Microsaccades Counteract Visual Fading during Fixation

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Summary

Our eyes move continually, even while we fixate our gaze on an object. If fixational eye movements are counteracted, our perception of stationary objects fades completely, due to neural adaptation. Some studies have suggested that fixational microsaccades refresh retinal images, thereby preventing adaptation and fading. However, other studies disagree, and so the role of microsaccades remains unclear. Here, we correlate visibility during fixation to the occurrence of microsaccades. We asked subjects to indicate when Troxler fading of a peripheral target occurs, while simultaneously recording their eye movements with high precision. We found that before a fading period, the probability, rate, and magnitude of microsaccades decreased. Before transitions toward visibility, the probability, rate, and magnitude of microsaccades increased. These results reveal a direct link between suppression of microsaccades and fading and suggest a causal relationship between microsaccade production and target visibility during fixation.

Introduction

Our visual system has a built-in paradox: we must fixate our gaze in order to inspect the minute details of the world, yet if we were to fixate perfectly, the entire world would fade from view due to neural adaptation. In the early 1950s, several independent groups showed that when all eye movements were eliminated (i.e., under retinal stabilization conditions), visual perception quickly faded to a homogeneous field (Ditchburn and Ginsborg, 1952; Riggs and Ratliff, 1952; Yarbus, 1967). Even though perfect retinal stabilization is most easily achieved under laboratory conditions, fading of stationary objects in our visual periphery occurs quite often in normal vision. Peripheral fading during visual fixation was first noticed by Troxler in 1804 (Figure 1A). In the late 1950s, Clarke connected Troxler's fading with the fading of stabilized images in the laboratory and attributed both phenomena to neural adaptation (Clarke, 1957, 1960, 1961; Clarke and Belcher, 1962). Interestingly, Troxler fading can also be observed while viewing displays containing kinetic edges (De Weerd et al., 1995; Ramachandran and Gregory, 1991; Spillmann and Kurtenbach, 1992). Flickering, moving, and even foveal stimuli are also susceptible to Troxler fading (Mag-

nussen et al., 2001; Schieting and Spillmann, 1987; Spillmann and de Weerd, 2003; Sturzel and Spillmann, 2001).

From the three types of fixational eye movements (microsaccades, drifts, and tremor), microsaccades are the largest and easiest to characterize; however, their role in counteracting adaptation during visual fixation has remained a matter of controversy for five decades. Some laboratories originally proposed that microsaccades may play an "important role in maintaining vision by counteracting retinal fatigue" (Ditchburn et al., 1959; Nachmias, 1961). It was found that, after stabilizing a retinal image in the laboratory, artificially superimposed movements of the stimulus similar to what one would expect from microsaccades could restore perception, whereas artificially superimposed movements of the image similar to drifts or tremor had a smaller effect in preventing fading (Carpenter, 1988; Ditchburn et al., 1959). Not all studies agreed with this result, however (Drysdale, 1975; Gerrits and Vendrik, 1970; Sharpe, 1972). Starting in the late 1960s and through the 1970s, a lively discussion on the importance of microsaccades for the maintenance of vision took place. Its main representatives were Ditchburn (microsaccades play an essential part in normal vision) and Steinman (microsaccades serve no useful purpose). The strongest arguments against the role of microsaccades in preserving visual perception were that (1) trained subjects can suppress their microsaccades for several seconds (Fiorentini and Ercoles, 1966; Steinman et al., 1967), and (2) microsaccades are naturally suppressed in normal vision while subjects perform high-acuity tasks, such as shooting a rifle or threading a needle (Kowler and Steinman, 1979; Winterson and Collewijn, 1976), even though the target of the fine-acuity task does not fade.

It was further argued that microsaccades were a laboratory artifact: i.e., that microsaccades do not occur in normal viewing conditions, but only occur in the laboratory during prolonged fixation and while the subject's head is restrained (for instance, with a bite bar). The reasoning was that in natural viewing conditions, normal head movements should be sufficient to maintain vision during fixation, and therefore very few or no microsaccades would be produced (Kowler and Steinman, 1980; Skavenski et al., 1979; Steinman and Collewijn, 1980). Thus, microsaccades might be nothing but an "evolutionary puzzle," that "contribute nothing of consequence to either oculomotor control or vision" (Kowler and Steinman, 1980). The arguments were that (1) when the head is unrestrained (even if the subject is trying to be "as still as possible"), average retinal image speed is at least twice as high as the average retinal speed when the head is restrained, and (2) if the subject is allowed to move his/her head normally, average retinal image speed climbs to more than 3°/s. In 1980, Kowler and Steinman concluded that microsaccades "solely […] aid vision (and aid it inefficiently) when human beings view equal-luminance chromatic borders while clenching a biteboard" (Kowler and Steinman, 1980), which effectively ended the debate for over 20 years. Recent papers (Malinov et al., 2000; Steinman et al., 2003) reiterate
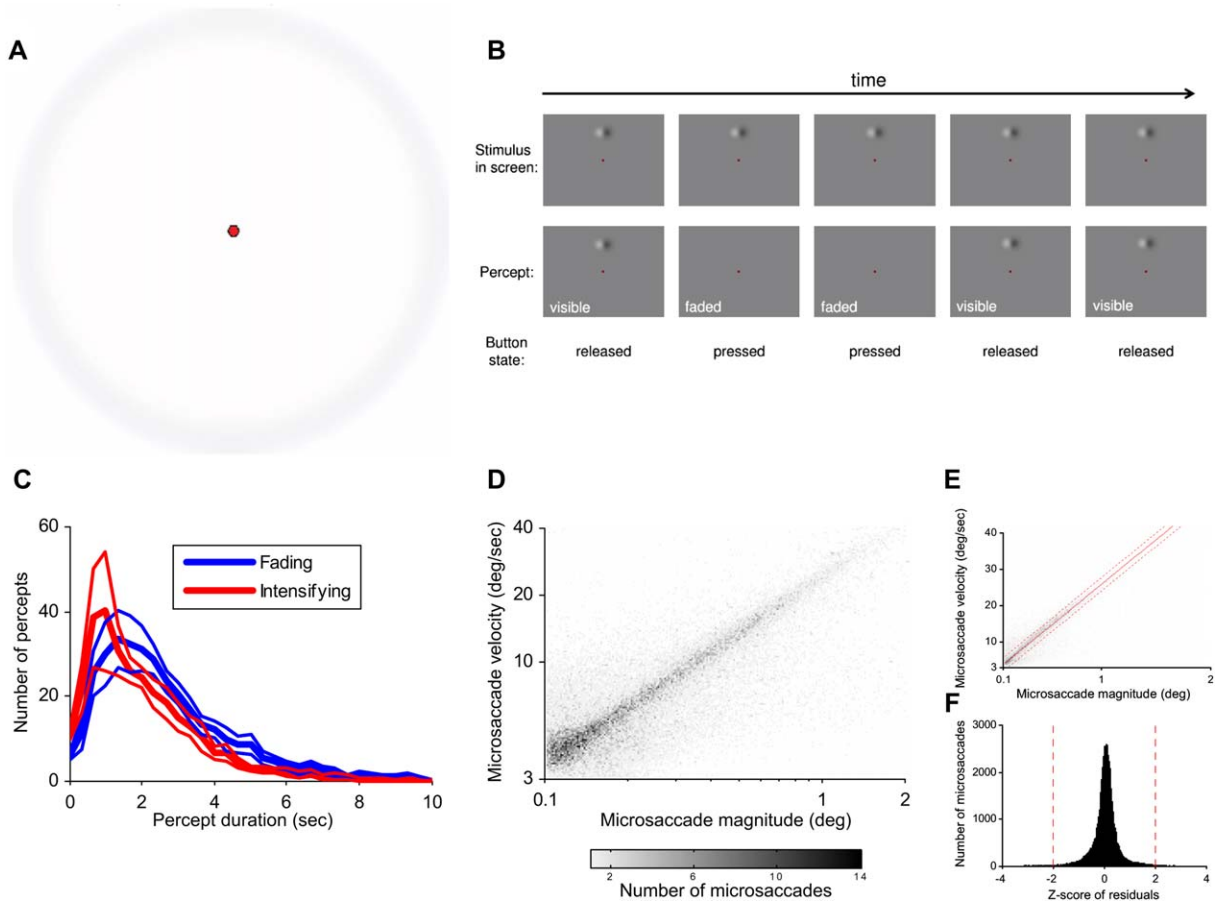
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Figure 1. The Measurement of Microsaccades during Troxler Fading

(A) Demonstration of peripheral visual fading, or Troxler's effect. Fixate very precisely on the red spot, while paying attention to the bluish annulus. After a few seconds of careful fixation, the annulus will fade, and the red spot will appear to be surrounded by a completely white field. Movements of the eyes will immediately bring the blue annulus back to perception. From Martinez-Conde et al. (2004). (B) An epoch from a trial during the experiment. The top row shows the stimulus, which does not change over time. The second row shows the percept of the stimulus, which is intermittently invisible due to Troxler fading. The third row shows the subjects' report of their perception. (C) Distribution of fading (blue) and intensifying (red) periods in experiment 1. Thin lines indicate SEM between subjects (n = 8 subjects). (D) Log-log main sequence of all microsaccades (n = 106,692 microsaccades) in experiment 1. (E) Linear plot of main sequence in (D). Dotted lines indicate the 95% prediction intervals. The 95% confidence intervals are obscured by the regression line. Microsaccade binning as in (D). (F) Distribution of linear regression residuals for microsaccadic main sequence. The vertical dotted lines contain 95% of all microsaccades and correspond to the prediction intervals in (E).

the conclusion that "[Microsaccades] were laboratory curiosities, confined to human adults, whose heads were supported artificially."

In the late 1990s and early 2000s, several laboratories found microsaccades to be correlated with increases in neuronal firing at all levels of the visual pathway (Bair and O'Keefe, 1998; Martinez-Conde et al., 2000, 2002; Reppas et al., 2002), which suggested a potentially important physiological role for microsaccades. In the last few years, microsaccades have been linked to fixation correction, control of binocular disparity (Engbert and Kliegl, 2004), and attentional shifts (Engbert and Kliegl, 2003a; Hafed and Clark, 2002; Rolfs et al., 2004), but not all studies agree (Tse et al., 2002, 2004). Although several studies have questioned the "usefulness [of microsaccades] for preserving vision by preventing fading" (Cunitz and Steinman, 1969; Kowler and Steinman, 1979; Steinman, 1975), the crucial experiment, to correlate microsaccades directly to the visibility of stimuli during fixation, has nevertheless not been done. Therefore, we

have based our present hypothesis, that microsaccades are sufficient for (and potentially causal to) visibility during fixation, on two previous independent observations: (1) microsaccades tend to be naturally suppressed during precise fixation (Winterson and Collewijn, 1976; Kowler and Steinman, 1979), and (2) Troxler fading tends to occur during precise fixation (Troxler, 1804). It follows that microsaccades may counteract Troxler fading. Due to recent eye movement measurement advances, microsaccades can now be accurately, objectively, and noninvasively sampled, and so their role in visual perception can finally be ascertained directly. Here, we set out to determine whether microsaccades prevent peripheral fading during visual fixation.

We also hypothesize that Troxler fading occurs because receptive fields in the periphery of our vision can be considerably larger than the amplitudes of drifts and tremor. Thus, when microsaccades are suppressed, drifts and tremor may not provide effective visual stimulation to prevent peripheral fading, especially in the case

of low-contrast stimuli. This is supported by Clowes (Clowes, 1962), who fixated the border between two equiluminant regions in such a way as to prevent fusion of the colors and found that both microsaccades and intersaccadic drifts were produced. However, Clowes did not quantify how well microsaccades prevented fusion or whether microsaccades were more or less effective than drifts.

In summary, it remains an open question whether microsaccades contribute significantly to the maintenance of visibility. Moreover, the comparative role of drifts is unknown. Here, we specifically probe for the role of microsaccades in maintaining/regenerating visual perception during Troxler fading (i.e., natural peripheral fading conditions) and compare them to drifts. It is also important to establish whether microsaccades occur, and counteract fading, under head-unrestrained conditions. If microsaccades are a laboratory artifact, as suggested previously, then their significance is vastly diminished, even if they are in fact correlated with visibility and neural activity when the subject's head is restrained. Thus, we also ask whether the role of microsaccades in counteracting visual fading depends on head-restraint conditions.

Results

Experiment 1

Subjects (five naive plus three authors) were tasked with fixating a small spot and continuously reporting whether a peripheral target was faded/fading (button pressed) versus visible/intensifying (button released) during a classical Troxler fading task, while their eye movements were recorded with high precision. Figure 1B describes a typical epoch during a trial. As with previous fading experiments (Spillmann and Kurtenbach, 1992), subjects reported that the perceptual state of the target appeared to oscillate between the faded/fading state and the visible/intensifying state (not unlike the oscillations of rivalrous and other bistable stimuli).

We categorized each millisecond in the trial as fading or intensifying, according to the subject's perceptual report. Then we calculated the probability of a microsaccade in-flight, the average microsaccade rate, and the average microsaccade magnitude before each millisecond of the experiment, as a function of whether the subject experienced the target as faded/fading or visible/intensifying during that millisecond. The results show a clear positive relationship between the probability, rate, and magnitude of microsaccades and visibility (Figure 2, red lines). Before and during a fading (invisible) period, the probability, rate, and magnitude of microsaccades decreased (Figure 2, blue lines). Equivalent results were found in the five naive subjects and in the three authors tested (Figure 2, rows 2–9). These findings reveal a direct link between microsaccade dynamics and visibility during fixation.

We did a second analysis to ask whether microsaccades may play a causal role in driving the dynamical structure of the stimulus's perceptual undulation. To do this, we looked at the various transition-triggered average microsaccade parameters for visible (intensifying) versus invisible (fading) periods. This analysis is similar to that in Figure 2, but here we examined the average

microsaccade dynamics in relationship to the very first millisecond after the button was pressed or released. The results show that, before each transition to a more visible state, there was, on average, an increase in microsaccade probability, rate, and magnitude (Figures 3A–3C, red lines), whereas just before transitions to a period of invisibility (fading), there was a decrease in microsaccade probability, rate, and magnitude (Figures 3A–3C, blue lines). To further establish the plausibility of a causal role of microsaccades in driving the perceptual transitions, we calculated the microsaccade-triggered average transition rates for fading versus intensifying percepts (Figure 4). The results show that microsaccade onsets are followed by increasing rates of transitions to visibility and decreasing rates of transitions to fading (i.e., a 64% increase and a 24% decrease from chance, respectively). This suggests that there may be a causal relationship between microsaccades and the dynamics of the undulations. At the very least, we cannot exclude a causal role of microsaccades in driving the perceptual transitions.

Just as the strength of the connection between a pre- and a postsynaptic neuron can be quantified from a cross-correlogram (Levick et al., 1972; Reid and Alonso, 1995; Usrey et al., 1999), one can also determine the impact of microsaccades in driving perceptual transitions. The *efficacy* of microsaccades can be defined as the percentage of microsaccades driving perceptual transitions to visibility. The *contribution* of microsaccades can be defined as the percentage of transitions to visibility driven by microsaccades. We generated a raw, unfiltered cross-correlogram (not shown) representing the number of coincidences between microsaccades and transitions. The *peak interval* was taken as 375 ms on either side of the maximum, which was constrained to fall within the 1000 ms preceding the transition. We found that 3.5% of microsaccades were followed by transitions to visibility. However, one must take into account that only those microsaccades that occur during the fading periods can potentially cause a transition to visibility. That is, microsaccades produced within a visible period cannot physically drive transitions to visibility (because the stimulus is already visible), although these microsaccades may extend the length of the visible period. After accounting for this fact, we found that 7% of the microsaccades that occur during fading periods were followed by transitions to visibility. Thus, the *efficacy* of microsaccades is 7%. However, the crucial quantification of microsaccade impact is not the percentage of microsaccades that cause transitions, but the percentage of transitions that are caused by microsaccades. We found that, on average, 60% of the transitions to visibility were preceded by one more microsaccade than was predicted by chance. Moreover, the *efficacy* and *contribution* of microsaccades are quantifications based solely on microsaccade rate. Our results show that microsaccade magnitude also plays a role, irrespective of rate (Figure 3C). Thus, even in the absence of a rate increase, increases in microsaccade magnitude can drive transitions to visibility. Consequently, the impact of microsaccades is even greater if both rate and magnitude are considered. Future analyses will determine the respective impact of microsaccade rate versus magnitude, as well as any potential interactions.
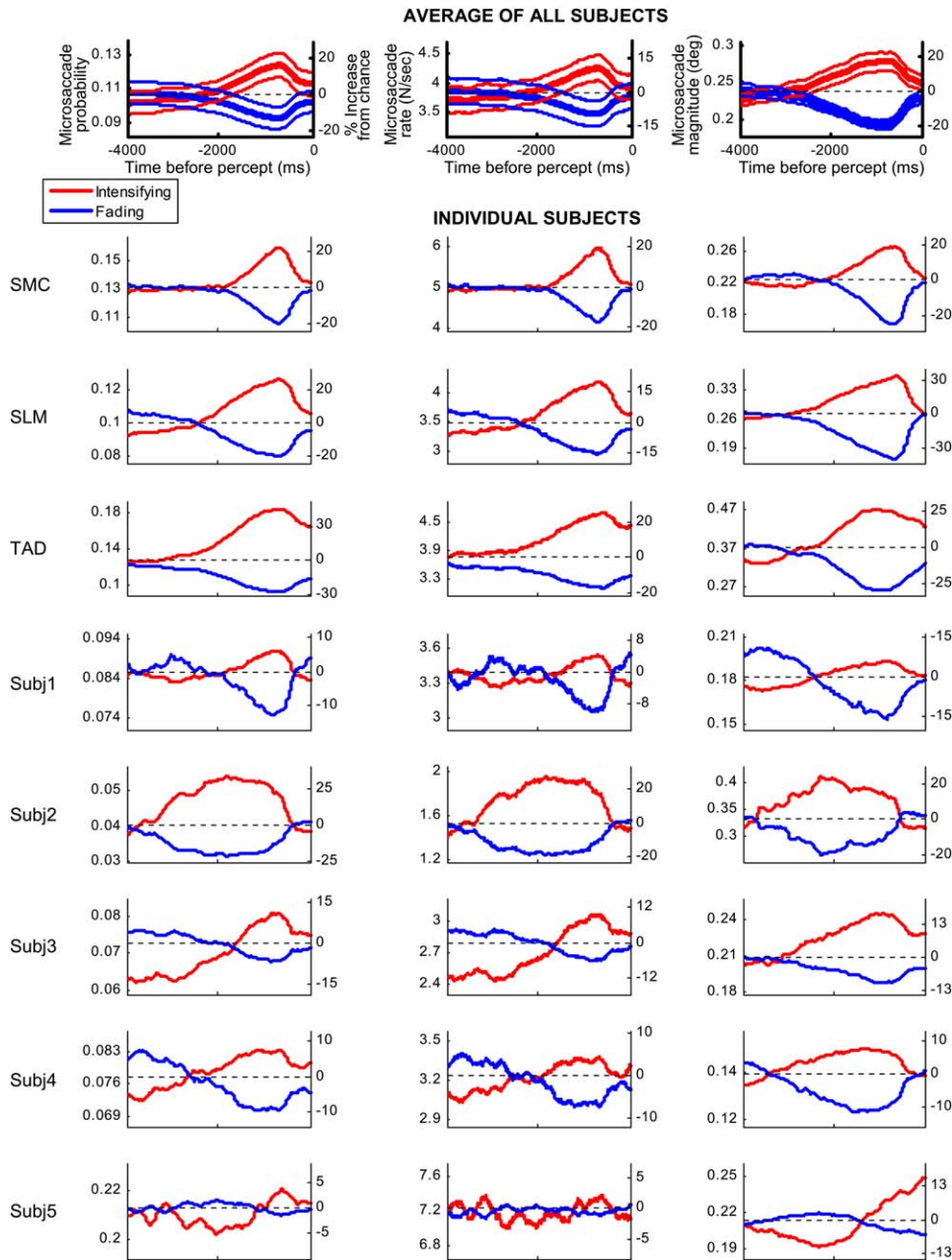
Figure 2. Microsaccade Parameters during Intensifying versus Fading Percepts

(Left column) Average probability of a microsaccade in-flight before each millisecond of the experiment, categorized as either intensifying (red) or fading (blue). (Middle column) Average microsaccade rate before each millisecond of the experiment, categorized as either intensifying or fading. (Right column) Average microsaccade magnitude before each millisecond of the experiment, categorized as either intensifying or fading. The horizontal black lines indicate overall average microsaccade probability/rate/magnitude. (Top row) Average of all subjects. Thin lines indicate SEM between subjects (n = 8 subjects). (Rows 2–9) Data from individual subjects. Authors are identified by their initials, and naive subjects are numbered from 1 through 5.

Figure 4 shows that peak increases and decreases in transition rates (indicated by button presses/releases) occurred at ~440 ms from microsaccade onsets. This value is in agreement with perceptual alternation studies in which subjects were asked to continuously report the perceptual status of a stimulus via button press (van Dam and van Ee, 2005). It should also be noted that the delay between microsaccade onset and button press is not equivalent to traditional reaction time mea-surements. Traditional reaction times indicate the delay between a physical change in the stimulus and the sub-ject's report; however, the delay between microsaccade onset and button press combines two different laten-cies: (1) the delay between microsaccade onset and per-ceptual change, and (2) the delay between perceptual change and the subject's report.

Recent studies have distinguished between monocu-lar and binocular microsaccades, with potentially
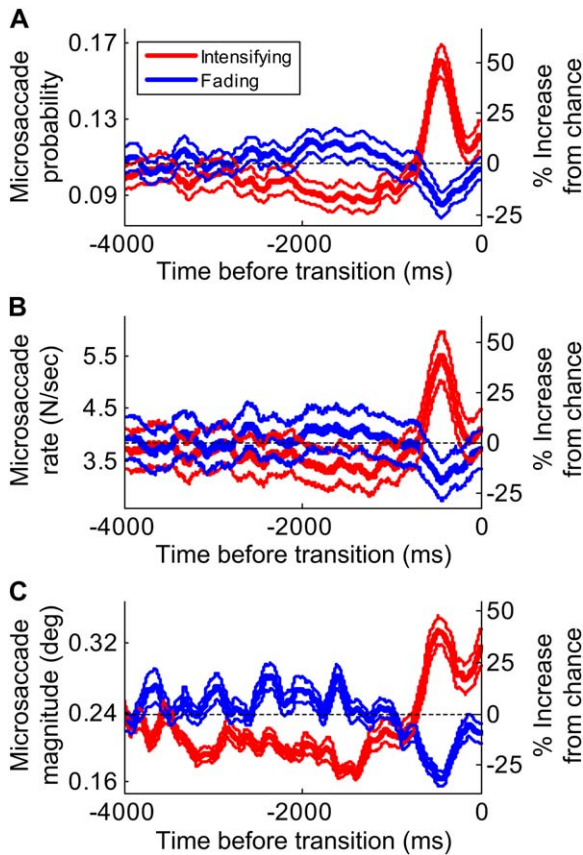
Figure 3. Microsaccade Dynamics before Transitions toward Perceptual Intensification versus Fading

(A) Average probability of microsaccades before transitions toward perceptual intensification (red) versus fading (blue). The horizontal black line indicates average probability of microsaccades during the recording session. (B) Average rate of microsaccades before perceptual transitions. (C) Average microsaccade magnitude before perceptual transitions. Thin lines indicate SEM between subjects (n = 8 subjects).

different functional roles (Engbert and Kliegl, 2003b). Figure 5 plots the transition-triggered average probability of monocular versus binocular microsaccades during Troxler fading. To construct these graphs, each millisecond that a microsaccade was in flight was classified as binocular (if both eyes were engaged in a microsaccade; 41% ± 2% of all microsaccade times) or monocular (if only one eye was engaged in a microsaccade; 59% ±
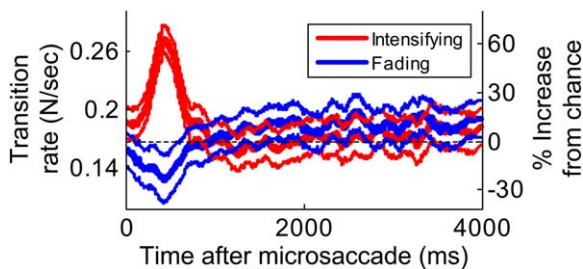

Figure 4. Average Rate of Perceptual Transitions toward Intensifying (Red) or Fading (Blue) after Microsaccade Onset

The horizontal black line indicates overall average rate of transitions. Thin lines indicate SEM between subjects (n = 8 subjects).
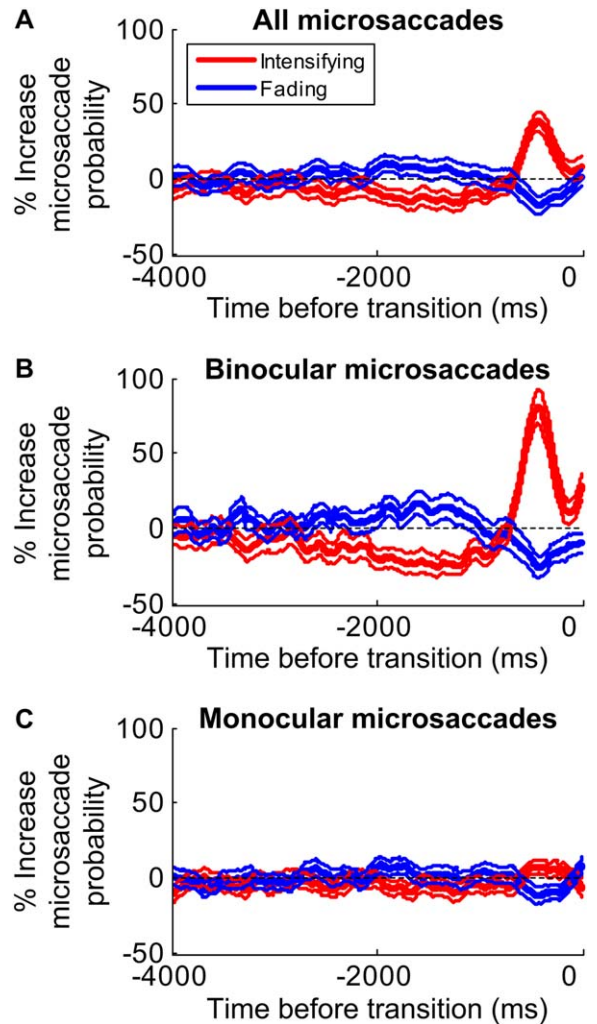

Figure 5. Average Percent Increase in Microsaccade Probabilities before Transitions toward Perceptual Intensification versus Fading

(A) All microsaccades (same data as in Figure 3A). (B) Binocular microsaccades. (C) Monocular microsaccades. Thin lines indicate SEM between subjects (n = 8 subjects).

2% of all microsaccade times). After normalizing by the number of microsaccades in each case, it becomes clear that binocular microsaccades have a stronger role in counteracting fading than monocular microsaccades.

The fact that monocular microsaccades have a lesser role in counteracting fading than binocular microsaccades suggested that drifts may not be important in preventing peripheral Troxler fading, because during monocular microsaccades, the nonmicrosaccadic eye was presumably drifting. Drifts are defined in the literature as the intervals between microsaccades, as even during strict fixation our eyes are never completely still (Yarbus, 1967). However, it is important to keep in mind that, as drift and tremor occur simultaneously (Carpenter, 1988; Pritchard, 1961), any effects associated with drifts (in this or other studies) could potentially be caused by tremor, or by a combination of drift and tremor. Figure 6 shows that, in our experimental conditions, drifts were correlated with transitions to fading and anticorrelated with transitions to visibility in the periphery.
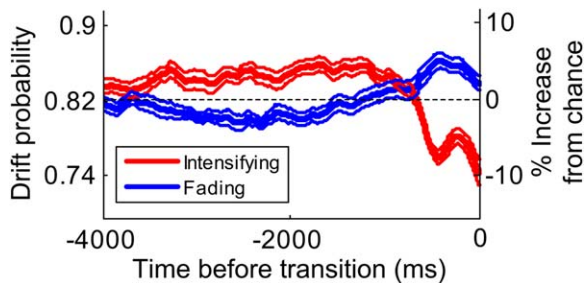
Figure 6. Average Drift Probabilities before Transitions toward Perceptual Intensification versus Fading

Thin lines indicate SEM between subjects (n = 8 subjects).

Experiment 2

Of the eight subjects tested in experiment 1 with a 9° eccentricity target, six subjects were available for retesting at two new target eccentricities: 6° and 3° from the fixation point. All other experimental details were as in experiment 1. The purpose of this experiment was to determine whether the results obtained at an eccentricity of 9° (medium-periphery) could be replicated at near-peripheral (6°) and parafoveal (3°) regions. Figure 7 shows that transitions to fading and visibility are preceded by corresponding decreases and increases in microsaccade probabilities at the three eccentricities tested, suggesting that microsaccades have a strong effect on visibility at a wide range of eccentricities. However, the effects of microsaccades on visibility were arguably larger for the 9° eccentricity target. These results suggest that the effects of microsaccades on visibility are at least partially determined by the relationship between the magnitude of microsaccades and the size of receptive fields at the eccentricities tested.

Experiment 3

Of the eight subjects tested with head restraint (experiment 1), seven were available for retesting without head restraints. Head-_unrestrained_ conditions were identical in every way to the head-restrained conditions, except that the subjects' chinrest was removed. Fading dynamics were strikingly similar in the two conditions (restrained versus unrestrained). Each faded/fading state lasted on average for 3.0 ± 0.4 s (56% ± 4% of the total fixation time) with heads _restrained_, and 2.9 ± 0.5 s (56% ± 4% of the total fixation time) with heads _unrestrained_. Each visible/intensifying state lasted on average for 2.5 ± 0.2 s (44% ± 4% of the total fixation time) with heads _restrained_, and 2.5 ± 0.3 s (44% ± 4% of the total fixation time) with heads _unrestrained_. Figure 8 plots the transition-triggered average probability of microsaccades during head-unrestrained Troxler fading. The results are equivalent to those obtained in head-restrained conditions. Differences in microsaccade parameters (peak probabilities, rates, magnitudes) before fading versus intensifying periods were highly significant both in head-restrained and head-unrestrained conditions (t test, $p \leq 0.01$). Equivalent results were moreover obtained when microsaccades were identified with a different automatic algorithm, freely available online at http://www.agnld.uni-potsdam.de/%7Eralf/micro/ and developed by an independent re-
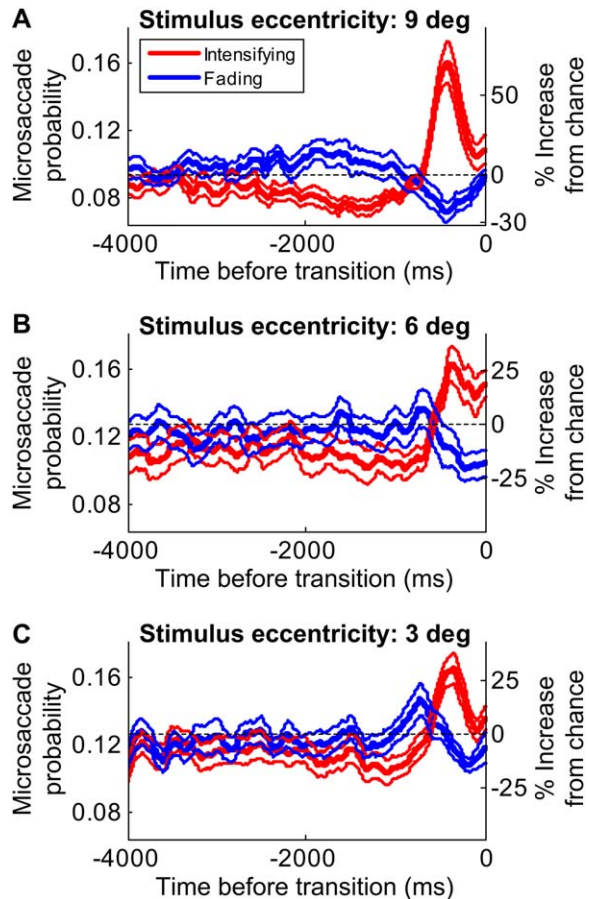

Figure 7. Average Probability/Rate/Magnitude of Microsaccades before Perceptual Transitions, at three Different Eccentricities

(A) 9°. (B) 6°. (C) 3°. Thin lines indicate SEM between subjects (n = 6 subjects).

search group (Engbert and Kliegl, 2003a) (data not shown). Thus, our results are not due to idiosyncrasies in our particular automatic and objective microsaccade characterization algorithm.

Discussion

The relationship between microsaccades and visibility during fixation had been previously inferred, but never directly measured perceptually. The disparate conclusions concerning the role of microsaccades during fixation, and the lack of direct measurement of the relationship between microsaccades and visibility, were no doubt largely due to the previous lack of high-quality and high-speed noninvasive eye-position measurement devices necessary to detect microsaccades. However, recent advances in eye movement recording technology allow us to measure microsaccades noninvasively in both eyes, with heads restrained or unrestrained. Another reason that it may have been difficult to measure the relationship between microsaccades and perception is that microsaccades can occur several times per second, which is on a timescale that can be difficult to address psychophysically (see Martinez-Conde et al. [2004] for a review of microsaccade and other fixational
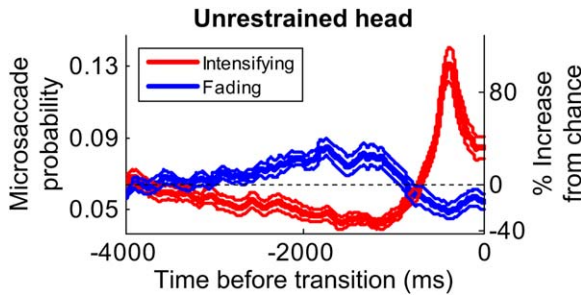
Unrestrained head

Figure 8. Average probability of Microsaccades before Perceptual Transitions under Head-Unrestrained Conditions

Thin lines indicate SEM between subjects (n = 7 subjects).

eye movement parameters). To directly correlate microsaccades to visibility may have thus seemed an untenable effort. Here, we have developed a psychophysical event-triggered paradigm in which the subject continually indicates the visibility condition of the stimulus in real time as it undulates during adaptation, while simultaneously measuring the dynamics of microsaccades.

This paradigm has allowed us to establish a direct relationship between stimulus visibility during fixation and various dynamics of microsaccades in the preceding seconds. We found significant increases in microsaccade probability, rate, and magnitude, before transitions to visibility, and opposite trends before transitions to invisibility. Thus, our results establish a potential causal relationship between microsaccades and periods of visibility during fixation. Our results also agree with predictions from physiological studies in which microsaccades were found to increase spiking rates in V1 and LGN neurons (Martinez-Conde et al., 2000, 2002).

One may wonder whether the subjective nature of the perceptual report (whether the stimulus is fading/faded versus intensifying/visible) could have an effect on the results reported here. We do not believe this is the case, as subjects have little voluntary control over microsaccade production (i.e., microsaccades can be suppressed voluntarily for a few seconds, but they cannot be voluntarily generated) or microsaccade magnitude, and so subjects cannot predict when the transition in perception will take place. This type of design is commonly used in experiments that assess the perception of subjects during binocular rivalry and other bi-stable transitions over which the subject similarly has no control. Therefore, the analyses presented in this paper correlate subjective reports with a completely independent measure (microsaccade dynamics), uncontrolled by the subjects and inaccessible to their awareness.

In 1980, Kowler and Steinman famously concluded (as stated in the title of their *Vision Research* reply to Ditchburn) that "Small saccades serve no useful purpose" (Kowler and Steinman, 1980). This view largely dominated the field of fixational eye movements for the next several decades. However, the role of microsaccades on visibility was never quantified directly. Here, we show that microsaccades counteract peripheral fading during fixation, whereas drifts appear to allow fading (at least for the stimuli and retinal eccentricities tested here, roughly spanning parafoveal, near-periphery, and medium-periphery regions). One should note that in our experi-

ments, when the peripheral stimulus was faded, the fixation spot always remained visible to the subjects. Thus, in the absence of microsaccades, drifts and tremor seem sufficient to maintain foveal visibility of certain stimuli. However, if one could eliminate drifts and tremor altogether, microsaccades might then suffice to sustain both foveal and peripheral vision during fixation. One should also keep in mind that even nonfixational eye movements (such as blinks or large voluntary saccades) may effectively prevent fading when they are produced.

Our results fundamentally agree with Ditchburn's assessment that microsaccades play an important role in maintaining/restoring visibility during fixation. However, our data are also compatible with the reports that accurate fixation tends to eliminate microsaccades (Kowler and Steinman, 1980; Skavenski et al., 1979; Steinman et al., 1967). Here, we show that accurate fixation does in fact reduce not only microsaccade rates but also microsaccade magnitudes. Microsaccade rates and magnitudes may also be reliable indicators of perceptual alternations during binocular rivalry (Sabrin and Kertesz, 1983) and other bi-stable percepts. Our results also show that microsaccades counteract fading in natural conditions (i.e., with heads unrestrained). Future studies should investigate the role of head movements in the prevention of Troxler fading.

Experimental Procedures

Subjects
Eight subjects (six females, two males) with normal or corrected-to-normal vision participated in this study. Each subject participated in a minimum of three sessions (one training session and two experimental sessions) of ~30 min each (experiment 1) and was paid $15/session. No eye movement data were collected during the training session. Six of the subjects participated in four additional sessions (two sessions for each of the additional eccentricities tested in experiment 2). Seven of the subjects participated in two additional head-unrestrained sessions (experiment 3). Five of the subjects were naive, and three subjects were authors in this paper. Experiments were carried out under the guidelines of the Barrow Neurological Institute's Institutional Review Board (protocol number 04BN039).

Experimental Design
Subjects rested their head on a chin-rest (except in the head-unrestrained experiment), 57 cm from a linearized video monitor (Barco Reference Calibrator V, 75 Hz refresh rate). Eye position was acquired noninvasively with a fast video-based eye movement monitor (EyeLink II, SR Research). The EyeLink II system records fixational eye movements simultaneously in both eyes (temporal resolution, 500 samples/s; instrument noise, 0.01° RMS), with either restrained or unrestrained heads, in its off-the-shelf configuration.

We conducted a continuously sampled two-alternative forced choice (2-AFC) task in which the subject fixated on a small spot on the center of the monitor's screen and simultaneously reported the visibility of a peripheral target via button press. Subjects pressed a key and the fixation spot and the target appeared simultaneously. Subjects were asked to fixate on the fixation spot and press a button if the target was fading (or completely faded) and to release the button if the target's visibility was intensifying (or the target was completely visible). Every millisecond was categorized into either fading (invisible) or intensifying (visible), according to the subject's report. That is, milliseconds during which the button was pressed were counted as pertaining to a fading period. Milliseconds during which the button was released were counted as pertaining to an intensifying period.

After 30 s, all stimuli disappeared and the trial ended. In order to disregard the potential effect of the initial target-onset transient on the visibility of the target, only those data recorded after the first

button press (which indicated the first perceptual transition toward target fading) were used in subsequent analyses. Each experimental session included 40 trials. The fixation spot was a small red dot (0.05°) on a 50% gray background. The target was a two-lobe gabor patch (Figure 1B) with a peak-to-trough width of 2.5° (Gaussian STD X, 1.5°; Gaussian STD Y, 1°; sinewave period, 0.36°; sinewave phase, 0 [odd gabor]), presented at an eccentricity of 9° (measured from the center of the fixation point to the center of the gabor patch). The target had a maximum contrast of 40% from peak to trough and the same average luminance (50%) as the background. Although the target remained stationary on each trial, its position varied pseudorandomly across trials at one of the eight points of the compass in order to control for possible contrast adaptation effects across trials. The orientation of the target also varied pseudorandomly in each trial (in 10° steps), to control for orientation-adaptation effects. The target's parameters were chosen so that it tended to either fade or intensify with an approximate 50-50 ratio during fixation. The distribution of fading and intensifying periods for experiment 1 (n = 8 subjects) is plotted in Figure 1C.

Eye Movement Analysis

We identified microsaccades automatically with a previously developed objective algorithm (Martinez-Conde et al., 2000, 2002). Eye position was continually recorded in both eyes at 500 Hz using Eyelink II, and eye-position data were resampled offline from 500 Hz to 1 kHz using linear interpolation. Linear resampling from 500 Hz to 1 kHz was conducted so that the previously published microsaccade-detecting algorithm (which was developed for a 1 kHz search coil system) could be applied unmodified to the present data. We computed the change in X and Y eye positions at each millisecond by subtracting the digitized measurement of eye position for each millisecond from the value of the previous millisecond. This amounted to taking the time derivative of movements and creating a table of change in X (dX) and Y (dY) for each millisecond. After differentiating the data, we smoothed them with a 31 ms-wide unweighted boxcar filter. We then prepared an array of vectors representing the size (r) and direction of motion (θ) of the eye at each millisecond of the recording. r may thus be thought of as the instantaneous velocity of the eye in degrees of visual space/ms. We declared the eye to be stopped any time the instantaneous velocity was less than 3°/s. From these measurements, we created three data points for each millisecond of the experiment, one that kept track of r, another that kept track of the instantaneous angle of the eye movement (θ), and a third that recorded when the eye was stopped.

We then employed a rate-of-turn indicator that checked whether the direction of movement had changed more than 15° since the last millisecond: if so, we considered the eye to have stopped and amended the eye-stopped array appropriately. When movement direction was constantly changing, we considered microsaccades to be absent. During a veritable microsaccade, on the contrary, the millisecond-vectors were aligned in the direction of the overall eye movement. In order to determine the overall distance and direction of each microsaccade, we stepped through the eye-stopped array, and whenever the eye was not stopped we integrated the millisecond-vectors over time with simple vector addition. This provided us with an array of eye movements containing, for each millisecond, the cumulative distance and directions of the movement up to that time. The values of integrated-r and integrated-θ of the final millisecond of each microsaccade were therefore equal to the final distance and direction of the entire eye movement. We could then apply upper and lower limits to the final value of integrated-r to be sure that we were investigating the effects of microsaccades and not very small artifacts or large saccades caused by voluntary movements. The microsaccades we used for the rest of the analysis were thus larger than 3 min of arc and smaller than 2°. Cumulative vectors larger than 2° were discarded as voluntary saccades.

The main sequence of all microsaccades in experiment 1 (n = 106,692 microsaccades) is plotted in Figure 1, both in log-log (Figure 1D) and linear scale (Figure 1E). In order to correct the digitization scalloping at the low end of the log-log plot, a random offset factor of ±0.2°/s in velocity and ± 0.01° in magnitude was applied to all microsaccades in the main sequence. Microsaccade distances from the regression line (Figure 1E) follow a normal distribution (Kolmo-

gorov-Smirnov test, p > 0.1; Figure 1F), confirming that the microsaccades studied had an orderly relationship between magnitude and speed (Zuber and Stark, 1965).

Drifts were defined as those fixational periods that were free from microsaccades, as well as occasional blinks and large saccades (Yarbus, 1967). Thus, the probability of drifts before perceptual transitions (Figure 6) was not the exact inverse of the probability of microsaccades before perceptual transitions (Figure 3A), due to the occasional presence of blinks and large saccades in the eye-position records (during which the eyes were neither producing microsaccades nor drifting).

Event-Triggered Averages

Event-triggered averages (Figures 2–8) were calculated by averaging a 4 s window of data (in a millisecond-by-millisecond manner), before (Figures 2–3 and 5–8) or after (Figure 4) the trigger event. Thus, the event-triggered average in Figure 3A represents the average probability that a microsaccade was in flight at any given millisecond during the 4 s window plotted. In the event that there were not 4 s of data before or after a given transition (due to the edge of the recording time during that specific trial), the 4 s window was dynamically reduced to fit the available data. Each subject's averages were calculated individually (as in Figure 2, rows 2–9). The population average and SEM error bars were then calculated across subjects. Microsaccade rate and probability plots include data periods before triggers in which microsaccades did not occur. Average microsaccade magnitudes were calculated by including only those periods during which microsaccades were made. Due to the nature of the plots, Figures 3–8 have significantly less data than Figure 2 and so they have been smoothed, for the purpose of plotting, with a first-order Savitzky-Golay filter (Savitzky and Golay, 1964) with a 151 ms window.

References

Bair, W., and O'Keefe, L.P. (1998). The influence of fixational eye movements on the response of neurons in area MT of the macaque. Vis. Neurosci. 15, 779–786.

Carpenter, R.H.S. (1988). Movements of the Eyes, Second Edition, (London: Pion Ltd).

Clarke, F.J.J. (1957). Rapid light adaptation of localised areas of the extra-foveal retina. Opt. Acta (Lond.) 4, 69–77.

Clarke, F.J.J. (1960). A study of Troxler's effect. Opt. Acta (Lond.) 7, 219–236.

Clarke, F.J.J. (1961). Visual recovery following local adaptation of the perpheral retina (Troxler's effect). Opt. Acta (Lond.) 8, 121–135.

Clarke, F.J.J., and Belcher, S.J. (1962). On the localization of Troxler's effect in the visual pathway. Vision Res. 2, 53–68.

Clowes, M.B. (1962). A note on colour discrimination under conditions of retinal image constraint. Opt. Acta (Lond.) 9, 65–68.

Cunitz, R.J., and Steinman, R.M. (1969). Comparison of saccadic eye movements during fixation and reading. Vision Res. 9, 683–693.

De Weerd, P., Gattass, R., Desimone, R., and Ungerleider, L.G. (1995). Responses of cells in monkey visual cortex during perceptual filling-in of an artificial scotoma. Nature 377, 731–734.

Ditchburn, R.W., and Ginsborg, B.L. (1952). Vision with a stabilized retinal image. Nature 170, 36–37.

Ditchburn, R.W., Fender, D.H., and Mayne, S. (1959). Vision with controlled movements of the retinal image. J. Physiol. 145, 98–107.

Drysdale, A.E. (1975). The visibility of retinal blood vessels. Vision Res. *15*, 813–818.

Engbert, R., and Kliegl, R. (2003a). Binocular coordination in microsaccades. In The Mind's Eyes: Cognitive and Applied Aspects of Eye Movements, R.R.J. Hyona and H. Deubel, eds. (Oxford: Elsevier), pp. 103–117.

Engbert, R., and Kliegl, R. (2003b). Microsaccades uncover the orientation of covert attention. Vision Res. *43*, 1035–1045.

Engbert, R., and Kliegl, R. (2004). Microsaccades keep the eyes' balance during fixation. Psychol. Sci. *15*, 431–436.

Fiorentini, A., and Ercoles, A.M. (1966). Involuntary eye movements during attempted monocular fixation. Atti. Fond. Giorgio. Ronchi. *21*, 199–217.

Gerrits, H.J., and Vendrik, A.J. (1970). Artificial movements of a stabilized image. Vision Res. *10*, 1443–1456.

Hafed, Z.M., and Clark, J.J. (2002). Microsaccades as an overt measure of covert attention shifts. Vision Res. *42*, 2533–2545.

Kowler, E., and Steinman, R.M. (1979). Miniature saccades: eye movements that do not count. Vision Res. *19*, 105–108.

Kowler, E., and Steinman, R.M. (1980). Small saccades serve no useful purpose: reply to a letter by R. W. Ditchburn. Vision Res. *20*, 273–276.

Levick, W.R., Cleland, B.G., and Dubin, M.W. (1972). Lateral geniculate neurons of cat: retinal inputs and physiology. Invest. Ophthalmol. *11*, 302–311.

Magnussen, S., Spillmann, L., Sturzel, F., and Werner, J.S. (2001). Filling-in of the foveal blue scotoma. Vision Res. *41*, 2961–2967.

Malinov, I.V., Epelboim, J., Herst, A.N., and Steinman, R.M. (2000). Characteristics of saccades and vergence in two types of sequential looking tasks. Vision Res. *40*, 2083–2090.

Martinez-Conde, S., Macknik, S.L., and Hubel, D.H. (2000). Microsaccadic eye movements and firing of single cells in the striate cortex of macaque monkeys. Nat. Neurosci. *3*, 251–258.

Martinez-Conde, S., Macknik, S.L., and Hubel, D.H. (2002). The function of bursts of spikes during visual fixation in the awake primate lateral geniculate nucleus and primary visual cortex. Proc. Natl. Acad. Sci. USA *99*, 13920–13925.

Martinez-Conde, S., Macknik, S.L., and Hubel, D.H. (2004). The role of fixational eye movements in visual perception. Nat. Rev. Neurosci. *5*, 229–240.

Nachmias, J. (1961). Determiners of the drift of the eye during monocular fixation. J. Opt. Soc. Am. *51*, 761–766.

Pritchard, R.M. (1961). Stabilized images on the retina. Sci. Am. *204*, 72–78.

Ramachandran, V.S., and Gregory, R.L. (1991). Perceptual filling in of artificially induced scotomas in human vision. Nature *350*, 699–702.

Reid, R.C., and Alonso, J.M. (1995). Specificity of monosynaptic connections from thalamus to visual cortex. Nature *378*, 281–284.

Reppas, J.B., Usrey, W.M., and Reid, R.C. (2002). Saccadic eye movements modulate visual responses in the lateral geniculate nucleus. Neuron *35*, 961–974.

Riggs, L.A., and Ratliff, F. (1952). The effects of counteracting the normal movements of the eye. J. Opt. Soc. Am. *42*, 872–873.

Rolfs, M., Engbert, R., and Kliegl, R. (2004). Microsaccade orientation supports attentional enhancement opposite to a peripheral cue. Psychol. Sci. *15*, 431–436.

Sabrin, H.W., and Kertesz, A.E. (1983). The effect of imposed fixational eye movements on binocular rivalry. Percept. Psychophys. *34*, 155–157.

Savitzky, A., and Golay, M.J.E. (1964). Smoothing and differentiation of data by simplified least squares procedures. Anal. Chem. *36*, 1627–1639.

Schieting, S., and Spillmann, L. (1987). Flicker adaptation in the peripheral retina. Vision Res. *27*, 277–284.

Sharpe, C.R. (1972). The visibility and fading of thin lines visualized by their controlled movement across the retina. J. Physiol. *222*, 113–134.

Skavenski, A.A., Hansen, R.M., Steinman, R.M., and Winterson, B.J. (1979). Quality of retinal image stabilization during small natural and artificial body rotations in man. Vision Res. *19*, 675–683.

Spillmann, L., and Kurtenbach, A. (1992). Dynamic noise backgrounds facilitate target fading. Vision Res. *32*, 1941–1946.

Spillmann, L., and de Weerd, P. (2003). Mechanisms of surface completion: perceptual filling-in. In Filling-In: From Perceptual Completion to Cortical Reorganization, L. Pessoa and P. de Weerd, eds. (New York: Oxford University Press), pp. 81–105.

Steinman, R.M. (1975). Oculomotor effects on vision. In Basic Mechanisms of Ocular Motility and Their Clinical Implications, G. Lennerstrand and P. Bach-Y-Rita, eds. (New York: Pergamon Press).

Steinman, R.M., and Collewijn, H. (1980). Binocular retinal image motion during active head rotation. Vision Res. *20*, 415–429.

Steinman, R.M., Cunitz, R.J., Timberlake, G.T., and Herman, M. (1967). Voluntary control of microsaccades during maintained monocular fixation. Science *155*, 1577–1579.

Steinman, R.M., Pizlo, Z., Forofonova, T.I., and Epelboim, J. (2003). One fixates accurately in order to see clearly not because one sees clearly. Spat. Vis. *16*, 225–241.

Sturzel, F., and Spillmann, L. (2001). Texture fading correlates with stimulus salience. Vision Res. *41*, 2969–2977.

Troxler, D. (1804). Ophthalmologische Bibliothek (Jena: Springer).

Tse, P.U., Sheinberg, D.L., and Logothetis, N.K. (2002). Fixational eye movements are not affected by abrupt onsets that capture attention. Vision Res. *42*, 1663–1669.

Tse, P.U., Sheinberg, D.S., and Logothetis, N.K. (2004). The distribution of microsaccade directions need not reveal the location of attention. Psychol. Sci. *15*, 708–710.

Usrey, W.M., Reppas, J.B., and Reid, R.C. (1999). Specificity and strength of retinogeniculate connections. J. Neurophysiol. *82*, 3527–3540.

van Dam, L.C., and van Ee, R. (2005). The role of (micro)saccades and blinks in perceptual bi-stability from slant rivalry. Vision Res. *45*, 2417–2435.

Winterson, B.J., and Collewijn, H. (1976). Microsaccades during finely guided visuomotor tasks. Vision Res. *16*, 1387–1390.

Yarbus, A.L. (1967). Eye Movements and Vision (New York: Plenum Press).

Zuber, B.L., and Stark, L. (1965). Microsaccades and the velocity-amplitude relationship for saccadic eye movements. Science *150*, 1459–1460.