion of horizontal microsaccades relative to centrally presented stimuli. However, these effects were not associated with the magnitude of crowding. This suggests that reduced fixation stability due to peripheral viewing does not contribute to crowding in peripheral vision.

# Introduction

Visual acuity deteriorates with increasing retinal eccentricity (Westheimer, 1979). This effect is most

pronounced when the acuity target is surrounded by flankers that induce crowding (Bouma, 1970) (Figure 1). The mechanisms that underlie visual crowding are of particular interest because crowding severely limits visual function in individuals with central vision loss who are forced to rely on peripheral vision (Chung, 2013; Wallace, Chung, & Tjan, 2017). In addition, amblyopia is associated with increased foveal crowding in the affected eye (Flom, Weymouth, & Kahneman, 1963; Hess, McIlhagga, & Field, 1997; Levi & Klein, 1985). Crowding may occur at an early stage (Chen, He, Zhu, Zhou, Peng, Zhang, & Fang, 2014; Nandy & Tjan, 2012) or at a late stage (Levi, 2008; Levi, 2011) of visual cortical processing. Higher level processes such as attention may also be involved (Chen et al., 2014; Levi, 2008).

The measurement of crowding in peripheral vision requires study participants to maintain central fixation while attending to peripheral stimuli, a process that involves covert allocation of attention (Carrasco, 2011). Both peripheral crowding and covert attention are associated with fixation stability-the magnitude and frequency of small involuntary eye movements such as microsaccades and ocular drifts that occur during fixation (Martinez-Conde, Macknik, & Hubel, 2004; Rolfs, 2009; Rucci & Poletti, 2015). As described below, fixational eye movements may contribute to crowding by increasing positional uncertainty (Bedell, Sideroy, Formankiewicz, Waugh, & Aydin, 2015; Macedo, Crossland, & Rubin, 2008), and covert allocation of attention may make fixation less stable (Sansbury, Skavenski, Haddad, & Steinman, 1973). The overall aim of this study was to explore the relationship between any changes in fixation stability induced by holding central fixation while attending to peripheral stimuli and the magnitude of crowding in peripheral vision.

Citation: Raveendran, R. N., Krishnan, A. K., & Thompson, B. (2020). Reduced fixation stability induced by peripheral viewing does not contribute to crowding. *Journal of Vision*, 20(10):3, 1–13, https://doi.org/10.1167/jov.20.10.3.

https://doi.org/10.1167/jov.20.10.3

Received March 2, 2020; published October 2, 2020

ISSN 1534-7362 Copyright 2020 The Authors





Figure 1. A demonstration of crowding in the peripheral vision. While fixating on the central target, it is easier to identify the letter "E" on the left-hand side than on the right-hand side, as the "E" on the left-hand side is not flanked.

With regard to crowding, fixational eve movements may complicate the segregation of a target from surrounding flankers by increasing positional uncertainty. Indeed, patients with macular degeneration experience both crowding (Wallace et al., 2017) and poor fixation stability (Kumar & Chung, 2014; Tarita-Nistor, Brent, Steinbach, & González, 2011; Tarita-Nistor, Gill, González, & Steinbach, 2017). Amblyopic eyes also exhibit increased crowding (Flom et al., 1963; Giaschi, Regan, Kraft, & Kothe, 1993; Hess et al., 1997; Levi & Klein, 1985), abnormally large fixational eye movements (González, Wong, Niechwiej-Szwedo, Tarita-Nistor, & Steinbach, 2012; Raveendran, Bobier, & Thompson, 2019a; Raveendran, Bobier, & Thompson, 2019b), and an association between reduced crowded visual acuity and reduced fixation stability (Bricolo, Salvi, Martelli, Arduino, & Daini, 2015: Chung & Bedell, 1995: Chung, Kumar, Li, & Levi, 2015; Raveendran et al., 2019b). When testing participants with normal vision, Macedo et al. (2008) observed a worsening of peripheral crowded visual acuity (but not uncrowded visual acuity) when the peripheral visual stimulus was moved to amplify normal fixational eye movements. In addition, Bedell et al. (2015) attributed exaggerated letter identification errors in long, crowded letter strings to fixational eye movements.

The properties of fixational eye movements when participants attend to a stimulus presented at fixation (overt allocation of attention) have been studied extensively (Martinez-Conde et al., 2004; Raveendran et al., 2019a; Rolfs, 2009; Steinman, 1965; Steinman, Cushman, & Martins, 1982). However, the effect of holding central fixation while attending to a stimulus in peripheral vision on fixational eve movements is less well understood. Sansbury et al. (1973) reported a deterioration of fixation stability associated with larger microsaccades and ocular drifts when a target was presented in the near periphery; however, the peripheral targets were presented in isolation, without a central fixation stimulus, and participants did not perform a visual task. The lack of a task may have limited the amount of attention directed to the periphery. More recently, several studies have measured the effect of covert attention on microsaccades (Engbert & Kliegl, 2003; Kulke, Atkinson, & Braddick, 2016; Laubrock, Engbert, & Kliegl, 2005; Lowet, Gomes, Srinivasan, Zhou, Schafer, & Desimone, 2018) and

reported that covert attention is associated with a stereotyped pattern of microsaccades toward and away from the attended stimulus (Engbert & Kliegl, 2003; Laubrock et al., 2005; Lowet et al., 2018). If covert attention does indeed influence the pattern and magnitude of fixational eye movements, it is possible that fixational eye movements associated with holding central fixation while performing a task in the visual periphery contribute to crowding in peripheral vision by increasing positional uncertainty.

The first objective of this study was to assess whether the pattern and/or magnitude of fixational eye movements differed when a visual discrimination task was performed at the point of fixation versus in peripheral vision. We employed a tumbling E discrimination task with the target presented in isolation or in the presence of bar flankers to induce crowding. Test stimuli were presented at fixation or at eccentricities of 5° or 10°. In addition, we tested whether the presence of flankers influenced fixation stability. The rationale was that the task with flankers is more difficult due to crowding and therefore may require greater attentional resources. The magnitude of crowding was defined as the decrease in task performance in the with-flanker condition compared to without-flanker condition (Ghahghaei & Walker, 2016; Musilová, Pluháček, Marten-Ellis, Bedell, & Siderov, 2018).

We found that holding central fixation while performing a task in peripheral vision was associated with less stable fixation and that the presence of flankers also reduced fixation stability. We then asked whether fixation stability was associated with the magnitude of crowding for the peripheral viewing task. Our hypothesis was that less stable fixation would be associated with greater crowding in peripheral vision. Contrary to our hypothesis, our results did not reveal evidence for a relationship between fixational eye movements associated with peripheral viewing and crowding in normal peripheral vision.

# Methods

#### Participants

Fourteen participants  $(38 \pm 7 \text{ years}; \text{nine females})$ took part in this study. All participants had bestcorrected visual acuity of at least 20/20 in both eyes and wore their habitual refractive correction while performing the task. All participants provided written, informed consent to take part in this study. The study was reviewed and approved by the Wichita State University research ethics committee. All study procedures adhered to the tenets of the Declaration of Helsinki.



Figure 2. Apparatus and visual stimuli.

#### Apparatus, visual stimuli, and procedure

Stimuli were presented on a liquid-crystal display monitor (24-inch; BenQ XL2420Z; BenQ Corporation, Taipei, Taiwan) with a resolution of  $1920 \times 1080$ pixels, placed at a distance of 114 cm from a chin and forehead rest (Figure 2). Black (3 cd/m<sup>2</sup>) tumbling E visual targets were presented with (crowded) and without (uncrowded) flankers (Figure 2, right panel) on a white background (69 cd/m<sup>2</sup>). Target contrast was approximately 95%.

Fixational eye movements were measured using a video-based infrared eyetracker, EyeLink 1000 Plus (SR Research, Ottawa, ON, Canada) at 1000 Hz. The eye tracker had an accuracy of 0.15°, a spatial resolution of 0.01° RMS, and microsaccade detection sensitivity of 0.05° (as indicated by the manufacturer). A nine-point calibration, validation, and drift correction process was completed at the start of each block of trials. The visual stimuli and the eye tracker were controlled by MATLAB software (MathWorks, Natick MA) using the Psychtoolbox (Brainard, 1997) and Eyelink Toolbox (Cornelissen, Peters, & Palmer, 2002) extensions.

All participants performed a monocular tumbling E orientation discrimination task at three different eccentricities: 0° (fovea), 5°, and 10°. Peripheral stimuli were presented to the temporal visual field of the dominant eye, which was determined using Porta's test (Zhang, Bobier, Thompson, & Hess, 2011). The non-viewing eye was occluded with an opaque black patch. Tumbling E targets were scaled individually for each participant based on their monocular near visual acuity measured using a standard crowded chart (near vision logMAR chart; Precision Vision, Woodstock, IL) at 40 cm. Target size was scaled to be 0.1, 0.6, and 0.8 logMAR larger than threshold visual acuity for the 0°, 5°, and 10° eccentricity viewing conditions, respectively (Westheimer, 1979). For flanked stimuli, four flanking bars surrounded the target. Flanking bars had the same stroke width as the target and were positioned at a

distance of one stroke width from the target. A central fixation cross, the same size as the 0° target ( $\approx 0.2^{\circ}$ ), was provided for the two peripheral viewing conditions (5° and 10°).

Monocular fixational eye movements were measured while the participants performed the orientation discrimination task. Participants were instructed to fixate on the central fixation cross ( $\approx 0.2^{\circ}$ ) for the two peripheral viewing conditions (5° and 10°). Trials were self-paced and blocked according to the factors of eccentricity (0°, 5°, or 10°) and flanker (present or absent). There were 15 trials in a block, and four blocks were presented per combination of eccentricity and flanker. Block presentation order was randomized.

#### Data analysis

For the tumbling E orientation discrimination data, the percentage of correct responses (CRs) was calculated for each combination of eccentricity and flanker status. The magnitude of crowding at each eccentricity was then calculated as a flanker effect (Ghahghaei & Walker, 2016) using the following equation:

Flanker effect (CR) = 
$$\frac{CR_{nf}}{CR_f} - 1$$
 (1)

where  $CR_{nf}$  and  $CR_f$  denote the percent correct responses in the without-flanker and with-flanker viewing conditions, respectively. A flanker effect of 0 indicates no effect of flankers on task performance. A positive flanker effect values indicate crowding (reduced task performance in the presence of flankers).

Horizontal and vertical eye movement traces were visually evaluated to eliminate saccades with an amplitude of  $\geq 2^{\circ}$ . We used a larger cutoff amplitude than most previous studies of fixation stability because

we wanted to ensure that we fully captured any effects of peripheral viewing on fixation stability. Therefore, the definition of microsaccades that we used for this study could potentially include both microsaccades and small-amplitude saccades. The eye movement data were converted to degrees and filtered using a Savitsky–Golay filter with an order of 3 and a frame length of 41 (Cherici, Kuang, Poletti, & Rucci, 2012; Ghasia & Shaikh, 2015; Ko, Snodderly, & Poletti, 2016). The filtered eye movement data were used for further analysis.

Fixation stability was quantified using global bivariate contour ellipse area (BCEA) (González et al., 2012; Raveendran, Babu, Hess, & Bobier, 2014; Steinman et al., 1982; Timberlake et al., 2005) defined by the following equation:

$$BCEA = \pi \chi^2 \sigma_x \sigma_y \sqrt{(1 - \rho^2)} \quad (2)$$

where  $\chi^2$  is the chi-square value (2 degrees of freedom) corresponding to a probability value of 0.682 (i.e.,  $\pm 1$  SD);  $\sigma_x$  and  $\sigma_y$  correspond to standard deviations of horizontal and vertical eye positions, respectively; and  $\rho$  corresponds to the Pearson correlation coefficient between horizontal and vertical eye positions. BCEA provides the area of the ellipse that encompasses 68% of eye positions within a trial; therefore, larger BCEA values indicate less stable fixation. Like percent correct responses, the flanker effect was also evaluated for fixation stability using a similar equation.

Flanker effect (BCEA) = 
$$\frac{BCEA_f}{BCEA_{nf}} - 1$$
 (3)

where  $BCEA_f$  and  $BCEA_{nf}$  denote fixation stability values for the flanker present and absent viewing conditions, respectively. A flanker effect of 0 indicates no effect of flankers on fixation stability. Positive flanker effect values indicate reduced fixation stability in the presence of flankers.

In addition, small saccades and microsaccades (hereafter referred to together as microsaccades) were detected using an unsupervised cluster detection method (Otero-Millan, Castro, Macknik, & Martinez-Conde, 2014). This method has been reported to reduce the errors in microsaccadic detection by 62% and does not require a criterion of microsaccades to be binocularly conjugate, meaning that it can be applied to monocular eye movements. Microsaccade direction was defined as horizontal if the angle ( $\theta$ ) was between 45° and 135° or 225° and 315°; otherwise, the direction was defined as vertical.

After logarithmic transformation, the values of percent correct responses, BCEA, and microsaccadic amplitudes were analyzed separately using repeated measures analysis of variance (ANOVA; raw values are presented in Figures 2 to 4) with factors of flanker (flanker present vs. flanker absent) and eccentricity (0°, 5°, and 10°). In addition to these analyses—correlations between the flanker effect on fixation stability and correct responses and for the with-flanker condition the change in percent correct from the central viewing condition and the change in BCEA/microsaccade amplitude from the central condition for both the 5° and 10° conditions were analyzed to determine the relationship between crowding and fixational eye movements. The distribution of microsaccade directions was analyzed using an unpaired Kruskal–Wallis test and Dunn's multiple comparison analysis to test for statistically significant differences between conditions.

#### Results

All 14 participants completed both viewing condition sessions. Figure 3 shows an example of heatmaps of gaze distribution for a representative participant.

#### **Correct responses**

Table 1 presents the mean percent correct responses and BCEA for each participant. For percent correct responses (Figure 4a), there was a significant main effect of flanker, F(1, 13) = 172.02, p < 0.001; a significant main effect of eccentricity, F(2, 26) =59.92, p < 0.001; and a significant interaction between these two factors, F(2, 26) = 67.53, p < 0.001. When flankers were present, percent correct was significantly lower at 5° (p < 0.001) and 10° (p < 0.001) eccentricity compared to 0° eccentricity. Percent correct was also significantly lower at 10° compared to 5° eccentricity (p < 0.001) when flankers were present. There were no differences in percent correct between any of the eccentricities when flankers were absent (p > 0.05). There was no significant difference between the with-flanker and without-flanker conditions for the 0° eccentricity condition (p > 0.05). However, as expected, there were large and significant differences between the with-flanker and without-flanker conditions for the 5° (p < 0.001) and 10° (p < 0.001) eccentricity conditions. This pattern of results is consistent with the well-documented properties of crowding in peripheral vision that our stimuli were designed to produce.

#### **Fixation stability**

For fixation stability quantified as logBCEA (Figure 4b), there was a significant main effect of flanker, F(1, 13) = 6.63, p = 0.02, and a significant





Figure 3. Representative heatmaps of gaze distribution during both with- and without-flanker viewing conditions for all three eccentricities. Color codes represent the probability distribution of gaze positions.

main effect of eccentricity, F(2, 26) = 43.29, p < 0.001. There was no significant interaction between these two factors, F(2, 26) = 0.24, p = 0.79. Post hoc analysis revealed significantly less stable fixation at 5° (p < 0.001) and 10° (p < 0.001) compared to 0° eccentricity when flankers were present. There was also significantly less stable fixation at 10° compared to 5° eccentricity when flankers were present (p = 0.04). Similarly, when flankers were absent, there was significantly less stable fixation at 5° (p < 0.001) and 10° (p < 0.001) compared to 0° eccentricity. Fixation stability did not differ between the 5° and 10° eccentricities when flankers were absent (p = 0.26). There was significantly less stable fixation in the with-flanker compared to the without-flanker condition at the eccentricities of 0° (p = 0.03) and 10° (p = 0.02), but not at 5° (p = 0.18).

A recent study by Krishnan, Agaoglu, and Chung (2017) reported that fixation duration influences fixation stability. Because our task was self-paced, it is was possible that the overall duration of fixation could vary between the different conditions. Indeed, an ANOVA conducted on the mean time taken to complete



Figure 4. Mean percentage of correct responses, fixation stability (BCEA), and flanker effect as a function of stimulus eccentricity. The mean  $\pm$  *SEM* of correct responses (a) and fixation stability calculated using all eye movement data from each trial block (b) or only the first 15 seconds of data from each trial block (c). The effect of flankers on correct responses (solid line) and BCEA (dotted line) are shown in (d). Data were collected while participants performed the tumbling E orientation discrimination task with (circular markers) and without (square markers) flankers.

|             |                 | Со    | rrect respo | onses (mear    | n, %)  |        |                 | Fixation stability |      |                | BCEA) (mean, deg <sup>2</sup> ) |      |  |  |
|-------------|-----------------|-------|-------------|----------------|--------|--------|-----------------|--------------------|------|----------------|---------------------------------|------|--|--|
| Participant | Flanker present |       |             | Flanker absent |        |        | Flanker present |                    |      | Flanker absent |                                 |      |  |  |
|             | 0°              | 5°    | 10°         | 0°             | 5°     | 10°    | 0°              | 5°                 | 10°  | 0°             | 5°                              | 10°  |  |  |
| P1          | 95.00           | 61.50 | 46.65       | 98.00          | 93.50  | 83.35  | 0.13            | 0.16               | 0.21 | 0.11           | 0.19                            | 0.22 |  |  |
| P2          | 96.94           | 80.00 | 53.33       | 100.00         | 100.00 | 98.33  | 0.40            | 0.65               | 0.80 | 0.39           | 0.64                            | 0.74 |  |  |
| P3          | 100.00          | 66.48 | 53.30       | 100.00         | 100.00 | 98.33  | 0.26            | 0.41               | 0.43 | 0.21           | 0.30                            | 0.37 |  |  |
| P4          | 100.00          | 55.00 | 56.68       | 100.00         | 95.00  | 89.90  | 0.24            | 0.53               | 0.82 | 0.16           | 0.48                            | 0.46 |  |  |
| P5          | 100.00          | 80.03 | 57.67       | 100.00         | 100.00 | 100.00 | 0.11            | 0.34               | 0.28 | 0.09           | 0.25                            | 0.29 |  |  |
| P6          | 100.00          | 46.68 | 55.00       | 100.00         | 93.33  | 98.33  | 0.24            | 0.31               | 0.47 | 0.17           | 0.16                            | 0.29 |  |  |
| P7          | 100.00          | 57.78 | 46.67       | 98.33          | 91.67  | 81.67  | 0.27            | 0.26               | 0.23 | 0.21           | 0.30                            | 0.24 |  |  |
| P8          | 100.00          | 73.33 | 61.67       | 98.33          | 98.33  | 73.34  | 0.21            | 0.41               | 0.56 | 0.17           | 0.31                            | 0.42 |  |  |
| Р9          | 98.33           | 61.67 | 35.00       | 96.67          | 93.34  | 86.67  | 0.26            | 0.28               | 0.45 | 0.18           | 0.35                            | 0.34 |  |  |
| P10         | 81.67           | 55.53 | 31.65       | 93.33          | 86.67  | 81.67  | 0.23            | 0.46               | 0.63 | 0.18           | 0.43                            | 0.55 |  |  |
| P11         | 100.00          | 63.34 | 40.00       | 100.00         | 80.00  | 73.34  | 0.11            | 0.13               | 0.14 | 0.11           | 0.19                            | 0.17 |  |  |
| P12         | 100.00          | 56.67 | 55.00       | 100.00         | 98.33  | 98.33  | 0.17            | 0.28               | 0.24 | 0.25           | 0.32                            | 0.30 |  |  |
| P13         | 80.00           | 83.34 | 63.34       | 85.00          | 100.00 | 95.00  | 0.21            | 0.40               | 0.48 | 0.19           | 0.23                            | 0.28 |  |  |
| P14         | 98.33           | 71.67 | 51.67       | 100.00         | 95.00  | 86.67  | 0.32            | 0.61               | 0.72 | 0.27           | 0.34                            | 0.42 |  |  |
| Mean        | 96.45           | 65.21 | 50.54       | 97.83          | 94.65  | 88.92  | 0.23            | 0.37               | 0.46 | 0.19           | 0.32                            | 0.36 |  |  |
| SEM         | 1.81            | 2.94  | 2.54        | 1.11           | 1.54   | 2.51   | 0.02            | 0.04               | 0.06 | 0.02           | 0.03                            | 0.04 |  |  |

Table 1. Percent correct responses and fixation stability for each participant.

a block of trials revealed a significant main effect of eccentricity, F(2, 26) = 6.11, p = 0.007, but not flanker, F(1, 13) = 1.70, p = 0.22, and no significant interaction, F(2, 26) = 0.61, p = 0.55. Tukey's honestly significant difference (HSD) test revealed that, when flankers were present, participants took significantly longer to complete blocks of 15 trials at 5° ( $24 \pm 1.5$  seconds; p = 0.01) and 10° ( $26 \pm 1.5$  seconds; p < 0.001) eccentricity compared to 0° ( $20 \pm 1.0$  seconds) eccentricity. There were no between-eccentricity differences in trial block duration when flankers were absent (p > 0.05).

To assess whether fixation duration influenced our fixation stability results, we repeated our analysis of fixation stability using only the first 15 seconds of data for each block to compute BCEA values (Figure 4c). The pattern of results remained largely unchanged. There was a significant main effect of flanker, F(1, 13) = 4.70, p = 0.049, and a significant main effect of eccentricity, F(2, 26) = 45.15, p < 0.001. There was no significant interaction between these two factors, F(2, 26) =0.61, p = 0.55. Post hoc analysis revealed significantly less stable fixation at 5° (p < 0.001) and 10° (p < 0.001) 0.001) eccentricity compared to 0° eccentricity in the with-flanker and without-flanker viewing conditions. Based on these results, we used BCEA values calculated using all data from each block for subsequent analyses.

# Association between flanker effect and fixation stability

The aim of this analysis was to test for an association between fixation stability and crowding as measured by the flanker effect. Flanker effects for percent correct and fixation stability were quantified using Equations 1 and 3, respectively. Group averages for the percent correct flanker effect and the BCEA flanker effect as a function of eccentricity are shown in Figure 4d. There was a significant effect of eccentricity on the percent correct flanker effect, F(2, 26) = 43.59, p < 0.001, and post hoc Tukey's HSD tests revealed that the flanker effect was significantly greater than 0 at 5° (p < 0.001) and 10° (p< 0.001). However, there was no effect of eccentricity on the flanker effect for BCEA, F(2, 26) = 0.16, p = 0.86. In addition, there was no significant correlation between the percent correct and BCEA flanker effects at the eccentricities of 5° (r = -0.1, p = 0.85) (Figure 5a) or 10° (r = 0.2, p = 0.41) (Figure 5b). There was also no significant correlation between the change in percent correct for the with-flanker stimuli from the central condition and the change in BCEA for 5° (r = 0.3, p = 0.12) (Figure 5c) and  $10^{\circ}$  (r = 0.3, p = 0.24) (Figure 5d).

# Microsaccadic amplitude, frequency and direction

Figure 6 shows microsaccade characteristics. The main sequence plots exhibited the anticipated linear relationship between microsaccadic amplitude and peak velocity, suggesting that microsaccades were identified correctly (Bahill, Clark, & Stark, 1975; Martinez-Conde, Macknik, Troncoso, & Hubel, 2009) (Figures 6a and 6b). The distribution of microsaccadic amplitudes for each combination of eccentricity and flanker (Figures 6c and 6d) revealed a larger proportion of smaller amplitude microsaccades for the foveal viewing conditions compared to the peripheral viewing conditions. Furthermore, despite the relatively large cutoff amplitude used to define microsaccades in this study, it is evident that very few saccades exceeded an amplitude of 1°.

Figure 6e shows mean microsaccadic amplitude. There was a significant main effect of eccentricity, F(2,(18) = 9.42, p < 0.001, on microsaccadic amplitude, but there was no significant effect of flanker, F(1, 9) = 1.06, p = 0.33, nor a significant interaction, F(2, 18) = 1.25, p = 0.31. Post hoc analyses revealed significantly larger microsaccadic amplitudes at 5° (p = 0.03) and at 10° (p < 0.001) compared to 0° eccentricity when flankers were present. Similarly, microsaccadic amplitude was significantly larger at 5° (p = 0.001) and at 10° (p =0.001) compared to 0° eccentricity when flankers were absent. Post hoc analyses did not reveal any significant differences between the with- and without-flanker conditions at any eccentricity. These results indicated that overall microsaccadic amplitudes were larger in the peripheral viewing conditions. Like BCEA, for the with-flanker condition there were no significant correlations between the change in percent correct from the central condition and the change in microsaccadic amplitude for the 5° (r = 0.1, p = 0.70) and 10° (r = 0.2, p = 0.87) eccentricity conditions.

Microsaccadic frequencies were calculated, and Figure 6f shows the mean microsaccadic frequency as a function of eccentricity and flanker. There was no significant main effect of eccentricity, F(2, 12) = 0.16, p = 0.85, or flanker, F(1, 6) = 0.078, p = 0.79, and no interaction between the two factors, F(2, 12) = 1.46, p = 0.27.

Table 2 shows the percentage of microsaccades that were horizontal in the with-flanker and without-flanker conditions for each eccentricity, and Figure 7 shows polar histograms of percent distribution of microsaccade direction for the withand without-flanker conditions. A Kruskal–Wallis test showed a significant difference between the conditions (p < 0.001). For the with-flanker condition, an increased percentage of horizontal microsaccades was observed for the 5° (MD = 148, p < 0.001) and  $10^{\circ}$  (MD = 171.5, p < 0.001) eccentricity conditions



Figure 5. Flanker effects for percent CRs and fixation stability (BCEA). (a, b) The relationship between the two flanker effects for the  $5^{\circ}$  (a) and  $10^{\circ}$  (b) eccentricity conditions. (c, d) The relationship between the change in correct responses and BCEA from the central fixation condition for the  $5^{\circ}$  (c) and  $10^{\circ}$  (d) eccentricity conditions for the with-flanker stimuli. Each point represents an individual participant.

|                                 | Eccentricity (%) |                            |                            |  |  |  |
|---------------------------------|------------------|----------------------------|----------------------------|--|--|--|
| Viewing condition               | 0°               | 5°                         | 10°                        |  |  |  |
| With-flanker<br>Without-flanker | 60.4<br>53.8     | 75.1 (48.4)<br>78.8 (50.9) | 75.5 (52.4)<br>77.1 (47.3) |  |  |  |

Table 2. Percentage of microsaccades that were horizontal for each eccentricity. The percentages of horizontal microsaccades that were directed toward the target are given inside the parentheses. Data are pooled across participants.

compared to the 0° eccentricity. Similarly, for the without-flanker condition, an increased percentage of horizontal microsaccades was observed for the 5° (MD = 286.4, p < 0.001) and 10° (MD = 264.2, p < 0.001) eccentricity conditions compared to the 0° eccentricity. There was no difference in the proportion of horizontal microsaccades between the with-flanker and without-flanker conditions at any eccentricity (p > 0.5).

## Discussion

The aims of this study were to determine (1) whether the characteristics of fixational eve movements differ depending on whether stimuli are presented at fixation or in peripheral vision, (2) whether the presence of flankers influences fixational eye movements, and (3) whether changes in fixational eve movements associated with peripheral viewing that may increase positional uncertainty are related to the magnitude of crowding. We measured fixational eve movements while participants performed an orientation discrimination task in central and peripheral vision, with and without flankers. This builds upon prior studies in the field such as those conducted by Macedo et al. (2008) and Bedell et al. (2015). The former study used simulation of eye movements, whereas the latter study measured eye movements and reading task performance in separate sessions. In the current study, the potential relationship between fixation stability and crowding was quantified directly.



Figure 6. Properties of microsaccades. (a, b) Main sequence plots for the with-flanker (a) and without-flanker (b) conditions indicating that the detected events were microsaccades. Each data point is an individual trial. (c, d) Frequency distributions for microsaccadic amplitudes for the with-flanker (c) and without-flanker (d) conditions. (e, f) Mean microsaccadic amplitudes (e) and mean microsaccadic frequency (f) as a function of eccentricity for the with- and without-flanker conditions. Error bars show *SEM*.

Fixation stability, quantified using BCEA, was significantly reduced (worse) for all peripheral viewing conditions compared to the central viewing conditions. As expected with less stable fixation (Chung et al., 2015; Raveendran et al., 2019a; Sansbury et al., 1973; Shaikh, Otero-Millan, Kumar, & Ghasia, 2016), microsaccades were also affected during peripheral viewing conditions such that the mean microsaccadic amplitude was significantly larger for peripheral viewing conditions compared to the central viewing condition.



Figure 7. Microsaccade direction. The polar histograms show the frequency distribution (%) of microsaccade direction in the with-flanker (top) and without-flanker (bottom) viewing conditions.

Our observation that the presence of flankers reduced fixation stability when viewing a centrally presented stimulus is consistent with the results of Bedell et al. (2015), who observed reduced fixation stability for long letter strings presented in central vision. For our study, this effect was likely due to an increase in the size of the fixation target (target plus flankers rather than target alone) (Steinman, 1965; Thaler, Schütz, Goodale, & Gegenfurtner, 2013). Importantly, this explanation cannot account for the effect of flankers on fixation stability for the peripheral viewing conditions because the task stimuli were remote from the fixation cross. Moreover, although the fixation target was changed to a cross for peripheral viewing conditions, the size of the cross was matched to the size of the tumbling E target presented during the central viewing conditions. For the peripheral viewing conditions, flankers increased task difficulty, as demonstrated

by the reduction in percent correct performance for the with-flanker versus without-flanker stimuli. Therefore, one possible explanation for the effect of flankers on fixation stability for the peripheral viewing conditions is that increased task difficulty increased attentional load and exacerbated the detrimental effect of peripheral viewing on fixation stability. A more direct manipulation of attentional load will be required to evaluate the validity of this explanation. We did not observe an effect of flankers on task accuracy for the central viewing condition. This was likely due to a ceiling effect because we used a supra-threshold target size.

Crowding may occur at an early (Chen et al., 2014; Nandy & Tjan, 2012) or late stage of visual cortical processing (Levi, 2008; Levi, 2011). Because fixational eye movements may contribute to crowding by increasing positional uncertainty (Bedell et al., 2015), we tested the association between the magnitude of fixational eve movements and crowding during peripheral viewing. To test the hypothesis that less stable fixation is associated with greater crowding, we quantified the magnitude of crowding as a flanker effect-that is, a comparison between the with-flanker and without-flanker conditions at each eccentricity for both percent correct task accuracy and fixation stability. As expected, we observed no flanker effect for task accuracy at 0° eccentricity and a pronounced flanker effect at 5° eccentricity that increased in magnitude at 10° eccentricity. However, the flanker effect for fixation stability remained unchanged across all eccentricities. Furthermore, there was no association between task accuracy and fixation stability at any eccentricity for the with-flanker stimuli. This observation is consistent with previous studies. For example, Falkenberg, Rubin & Bex (2007) reported that crowding was unaffected by different levels of simulated fixation instability in normal peripheral vision, and Greenwood, Szinte, Sayim, & Cavanagh (2017) found no association between the magnitude of crowding and saccadic precision in peripheral vision. Moreover, there are models that consider positional uncertainty or confusion between target and flankers (Strasburger & Malania, 2013) or a combination of both uncertainty and confusion (Harrison & Bex, 2017) to explain the effect of crowding. The lack of an association between reduced fixation stability and crowding suggests that fixational eye movements are not a source of positional uncertainty in observers with normal vision. This may be because changes in fixation stability induced by overt attention are not sufficiently large to move visual stimuli across the large receptive fields that characterize peripheral vision.

Reduced fixation stability induced by peripheral viewing was associated with an increased proportion of horizontal microsaccades. This may have been due to an increase in the number of microsaccades made toward and away from the peripheral target stimulus, as has been reported in previous studies of covert attention (Engbert & Kliegl, 2003; Laubrock et al., 2005; Rolfs, Engbert, & Kliegl, 2004). Alternatively, this result may simply reflect that fact the microsaccades tend to be horizontal (Costela et al., 2015) and that a larger proportion of horizontal microsaccades may occur as fixation stability decreases.

A limitation of our study is that we used flanker bars to induce crowding rather than flanking letters. Our motivation for using flanker bars rather than flanking letters was to minimize the difference in overall stimulus size between the with- and without-flanker conditions. Larger stimuli reduce fixation stability (Steinman, 1965; Thaler et al., 2013); therefore, the use of letter flankers may have made it difficult to detect changes in fixation stability associated with flankers from those associated with large changes in stimulus size. We observed a significant reduction in task accuracy when bar flankers were added to peripheral stimuli, and crowding effects induced by bar flankers may share similar low-level mechanisms with crowding induced by more complex flanking stimuli (Doron, Spierer, & Polat, 2015; Lev & Polat, 2015; Musilová et al., 2018). However, we acknowledge that our results may not extend to crowding induced by letter flankers. In particular, bar flankers produce less pronounced reductions in task accuracy than letter flankers, perhaps because bars are dissimilar to letters (Song, Levi, & Pelli, 2014). Furthermore, bar flankers may reduce positional uncertainty by cuing the position of the flanked target, an effect that does not occur for flanking letters (Harrison & Bex, 2017), which represent viable alternative responses. Reduced positional uncertainty induced by bar flankers may have counteracted any effect of fixational eye movements on the recognition of flanked stimuli because fixational eye movements are likely to increase positional uncertainty. Finally, even though no association between crowding and fixational eye movements was observed in this study of observers with normal vision, larger fixational eye movements such as those that occur in amblyopia (Chung et al., 2015; Raveendran et al., 2019b) and macular degeneration (Macedo et al., 2008) may contribute to crowding.

## Conclusions

Holding central fixation while performing a discrimination task in peripheral vision was associated with reduced fixation stability and increased microsaccade amplitude compared to performing the same task at fixation. However, the effects of peripheral viewing on fixation stability did not influence crowding in normal peripheral vision, at least for the specific stimuli we employed.

Keywords: crowding, contour interaction, fixational eye movements, BCEA, covert attention

### Acknowledgments

The authors thank Mehmet Ağaoğlu, PhD, for providing MATLAB code for eye movement analysis, as well as Sharavanan Ganesan, MSc; Güler Arsal, PhD; and Joel Suss, PhD, for their valuable suggestions and assistance.

This research is funded by an Envision fellowship awarded by LCI Foundation to RNR. BT is supported by Natural Sciences and Engineering Research Council of Canada Grants RPIN-05394 and RGPAS-477166.

Commercial relationships: none.

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