

# Current Biology

## Neuronal Response Gain Enhancement prior to Microsaccades

### Highlights

- SC and FEF neurons exhibit stronger visual bursts for stimuli before microsaccades
- Suppressed visual bursts occur for stimuli after microsaccades
- The modulations are highly dependent on microsaccade direction
- Response gain enhancement is not accompanied by increased noise in representations

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### In Brief

Chen et al. show that superior colliculus (SC) and frontal eye fields (FEFs) exhibit stronger responses when visual stimuli appear immediately before tiny fixational eye movements called microsaccades. The enhancement is spatially specific and independent of behavioral tasks, showing that microsaccades can have strong impacts on neuronal activity.



# Neuronal Response Gain Enhancement prior to Microsaccades

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<http://dx.doi.org/10.1016/j.cub.2015.06.022>

## SUMMARY

Neuronal response gain enhancement is a classic signature of the allocation of covert visual attention without eye movements. However, microsaccades continuously occur during gaze fixation. Because these tiny eye movements are preceded by motor preparatory signals well before they are triggered, it may be the case that a corollary of such signals may cause enhancement, even without attentional cueing. In six different macaque monkeys and two different brain areas previously implicated in covert visual attention (superior colliculus and frontal eye fields), we show neuronal response gain enhancement for peripheral stimuli appearing immediately before microsaccades. This enhancement occurs both during simple fixation with behaviorally irrelevant peripheral stimuli and when the stimuli are relevant for the subsequent allocation of covert visual attention. Moreover, this enhancement occurs in both purely visual neurons and visual-motor neurons, and it is replaced by suppression for stimuli appearing immediately after microsaccades. Our results suggest that there may be an obligatory link between microsaccade occurrence and peripheral selective processing, even though microsaccades can be orders of magnitude smaller than the eccentricities of peripheral stimuli. Because microsaccades occur in a repetitive manner during fixation, and because these eye movements reset neurophysiological rhythms every time they occur, our results highlight a possible mechanism through which oculomotor events may aid periodic sampling of the visual environment for the benefit of perception, even when gaze is prevented from overtly shifting. One functional consequence of such periodic sampling could be the magnification of rhythmic fluctuations of peripheral covert visual attention.

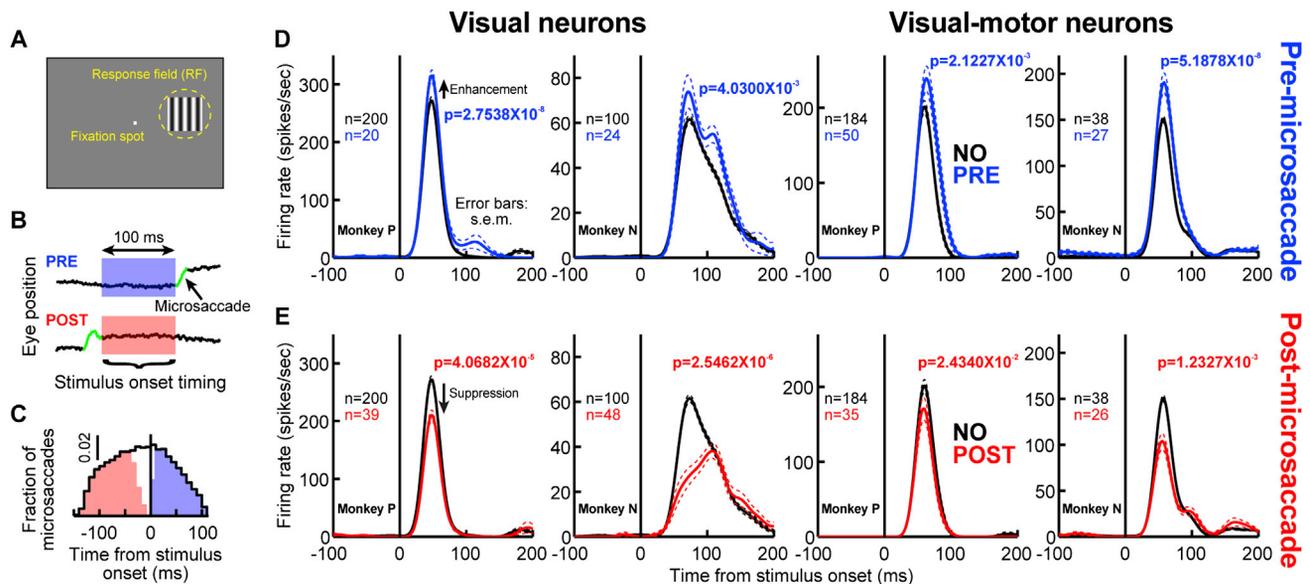
## INTRODUCTION

Covert visual attention refers to the brain's ability to selectively process behaviorally relevant stimuli [1, 2]. Such selective pro-

cessing arises through changes in stimulus representation. For example, neuronal response is enhanced if a stimulus was attended [3–12]. Concomitant reductions in variability also take place [13], and when attention deviates away from the stimulus, during inhibition of return (IOR) [2, 14, 15], suppression occurs [6, 11, 16]. These sensory modulations are signatures of selective covert visual attention.

Inherent in covert attention is a requirement to fixate. However, subliminal gaze shifts continuously occur [17–19]. Microsaccades are modulated in an automatic manner by any stimulus, whether or not attentionally relevant [19, 20]. Moreover, these eye movements are generated using similar mechanisms to larger saccades [21, 22], and they are also associated with peri-movement changes in vision, similar to those accompanying saccades [23, 24]. Given these peri-movement changes, it may be expected that at least some changes in stimulus representation during gaze fixation (for example, during attentional allocation) might be time locked to microsaccades, reflecting peri-movement changes. It might also be the case that these changes share characteristics with changes observed when attentional allocation is instructed. For example, if microsaccade-related preparatory activity in the superior colliculus (SC) [21] were to provide a “gain” modulation signal for visually evoked neuronal activity [24], similar to how it might do with large saccades [25–30], then response enhancement could potentially be observed for stimuli appearing before microsaccades, independent of whether a task involved attention. Thus, response enhancement, an attentional signature, can also occur in tight synchrony with individual microsaccades. Starting from this hypothesis, using behavioral and computational studies, we recently found that spatial attentional performance was modulated peri-microsaccadically [19, 24]; the largest attentional effects occurred when targets appeared around microsaccades, during a period in which visual perception is altered [24]. Here, we investigated possible neuronal correlates of these findings.

We describe robust response enhancement if stimuli appear before microsaccades, independent of whether or not an attentional task is used. Moreover, there is often sustained activity elevation, similar to sustained attentional modulations [5]. Finally, such enhancement is not associated with increased neuronal variability, but rather decreased variability in some cases. Thus, pre-microsaccadic alterations in visual representations both contribute to and modulate neuronal signatures of covert attention. While these results have strong implications on the interpretation of a large body of literature [24], they do not deny the concept of attention. Instead, they uncover a tight temporal



**Figure 1. Pre-microsaccadic Response Gain Enhancement**

(A) Monkeys fixated on a spot, and a sine wave grating appeared inside a neuron's response field (RF). (B) Our analysis approach is as follows: if the grating appeared <100 ms before microsaccade onset, the trial had a pre-microsaccadic stimulus (pre); if it appeared <100 ms after microsaccade end, it was a post-microsaccade trial (post). "Baseline" trials had no microsaccades  $\pm 100$  ms from the grating onset. (C) Across all trials and sessions, microsaccades occurred around stimulus onset, allowing us to explore pre- and post-microsaccadic modulations. Red denotes microsaccades in which the stimulus appeared after microsaccade end (post); blue denotes microsaccades with stimuli appearing before microsaccade onset (pre). We did not include trials with stimulus onset during microsaccades (unshaded region). (D) Four sample superior colliculus (SC) neurons (two from each monkey), in which responses were enhanced for stimuli appearing before microsaccades directed toward their hemifield. Black curves show no-microsaccade responses; blue curves show enhanced responses for pre-microsaccadic stimuli (t test; p values and numbers of trials are shown in the figure; [Experimental Procedures](#)). (E) The same neurons were suppressed on post-microsaccade trials. This figure shows responses to 80% contrast. [Figure 5](#) shows results from full contrast sensitivity curves. Error bars denote SEM.

relationship between attentional effects and individual microsaccades. Thus, even during fixation, perception is periodically interjected with momentary increases or decreases in visual sensitivity, which are time locked to individual microsaccades, and which will not only affect attentional performance [24] but also generally influence perception [24, 31] and action [23].

## RESULTS

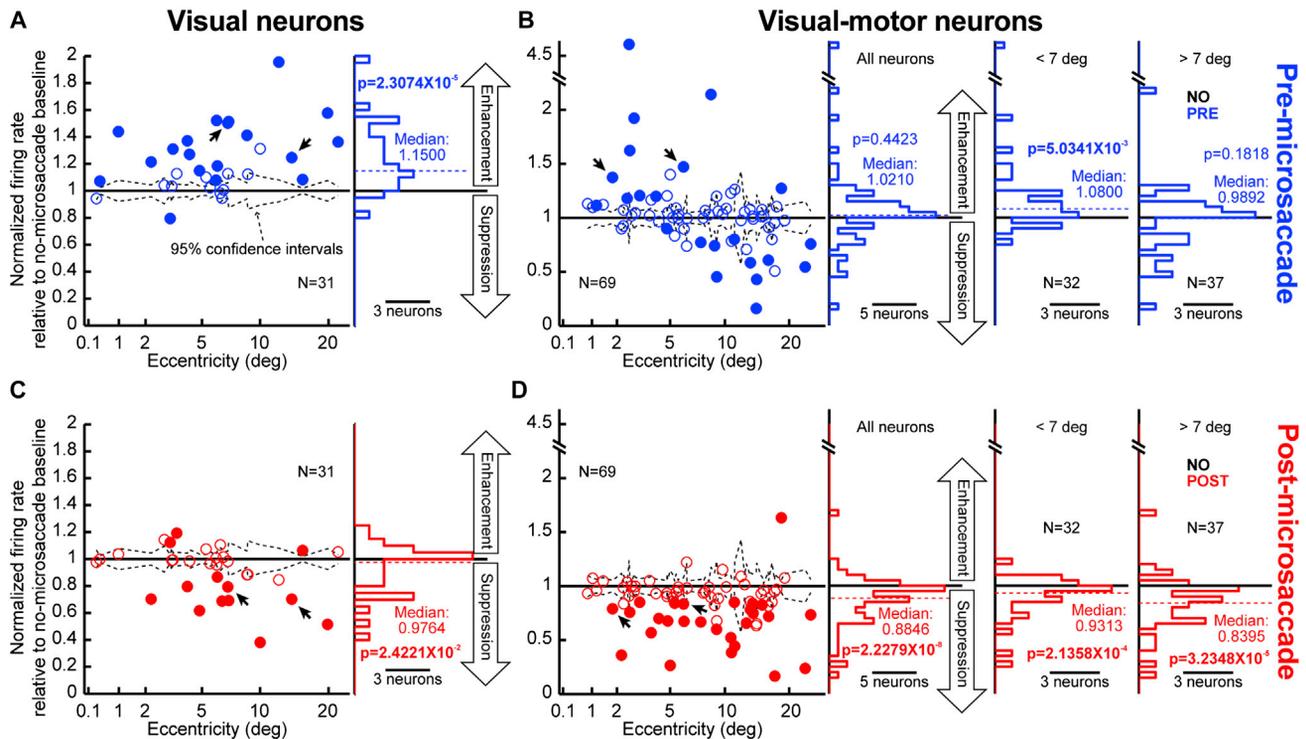
### Response Enhancement for Stimuli before Microsaccades

We first describe results from two monkeys, P and N, performing a pure fixation task. After fixating on a spot for 400–550 ms, the spot transiently dimmed for 50 ms, which reset microsaccadic rhythms [19] without inducing a spatial bias in microsaccades. After 110–320 ms, a vertical sine wave grating (2.22 cycles/°) appeared for 300 ms within a neuron's response field (RF) ([Figure 1A](#)). Monkeys were rewarded only for maintaining fixation, and we investigated how grating-induced visual responses were modulated around microsaccades ([Figure 1B](#)): we analyzed response strength when the grating appeared without any microsaccades within  $\pm 100$  ms from stimulus onset or when it appeared <100 ms before (blue) or after (red) microsaccades. Across all trials, microsaccades occurred at different times relative to stimulus onset ([Figure 1C](#)), allowing us to map the time course of peri-microsaccadic changes in neuronal activity.

Visually responsive SC neurons showed enhanced responses for stimuli appearing before microsaccades, even though these microsaccades never placed the monkey's gaze at the stimuli. [Figure 1D](#) shows the activity of four example neurons and demonstrates such enhancement for a high-contrast (80%) grating. When the grating appeared <100 ms before a microsaccade directed toward its hemifield (blue), enhancement occurred, similar to SC enhancement in covert attention tasks [6, 7, 9, 11, 29], but we observed it merely during fixation.

Response enhancement was restricted to pre-movement intervals. If the same stimulus appeared <100 ms after microsaccades, suppression occurred ([Figure 1E](#), red), analogous to microsaccadic suppression [23]. Thus, both visual and visual-motor SC neurons showed pre-microsaccadic enhancement and post-microsaccadic suppression, consistent with behavioral evidence [24] and reminiscent of SC neuronal response gain changes during covert attention [6, 7, 9, 11, 16, 29].

Across the population, we computed a modulation index normalizing activity on trials with microsaccades to activity on trials without. [Figure 2A](#) plots this index for all visual neurons as a function of their preferred eccentricity. For stimulus onsets <100 ms before microsaccades, there was  $\sim 15\%$  (median) enhancement ([Figure 2A](#), blue histogram;  $p = 2.3 \times 10^{-5}$ , paired signed-rank test); 18/31 (58%) neurons were individually significant ( $p < 0.05$ ). For stimulus onsets <100 ms after microsaccades ([Figure 2C](#), red),  $\sim 2.4\%$  (median) suppression occurred



**Figure 2. SC Population Summary**

(A) Normalized activity on pre-microsaccade trials against neuronal preferred eccentricity. Eccentricity is plotted on a logarithmic scale representing SC topography (Experimental Procedures). Points above one are neurons with enhanced responses. Filled symbols indicate significant modulations ( $p < 0.05$ ; t test; Experimental Procedures). Dashed lines around one are 95% confidence intervals for the no-microsaccade baselines computed for each neuron. The marginal histogram summarizes the population result. The two arrows point to the two-sample visual neurons of Figure 1.

(B) The same as in (A), except for visual-motor neurons. Similar observations were made, except that eccentric neurons ( $>7^\circ$ ) do not show enhancement. The marginal histograms show neuronal modulation indices for all neurons (leftmost histogram) or for either central (middle histogram) or eccentric (rightmost histogram) neurons.

(C and D) The same as in (A) and (B), except for post-microsaccade trials. Suppression occurred and was strongest for peripheral visual-motor neurons. In all panels, neuron numbers are indicated, and p values are from paired signed-rank tests (Experimental Procedures). Colored dashed lines indicate median values. See also Figures S1–S3.

( $p = 2.4 \times 10^{-2}$ , paired signed-rank test). Importantly, pre-microsaccadic enhancement occurred in neurons at all tested eccentricities; microsaccades were associated with response enhancement even for neurons at  $>20^\circ$ .

Visual-motor SC neurons behaved similarly (Figure 2B), but pre-microsaccadic enhancement was now eccentricity dependent. Neurons with RF centers  $<7^\circ$  exhibited enhancement; more eccentric neurons showed no modulation or suppression. The leftmost histogram in Figure 2B describes all neurons ( $n = 69$ ), and the middle and rightmost histograms show modulation indices for RF centers less ( $n = 32$ ) or greater ( $n = 37$ ) than  $7^\circ$ . More central neurons exhibited  $\sim 8\%$  (median) enhancement ( $p = 5 \times 10^{-3}$ , paired signed-rank test); more eccentric ones did not exhibit enhancement ( $p = 0.1818$ ). Suppression occurred for post-microsaccadic stimuli (Figure 2D).

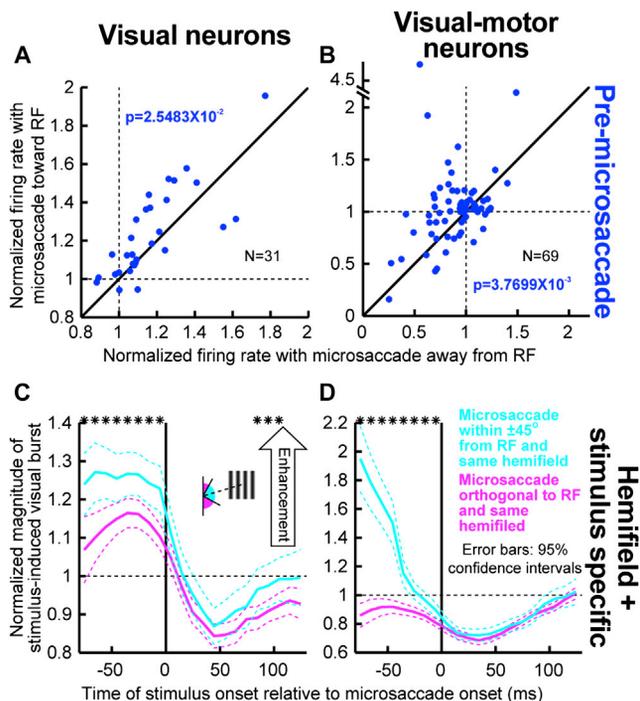
Therefore, we found pre-microsaccadic enhancement in both visual and visual-motor SC neurons, only under simple fixation. We also checked whether the monkeys may have sustained attention at the RF location by analyzing pre-stimulus microsaccade directions. If monkeys sustained attention at that location, because of its predictability, previous work [17, 18] suggests strong microsaccade direction biases toward it. This was not

the case (Figure S1A). Moreover, if the stimulus appeared after a microsaccade (Figures 1 and 2), there was suppression; thus, the modulations were time locked to movement generation, rather than reflecting a sustained RF-directed bias. Post-stimulus microsaccades were also not affected by stimulus location (Figure S1B), consistent with their short onset times (Figure 1C) and suggesting that they were not visually triggered by the grating.

Our results are also not due to peri-microsaccadic modulations, either in the absence (Figure S2A) or presence (Figure S2B) of RF stimuli, and they still occurred with brief RF stimuli (Figure S2C). We also confirmed that our results are not due to displacements of stimuli by microsaccades relative to RF centers (Figure S3). Finally, no stimulus-foveating saccades occurred. Microsaccade amplitude was  $<0.25\times$  the nearest stimulus eccentricity and much more often  $>10\times$  smaller.

#### Dependence on Microsaccade Direction

We asked whether microsaccade direction relative to the RF matters, as predicted recently [24]. We plotted (Figures 3A and 3B) each neuron's response if a stimulus appeared before a microsaccade toward (y axis) or away from (x axis) the stimulus



**Figure 3. Differential Influence of Microsaccade Direction on Pre-microsaccadic Enhancement in the SC**

(A and B) Normalized firing rate (relative to no-microsaccade baselines) on trials with a stimulus before a microsaccade toward the hemifield (y axis) of the stimulus versus the opposite (x axis) is shown. For pure visual neurons (A), even though opposite trials still showed enhancement (x axis points above one), the enhancement was stronger if the microsaccade was toward the hemifield of the stimulus. This effect was even stronger for visual-motor neurons (B), which often showed suppression before “opposite” microsaccades (x axis points below one) but enhancement before “toward” movements (y axis points above one). p values are from paired signed-rank tests.

(C and D) Time courses of peri-microsaccadic response modulation. Both visual (C) and visual-motor (D) neurons show pre-microsaccadic enhancement, which was strongest for microsaccades within  $<45^\circ$  in direction relative to the RF stimulus location. Notice how for visual-motor neurons (D), even movements within the same hemifield but orthogonal to the stimulus location exhibited pre-microsaccadic suppression (magenta). Thus, pre-microsaccadic enhancement (in both visual and visual-motor neurons) was best for microsaccades specifically “pointing” toward the stimulus location. Asterisks illustrate times with a significant difference between toward and “away” ( $p < 0.05$ ). The icon in (C) indicates the analysis logic: we considered all microsaccades toward (cyan) the hemifield of the grating and within  $<45^\circ$  in direction from grating location, and we compared them to microsaccades within the same hemifield but pointing “away” from the grating location (magenta). Error bars in (C) and (D) denote 95% confidence intervals. See also Figure S4.

hemifield. For visual neurons, microsaccades toward were associated with stronger enhancement than microsaccades opposite (Figure 3A;  $p = 2.5 \times 10^{-2}$ , paired signed-rank test), even though opposite trials still showed enhancement (x axis points lying above one). Visual-motor neurons showed an even stronger directional effect: there was suppression before opposite microsaccades (x axis points lying below one) but enhancement toward (y axis points lying above one) (Figure 3B;  $p = 3.8 \times 10^{-3}$ , paired signed-rank test). Thus, an upcoming microsaccade was associated with sensitization of visual responses to

stimuli in the same direction, but weaker sensitization or suppression opposite. These results are reminiscent of direction-dependent pre-microsaccadic behavioral effects [24].

The full time course of response modulation further demonstrated direction dependence. We measured responses as a function of when stimuli appeared relative to microsaccade onset [23], and we asked whether even movements within the same hemifield but orthogonal to the RF location had differential effects from movements specifically directed toward the RF location. There was a distinct time course of pre-microsaccadic enhancement followed by post-microsaccadic suppression, and the enhancement was always stronger (visual neurons) or only present (visual-motor neurons) for movements directed toward the stimulus (Figures 3C and 3D). Note that our time range in this analysis was dictated by having sufficient trials with a stimulus appearing within a given time window. Because stimulus onsets result in microsaccadic inhibition  $\sim 75$ – $100$  ms later [19, 20] (Figure 1C), we could not map times  $< -75$  ms. Nonetheless, the analysis sufficiently demonstrated pre-microsaccadic enhancement. Most interestingly, visual and visual-motor neurons showed qualitative differences, with visual-motor neurons showing an earlier effect. In fact, Figure S4 suggests that even visual-motor neurons at large eccentricities can still exhibit enhancement (an effect masked in Figure 2 with a less sensitive time-window analysis), indicating that visual-motor enhancement was not due to a “microsaccade-related” motor discharge restricted in the foveal SC (Figure S2A).

Thus, microsaccades were associated with spatially specific SC response enhancement. Next, we explore the generalizability of this phenomenon and describe additional corroborations of it.

### Generalizability across Tasks and Areas

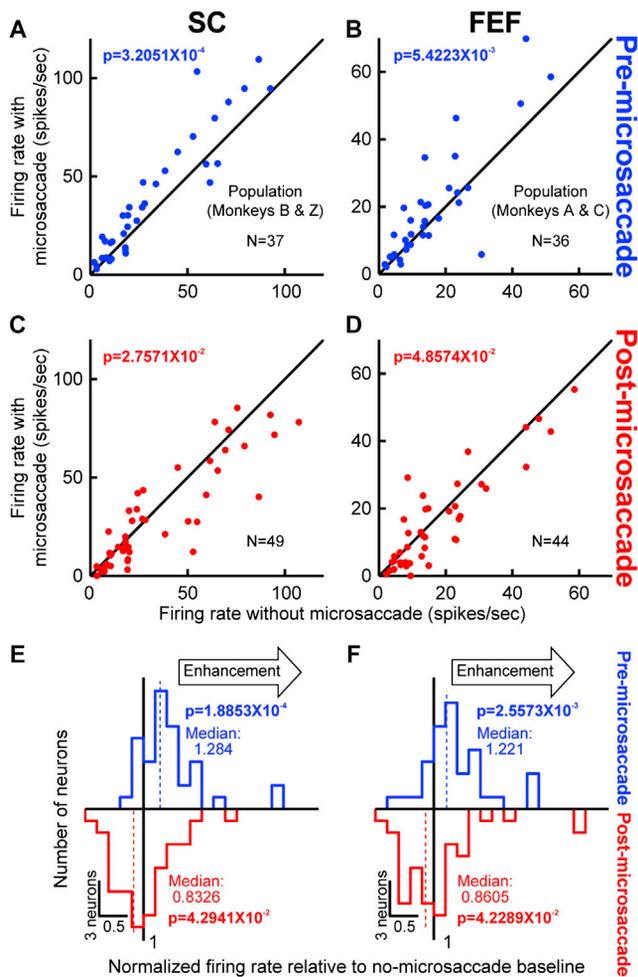
In a study of the SC’s role in covert attention [7], activity was modulated after attentional cue onset. We re-analyzed 60 neurons from this study and asked whether cue-induced activity was also modulated around microsaccades. Even though these experiments were not designed to focus on microsaccades, thus not allowing individual-neuron statistics (Experimental Procedures), we still found robust population results: two additional monkeys (B and Z) showed similar pre-microsaccadic enhancement (Figures 4A and 4E, blue) and post-microsaccadic suppression (Figures 4C and 4E, red). Thus, all four monkeys, regardless of whether or not a stimulus was an attentional cue, showed enhancement.

We also ran the same task [7] using two additional monkeys (A and C), now recording in the frontal eye fields (FEFs) [10, 32, 33]. Once again, qualitatively and quantitatively similar modulations occurred (Figures 4B, 4D, and 4F), and these results were also similar when we analyzed visual and visual-motor neurons separately.

Therefore, in six monkeys and two areas implicated in attention [6, 7, 9–11, 33, 34], pre-microsaccadic enhancement occurred, and with different stimulus types (gratings versus spots). These results confirm that pre-microsaccadic enhancement can occur in attentional tasks [24].

### Changes in Contrast Sensitivity

In monkeys P and N, we also presented different contrasts. Figure 5A (left) shows contrast sensitivity curves for an example



**Figure 4. Generalizability of Pre-microsaccadic Enhancement across Monkeys, Areas, and Tasks**

(A) Cue-induced SC visual bursts from a previously published [7] attentional cueing task. We plotted activity on trials with cue onset before microsaccades versus activity without microsaccades (as in Figures 1 and 2; Experimental Procedures). Across the population, significant enhancement occurred (paired signed-rank test). Thus, pre-microsaccadic SC enhancement occurred in four monkeys, in different tasks (fixation in Figures 1, 2, and 3; attentional cueing in this figure), and with different stimuli (gratings in Figures 1, 2, and 3; spots in this figure).

(B) Similar results from the same cueing task but in the FEFs and with two additional monkeys are shown. The neurons in this analysis had similar eccentricities as those in (A) and also similar proportions of visual and visual-motor neurons (Experimental Procedures).

(C and D) If the cue appeared after microsaccades, both SC and FEF neurons were suppressed.

(E and F) Neuronal modulation indices are shown in a manner similar to Figure 2, except for the data in (A)–(D). Blue histograms show pre-microsaccadic indices and demonstrate enhancement. Red histograms show post-microsaccadic indices and demonstrate suppression. All population-level statistics are from paired signed-rank tests. Only neurons that had enough measurements of both no-microsaccade and either pre- or post-microsaccade trials were included (Experimental Procedures). Colored dashed lines indicate median values.

neuron from Figure 1B. For stimuli <100 ms before microsaccades toward their hemifield (blue), the curve was scaled upward. Also in Figure 5A, the right curves show population results

(Experimental Procedures), and Figure 5B repeats this for visual-motor neurons. In all cases, whenever enhancement occurred, the multiplicative gain parameter in our psychometric curve fits (Experimental Procedures) was the parameter that was significantly altered compared to no-microsaccade psychometric curves ( $p < 0.05$ , bootstrapping). For stimuli after microsaccades, contrast sensitivity curves were scaled downward (Figures 5C and 5D). Whether pre- or post-microsaccade, there was no statistically significant shift in semi-saturation sensitivity points ( $p > 0.05$ , bootstrapping). For microsaccades opposite the stimulus, pre-microsaccadic enhancement was reduced or eliminated (Figure S5), consistent with Figure 3. Therefore, response gain enhancement for our stimuli appeared to be primarily governed by multiplicative modulation, although we acknowledge that enhancement at low contrasts was less strong in our data compared to cortical studies of attention.

### Lack of Variability Increases

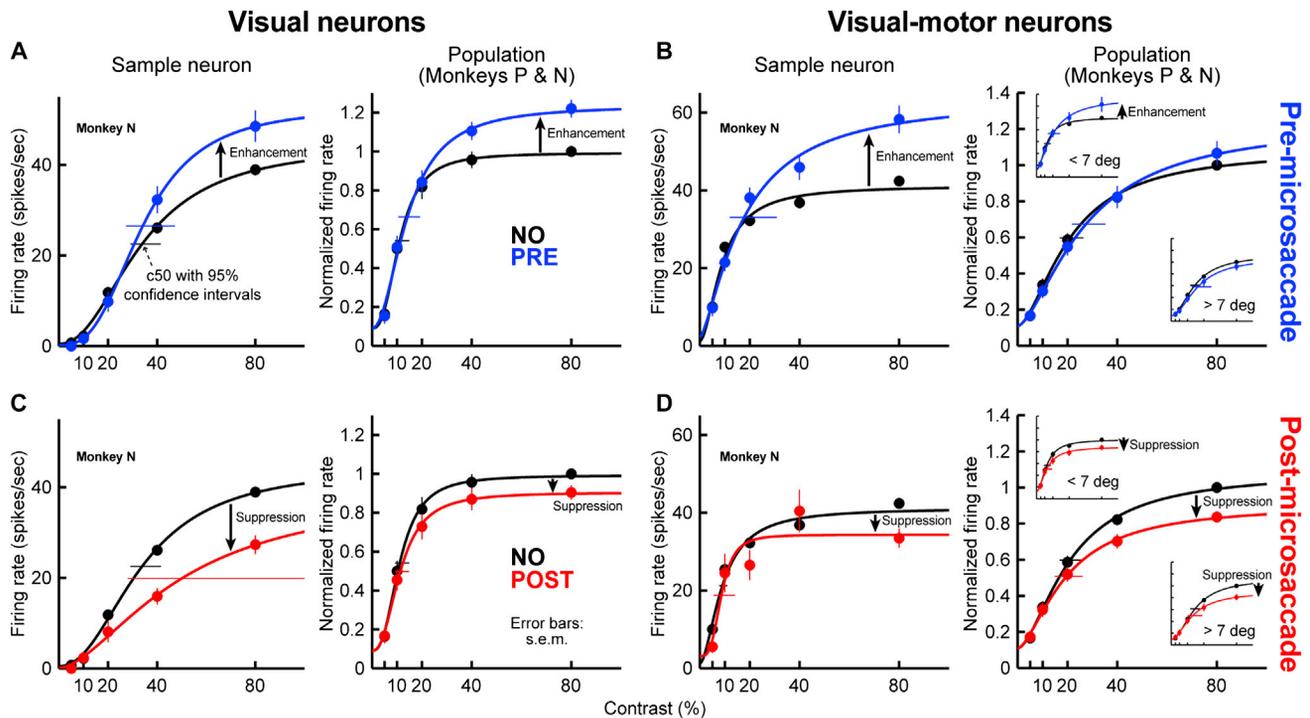
If enhancement is accompanied by increased variability, readout of neuronal populations could be muddled by noise [13]. In monkeys P and N, from which we had enough data to explore this, we performed receiver operating characteristic curve (ROC) analyses, to assess whether enhancement resulted in significant discriminability of neuronal responses between no-microsaccade and microsaccade trials. Figures 6A and 6B show the area under the ROC curve for trials with 80% gratings appearing before a microsaccade toward RF hemifield. In both visual and visual-motor neurons, enhanced responses were highly discriminable from baseline (and across contrasts; Figure S6).

We also analyzed fano factor and plotted data as performed previously in the SC [35]. Figure 6C shows results from visual neurons, comparing trials with a stimulus before a microsaccade toward the RF hemifield (y axis) to trials without microsaccades (x axis). Each color represents a single contrast, and each faint dot represents data from a single neuron; dots with saturated colors summarize population results. Visual neurons showed reduced fano factors ( $p = 0.015817$ ), which was also observed previously for large saccades (albeit anecdotally) [9]. Visual-motor neurons (Figure 6D) showed no modulation.

Thus, pre-microsaccadic enhancement was accompanied by putatively equal- or higher-fidelity sensory representations. In our case, this happened without attentional tasks and demonstrated instead tight synchrony between microsaccades and altered visual representations.

### A Sustained Enhancement

Some of our SC neurons possessed sustained activity (Experimental Procedures). For these neurons in monkeys P and N, we asked whether sustained enhancement could still be observed. Figure 7A shows data from one such neuron (80% grating). For stimuli before microsaccades toward the RF hemifield, the neuron showed both burst enhancement and sustained elevation (blue; shaded region), similar to sustained elevations with attention [5]. For post-microsaccadic stimuli (Figure 7C), the effect disappeared. These observations were consistent across 30 neurons (27/100; plus three neurons recorded for this analysis) (Figures 7B, 7D, and S7), and they were again accompanied by significant ROC discriminability (Figure 7E). Moreover, fano factor analyses revealed a subtle variability



**Figure 5. SC Contrast Sensitivity Changes around Microsaccades**

(A) Left shows responses of the visual neuron of Figure 1B from monkey N. Black shows responses on no-microsaccade trials. Blue shows responses for stimuli <100 ms before a microsaccade toward the hemifield of the stimulus. Error bars denote SEM, and horizontal bars show 95% confidence intervals for the semi-saturation contrasts (c50) (Experimental Procedures). Right shows results from the population of visual neurons in monkeys P and N. Before combining neurons, each neuron's curve was normalized based on the no-microsaccade baseline curve (Experimental Procedures).

(B) The same as in (A), except for visual-motor neurons from monkeys P and N. The sample neuron shown is the visual-motor neuron of Figure 1B from monkey N. Visual-motor neurons also show enhancement, but the effect was strongest for more central neurons (insets).

(C and D) Both visual (C) and visual-motor (D) neurons show significant suppression for stimuli after microsaccades.

See also Figure S5.

decrease ( $p = 0.00143$ ) (Figure 7F). We did not have enough trials from monkeys B, Z, A, and C to repeat these analyses, but we did notice population-level evidence that cueing trials with sustained post-cue activity elevations [7] were ones with pre-microsaccadic cue onsets.

Thus, previously observed single-neuron correlates of covert attention can also be observed during simple fixation. Because microsaccades occur systematically during spatial attention tasks, this indicates that pre-microsaccadic processes may be tightly correlated with covert attentional modulations.

### Relationship to Behavior

Previous behavioral work strongly motivated our study [24]. More recently, we tested monkeys P and N on a prediction of the current data: if visual bursts are modulated on pre-microsaccade trials in a spatially specific manner (Figure 3), then reaction times (RTs) to stimuli might also be affected. We indeed found that RTs were faster if a stimulus appeared before microsaccades toward the stimulus than away from it (X. Tian, M. Yoshida, and Z.M.H., unpublished data; data not shown).

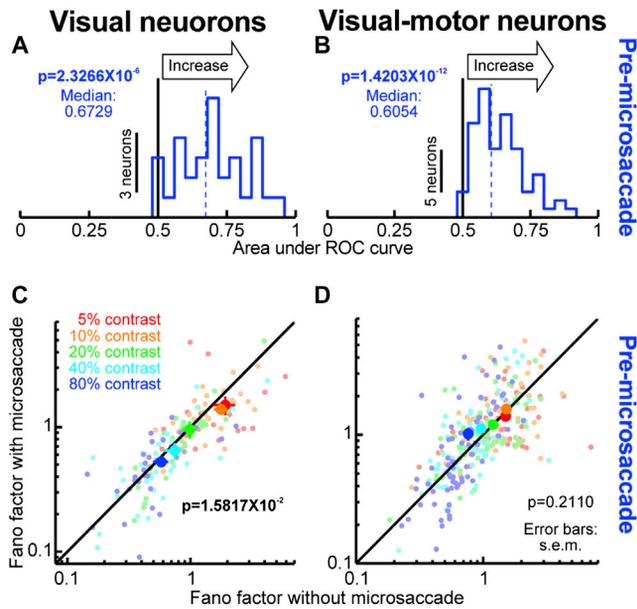
We also analyzed behavior from monkeys B, Z, A, and C. We reasoned that if cue-induced activity was modulated as we observed, then final performance might also change. We indeed

found that if the cue appeared <100 ms before a microsaccade toward its direction, performance was 80% correct; if the microsaccade was away, performance was 66.4% ( $p = 0.0185$ ;  $\chi^2$  test;  $\chi^2$  statistic: 5.5489;  $n = 143$  trials for toward and 105 trials for opposite). Performance on no-microsaccade trials was in between (73.4%). It is truly remarkable that this result was obtained at all, especially because in these attentional tasks, task difficulty was continuously adjusted from trial-to-trial [7], which likely muted our effect.

Thus, combined with these and earlier behavioral [24] and computational (X. Tian, M. Yoshida, and Z.M.H., unpublished data; data not shown) studies, our results suggest that behavioral and neuronal signatures of attention can be observed around microsaccades. Peri-microsaccadic alterations in vision, regardless of their origin, can modulate and potentially magnify [24] behavioral and neuronal signatures of covert attention.

### DISCUSSION

Because microsaccades occur systematically in attentional tasks [17–20], our results suggest that attentional modulations may be modified around microsaccades. These results do not in any way deny the concept of attention, but they highlight a



**Figure 6. SC Neuronal Discriminability and Variability with Pre-microsaccadic Stimuli**

(A) For pure visual neurons of monkeys P and N, we plotted area under the ROC curve comparing pre- and no-microsaccade trials. Values  $>0.5$  indicate above-chance discriminability. Data from 80% gratings are shown. See also Figure S6 for other contrasts and data for microsaccades opposite the RF hemifield.

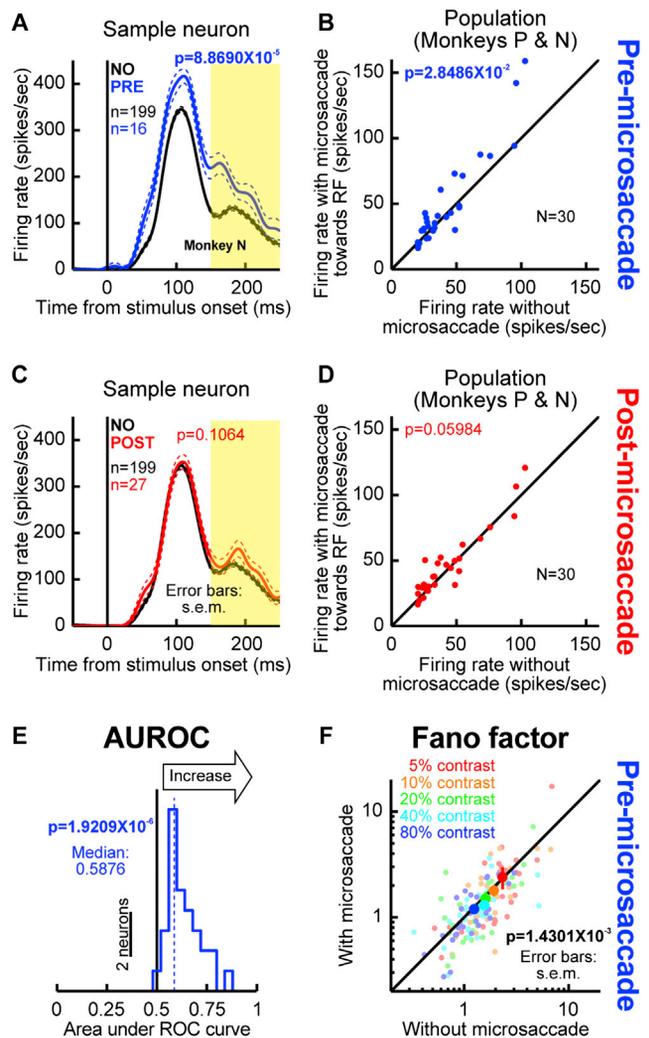
(B) The same as in (A), except for visual-motor neurons.

(C and D) Fano factors on trials with and without microsaccades. Each dot shows data from a neuron, and each color denotes a single contrast. The dots with saturated colors show means (and SEM) across neurons for a given contrast. The p value shows statistical test results across all neurons and all contrasts, similar to the approach of [35]. Visual neurons show reduced fano factors when response gain was increased (for microsaccades toward the RF hemifield); visual-motor neurons show neither a reduction nor increase. All statistics are from paired signed-rank tests.

possible mechanism through which attentional effects may be magnified.

While our results do not establish causality in either direction, one possible mediator of synchrony between microsaccades and neuronal or behavioral [24] signatures of selective processing could be corollary discharge. For example, SC activity for large saccades is sent to cortex to update spatial representations [36]. Given that models of such updating invoke an oculomotor-derived “gain” signal [24, 26], our results could reflect the influence of such a signal [24]. Indeed, within the SC, an excitatory pathway from motor to sensory layers exists [37]. Interestingly, in this pathway, there is widespread influence, akin to a saccade toward one eccentricity influencing visual representations at different eccentricities. This is consistent with our observation of peripheral enhancement more eccentric than the microsaccade endpoint and also consistent with large-saccade dissociations of enhancement [9, 29].

Alternatively, or perhaps additionally, continuous brain-state fluctuations [38] likely also contribute to our results. These fluctuations happen independently of attentional task requirements and only get reset by attentional cues. Since cues reset microsaccadic rhythms [19, 39], and since microsaccades themselves



**Figure 7. A Sustained Influence of Pre-microsaccadic SC Modulations**

(A) A sample visual neuron from monkey N with 80% contrast is shown. The neuron had a sustained response (black curve, shaded region). If the stimulus appeared before a microsaccade toward its hemifield, this response was enhanced (blue) even though the microsaccade had long ended. Error bars denote SEM.

(B) Summary of sustained interval measurements from neurons with sustained activity in the no-microsaccade condition. This sustained activity was consistently enhanced for pre-microsaccadic stimuli (paired signed-rank test). (C and D) This effect disappeared when the stimulus appeared after microsaccades. See also Figure S7.

(E and F) Summaries of ROC (similar to Figures 6A and 6B) and fano factor (similar to Figures 6C and 6D) analyses performed on the sustained interval highlighted in (A). Pre-microsaccadic enhancement was accompanied by significant discriminability (ROC) and (a subtle) decreased variability (fano factor) even in the sustained response interval.

reset brain fluctuations [39] (probably through the pre- and post-motor changes we report here), synchrony between microsaccades and attentional modulations is expected [24]. Importantly, such synchrony suggests that a saccadic-rhythmicity model only employing pre-microsaccadic sensitivity changes is sufficient to generate “attentional capture” and “IOR” in Posner

cueing (X. Tian, M. Yoshida, and Z.M.H., unpublished data; data not shown). Finally, synchrony between neuronal excitability and microsaccades makes functional sense: saccades and attention are obligatorily synchronized under natural conditions, and microsaccades are a subset of saccades.

The idea of pre-motor links to attention has a rich history, with behavioral [40] and neurophysiological [25] support. Structures critical for saccades, like SC [34] and FEFs [32], are influential for attention. Our results extend these observations, suggesting that even under fixation, pre-motor modulations may contribute to neuronal and behavioral [24] modulations. In fact, microsaccades, like saccades, disrupt visual information flow. Thus, as part of a generalized perceptual stability mechanism, the brain could “attentionally sample” the world just before microsaccades. Indeed, microsaccades cause perceptual mislocalizations that are believed to be a hallmark of perceptual stability mechanisms [24]. Therefore, attention may be a general component of peri-saccadic perceptual stability [27].

Our sustained activity elevations are particularly intriguing (Figures 7 and S7). In this case, the microsaccade had long ended. This suggests that neuronal analyses of attentional modulations may miss possible influences of earlier microsaccades and that a microsaccade can have prolonged impact [24].

Equally interesting is the role of microsaccade directions. Pre-microsaccadic enhancement is spatially specific and strongest for stimuli congruent with microsaccade direction (Figure 3). We think that this effect, reminiscent of the focal nature of spatial attention, could arise because of an interaction between two signals: a gain-modulation signal that is potentially provided by corollary discharge [24, 37] and a spatially specific stimulus-induced burst. It would be interesting to further test this hypothesis with multiple simultaneous stimuli. In this case, for visual-motor SC neurons, microsaccades need to be congruent with one stimulus at a time to be associated with enhancement for each of the stimuli, reminiscent of sequential attentional sampling [38, 41]. If a pre-microsaccadic “gain” signal were to now be broadcast to visual areas at multiple hierarchies (e.g., to V1 with small RFs and V4 with larger ones), then this mechanism could also result in additional RF modulations: RF size in a higher area might appear to “shrink” around the stimulus location congruent with a microsaccade because with multiple stimuli, earlier visual areas with small RFs (each “seeing” only one of the stimuli) would either be enhanced or suppressed based on the microsaccade direction relative to its RF stimulus. This effect would then trickle toward the higher visual area, now pooling an enhanced response from one stimulus and a suppressed response from another. As for superficial SC layers, we found pre-microsaccadic enhancement regardless of microsaccade direction, albeit with direction-dependent differences (Figure 3A). Thus, a single microsaccade could subserve simultaneous enhancement, as with “divided attention.”

Finally, we observed consistent FEF modulations, which are interesting in light of the role of FEFs in attention [33]. In fact, V4 exhibits similar modulations before saccades to their modulations during attention [42], presumably mediated by FEFs. Our results add to these findings the observation that FEFs may also mediate synchrony between microsaccades and visual cortical neuronal modulations. Even when target selection oc-

curs without overt actions, covert processing may nonetheless intrinsically remain an “active perception” phenomenon.

## EXPERIMENTAL PROCEDURES

Ethics committees approved all experiments.

### Animal Preparation

Monkeys P and N (male, *Macaca mulatta*, aged 7 years) were prepared for behavior earlier [43]. We placed SC chambers on the midline, aimed at 1 mm posterior of and 15 mm above the inter-aural line. Chambers were tilted posterior of vertical (38° and 35° for P and N, respectively).

The methods used for monkeys B and Z were described earlier [7]. Monkeys A and C (C, female, aged 10 years; A, male, aged 9 years) were prepared in the same laboratory [7].

### Behavioral Tasks

Monkeys P and N fixated only. In each trial, a white spot (~8.5 × 8.5 min arc) appeared over a gray background [43]. We presented a vertical sine wave grating [30]. Grating contrast ( $L_{max} - L_{min} / L_{max} + L_{min}$ ) was 5%, 10%, 20%, 40%, or 80%, and phase was randomized. Grating size (filling the RF) was large enough to avoid a potential “micro” form of changing/shifting RFs around saccades [27, 28]. If such changes occur around microsaccades, they would be small and canceled with large stimuli.

We analyzed 103 SC neurons (1,075 ± 326 SD trials per neuron). We collected >20 no-microsaccade trials per contrast per neuron (average: 97.8 ± 68.1 SD) and >9 pre- or post-microsaccade trials (average: 21.9 ± 8.5 SD).

For monkeys B and Z, we re-analyzed data from [7], in which monkeys performed a covert spatial attention task. They fixated on a spot, while a peripheral cue appeared, followed by a landolt C at the cued location. Monkeys reported the direction of C opening.

Monkeys A and C performed the same peripheral cueing task [7], except during FEF recordings. We placed the cue inside a neuron’s RF and collected 85.7 trials ± 32.4 SD per neuron.

Before the main experiment, we classified SC neurons from monkeys P and N using saccade-related tasks. For delayed saccades [44], a spot appeared, after which time a target was presented. After 500–1,000 ms, the spot disappeared, releasing fixation. Across trials (>23; average: 136 ± 82.2 SD), we moved the target location to map visual and motor RFs. We also used a memory-guided saccade task. The target only appeared for ~50 ms. A memory interval (300–1,100 ms) then ensued before fixation was released. The monkey made a memory saccade (to within 3°) and maintained gaze for 200 ms, after which time the target re-appeared. We ran this task with saccades to the RF center (>35 trials).

Neurons from monkeys B, Z, A, and C were classified based on memory-guided saccades [7].

### Identifying SC and FEFs

We identified SC and FEFs using anatomical and physiological markers. For FEFs, during the last eight sessions from monkey C and three sessions from monkey A, we confirmed electrode locations by applying (on random trials) bipolar electrical stimulation (25 pulses at 350 Hz) to evoke short-latency saccades (38–63 ms after stimulation onset). In all experiments, we evoked saccades on >61% of stimulation trials using currents ≤40 μA [45].

### Neuron Classification

We recorded from all visually responsive SC neurons. A neuron was “visual” if activity 0–200 ms after target onset in the delayed saccade task was higher than 0–200 ms before target onset ( $p < 0.05$ , paired t test). The neuron was “visual motor” if its pre-saccadic activity (within 50 ms) was additionally elevated for either delayed or memory-guided saccades relative to an earlier interval (100–175 ms pre-saccade) [30].

We classified 60 SC neurons from monkeys B and Z in the same way, but using memory-guided saccades [7]. Using the current classification, “visuo-memory” and “visuo-motor” neurons in [7] were now visual-motor (35/60).

For FEFs, we analyzed four time windows for the location eliciting maximal visual response in memory-saccade trials: baseline (100 ms after fixation onset

to 100 ms before target onset), visual (70 ms after target onset to 70 ms after target offset), memory (100 ms after target offset to fixation-spot offset), and motor (0–200 ms after fixation-spot offset). Activity within each interval was normalized to the maximum. A neuron was visual if only visual-interval activity was >0.5. Visual-motor neurons had both visual and motor intervals >0.5. We found similar results for visual (16) and visual-motor (38) neurons and thus combined them to improve statistics. RFs had 8°–16° eccentricities (10.8 ± 2.1 SD), which was within the range tested in SC. Moreover, the relative proportions of visual and visual-motor neurons were similar to those in the SC data re-analyzed from [7]. Thus, Figure 4 data from the same laboratory [7] were comparable as much as possible.

### Data Analysis

In visual burst analyses from monkeys P and N, we measured activity 50–150 ms after grating onset. Our choice of a visual burst interval ensured measuring responses to stimulus onset, regardless of microsaccades. If a microsaccade occurred while a stimulus was on (e.g., pre-microsaccade trials), we were still measuring response to stimulus onset and not to microsaccade-induced image motion of the stimulus, because afferent delays would need to ensue after the microsaccade before image motion could influence neurons. Thus, potential re-afference would appear after our measured bursts. Moreover, we replicated our main results in some neurons with only brief stimulus flashes (Figure S2), and we also checked that microsaccade-related modulations with or without an RF stimulus were not sufficient to explain our results (Figure S2).

We compared activity with no microsaccades to activity from pre- or post-microsaccade trials using two-tailed t tests. For population summaries, we computed a modulation index normalizing activity on pre- or post-microsaccade trials to no-microsaccade trials. For Figure 2, we plotted eccentricity logarithmically using the afferent mapping of the SC [46].

For fano factors, we counted spikes in a 70-ms interval starting at 30 (visual neurons) or 40 ms (visual-motor neurons), and we normalized spike count variability by firing rate. We also created ROC curves based on firing rates from no-microsaccade and pre- or post-microsaccade trials.

Population summaries were tested using paired signed-rank tests. We performed analyses for microsaccades toward the stimulus or away from it. For time courses (Figures 3C and 3D), we used previous procedures [23].

For contrast sensitivity curves, we fit visual burst measurements to

$$f.r.(c) = R * \frac{c^n}{c50^n + c^n} + B, \quad (\text{Equation 1})$$

where  $c$  is contrast,  $R$  is a multiplicative term,  $c50$  is semi-saturation contrast,  $n$  determines curve steepness, and  $B$  is baseline activity (obtained from a 50-ms pre-stimulus interval). To obtain 95% confidence intervals for fit parameters, we used bootstrapping (1,000 bootstraps). When combining neurons, we first normalized activity to that of no-microsaccade trials with the highest contrast.

For sustained analyses (Figure 7), we analyzed activity 150–250 ms after grating onset. We only included neurons if activity 150–250 ms after 80% grating onset was >20 spikes/s on no-microsaccade trials.

For monkeys B, Z, A, and C, we computed a similar modulation index to above (Figure 4), averaging activity 30–80 ms (SC) or 60–120 ms (FEFs) after cue onset. These experiments were not originally designed for microsaccade analysis; they employed significantly fewer trials per neuron. Thus, we restricted analyses to population levels with no claims about individual neuron significance. This approach is equivalent to employing a multi-unit activity (e.g., [5]). Individual neurons were only analyzed if they had >1 trial with either pre- or post-microsaccade stimulus (average pre-stimulus: three trials; post-stimulus: five trials).

### SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.06.022>.

### AUTHOR CONTRIBUTIONS

C.-Y.C. and Z.M.H. implemented and analyzed the SC experiments. C.-Y.C., A.I., and Z.M.H. analyzed the second SC data. A.I. and P.T. implemented the FEF experiments. A.I. analyzed the FEF data. Z.M.H. wrote the paper.

### ACKNOWLEDGMENTS

We thank P.W. Dicke for help with the FEF experiments. We were supported by the Werner Reichardt Centre for Integrative Neuroscience (CIN), which was funded through Excellence Cluster funds (EXC 307) from the German Research Association (DFG).

Received: April 8, 2015

Revised: June 1, 2015

Accepted: June 9, 2015

Published: July 16, 2015

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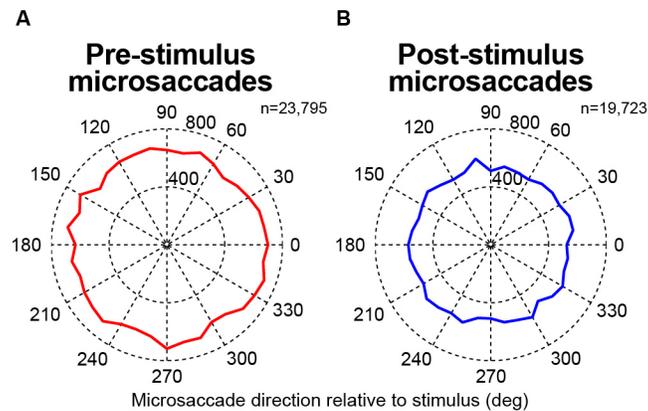
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**Supplemental Information**

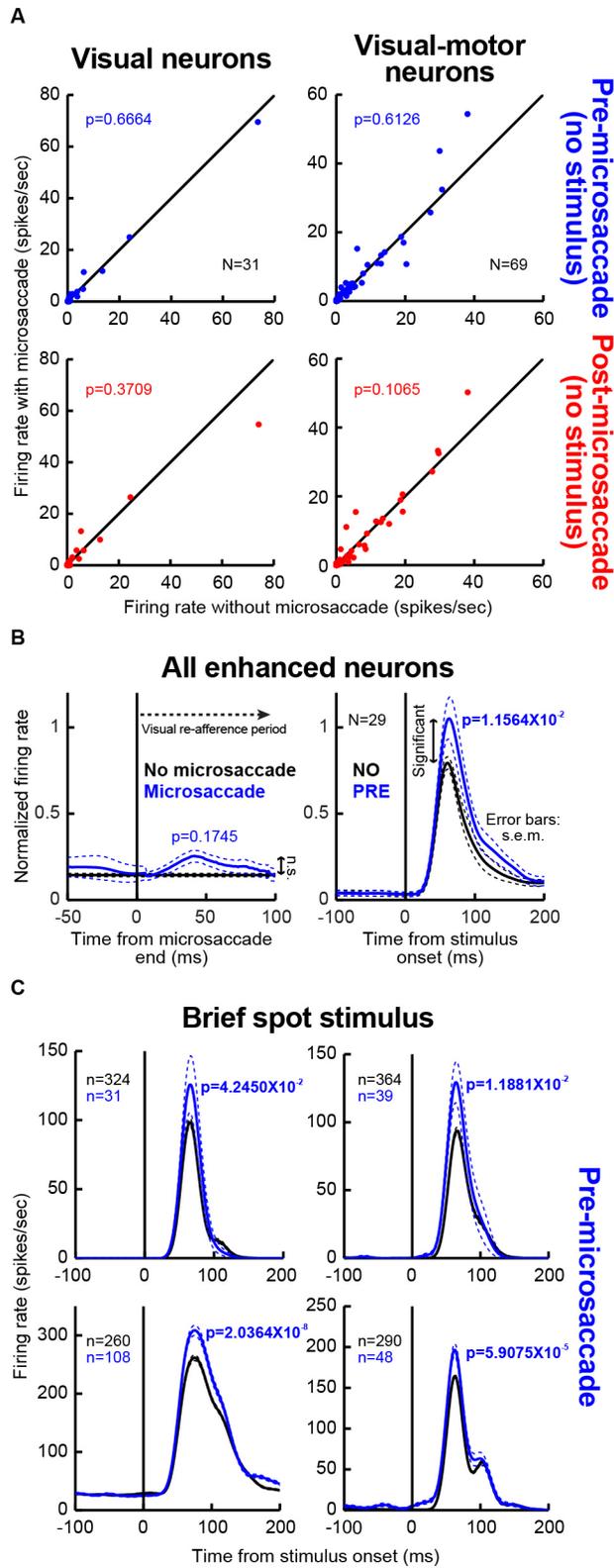
**Neuronal Response Gain Enhancement  
prior to Microsaccades**

**Chih-Yang Chen, Alla Ignashchenkova, Peter Thier, and Ziad M. Hafed**

## Supplemental Figures

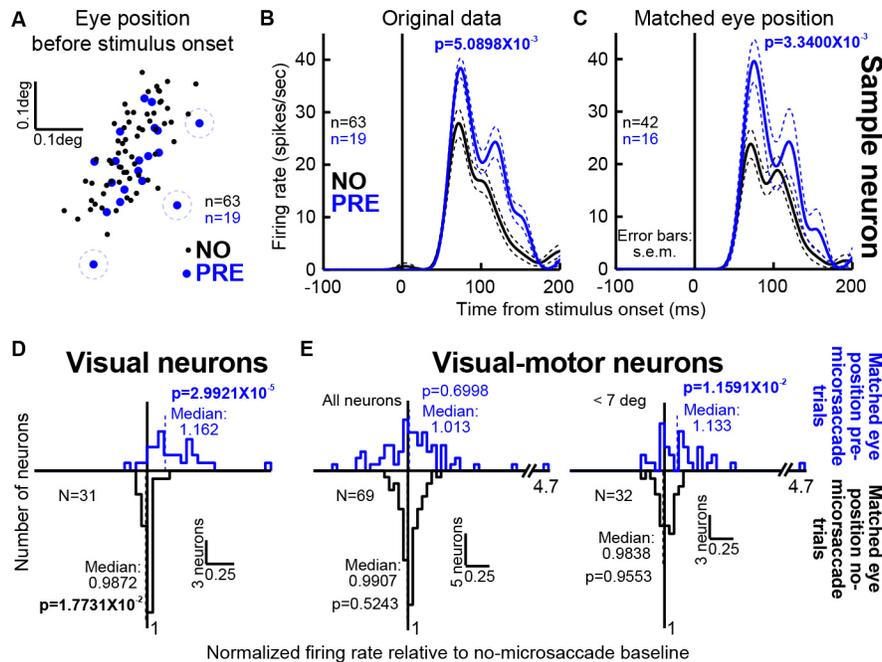


**Figure S1, Related to Figure 2** Distribution of pre-stimulus and post-stimulus microsaccade directions, demonstrating a lack of endogenous (A) or stimulus-driven (B) biases towards RF locations. Microsaccade directions can be very strongly biased towards peripheral locations. (A) To test whether our neuronal modulations were due to a sustained endogenous attentional bias towards the RF stimulus location, we analyzed the directions of microsaccades occurring <100 ms before stimulus onset. If there was a sustained endogenous attentional bias towards the RF stimulus location, then these microsaccades should be strongly directed towards that location. For each session, we rotated all data such that stimulus location was represented at 0 deg, and we then pooled all pre-stimulus microsaccades from all sessions. There was no strong peak of pre-stimulus microsaccade directions near 0 deg, as might be expected if there was a generalized sustained attentional bias towards the RF location. (B) Similarly, there was no directional bias for the microsaccades occurring immediately after stimulus onset (i.e. the microsaccades that were associated with neuronal response gain enhancement), suggesting that these microsaccades were not visually-triggered.



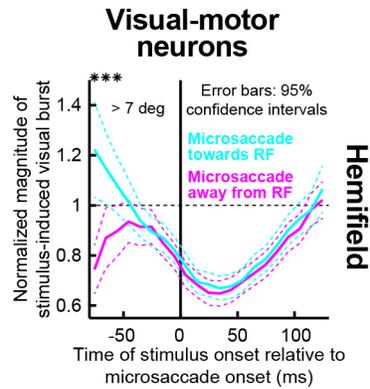
**Figure S2, Related to Figure 2** Exploring *microsaccade-only* (A) or *microsaccade-stimulus* (B, C) interactions in our results. (A) Lack of influence of microsaccades on

neuronal activity *without* the presence of an RF stimulus. To rule out the possibility that pre-microsaccadic enhancement in the main text was observed because our neurons were simply sensitive to microsaccade generation, we analyzed peri-microsaccadic changes in neuronal activity during a pre-stimulus fixation epoch. For each neuron, we analyzed microsaccades occurring 0-300 ms before stimulus onset. For each microsaccade, we measured neuronal activity in the interval 0-100 ms before microsaccade onset (top two panels), and we plotted it against activity from a similar interval but containing no microsaccades (0-100 ms before stimulus onset). We also repeated the same analysis but for an interval 0-50 ms after microsaccade end (bottom two panels), and compared it to a 50-ms interval without microsaccades (0-50 ms before stimulus onset). Most neurons were inactive without an RF stimulus, whether there was a microsaccade or not. For the few that did possess *baseline* activity, the activity was statistically unaltered by microsaccades. Each panel shows the number of neurons and p-value for the shown comparison (paired signed rank test, Experimental Procedures). Note that this figure includes data from *all* neurons. However, because a lot of these neurons were silent without a stimulus, a majority of them in the present analysis (i.e. without a stimulus in the RF) lied on the origin of the plots shown in this figure. **(B)** Comparing peri-microsaccadic modulations in the *presence* of an RF stimulus to the enhancement effects that we saw in the main text. For all neurons that individually exhibited statistically significant enhancement (Figs. 1-2), we normalized activity to the peak firing rate after stimulus onset on no-microsaccade trials (black, right panel), and we then averaged across neurons. The right panel shows population results, demonstrating strong visual-burst enhancement if a stimulus appeared <100 ms before a microsaccade towards the stimulus' hemifield (consistent with the main text). In the left panel, for the *same* neurons, we picked an interval 150-250 ms after stimulus onset and investigated possible visual re-afferent responses to microsaccades occurring within this interval (i.e. with the stimulus still present over the RF). The blue curve shows neuronal activity aligned on microsaccade end (for microsaccades towards the RF stimulus' hemifield; similar results were obtained for opposite microsaccades). The black curve shows neuronal activity from an interval of the same length (200-300 ms) when no microsaccades occurred. There was no statistically significant visual re-afferent response in the interval 0-100 ms after microsaccade end compared to no-microsaccade baselines; on the other hand, the right panel shows robust visual burst enhancement with an analysis interval of identical length. Thus, visual burst enhancement was not accounted for by visual re-afferent neuronal responses. **(C)** To further support this idea, we recorded from SC visual-motor neurons while we presented only *brief* flashes (50-ms spots) rather than our longer-duration gratings. We still observed strong enhancement, as can be seen from four sample neurons illustrated in this panel. In fact, with these brief flashes, we were able to pick specific times of microsaccade onsets that resulted in *no* overlap with a stimulus presentation over the RF (i.e. the stimulus had appeared and disappeared *before* the eye movement began). We still observed strong enhancement. Thus, **B-C** demonstrate that pre-microsaccadic response gain enhancement was not accounted for by visual re-afferent neuronal responses. All conventions in **B-C** are like in the main text (Figs. 1-2).

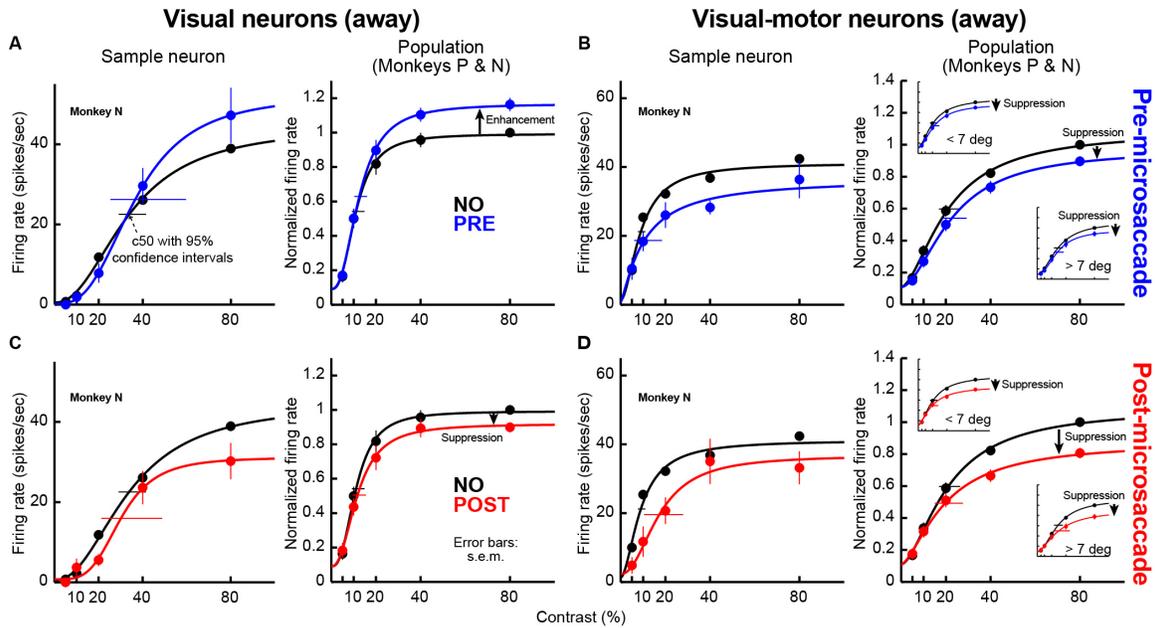


**Figure S3, Related to Figure 2** Independence of our modulations from a potential role of instantaneous eye position at stimulus onset. It could be argued that the modulations we saw were explained by changes in eye position across trials, which can change the position of the presented stimulus over the RF. For example, if no-microsaccade trials had a certain eye position at stimulus onset that was different from the eye position on pre-microsaccade trials, then it could be argued that eye position on pre-microsaccade trials was always such that when the stimulus appeared, it was placed at a more preferred RF location than when it appeared for no-microsaccade trials (thus giving higher firing rates). While this is highly unlikely given the specific patterns of effects that we saw (e.g. Fig. 3), we ruled it out by repeating our analyses but after “matching” eye position on no-microsaccade and microsaccade trials. **(A)** Data from an example session demonstrating a large overlap in eye position on no-microsaccade and pre-microsaccade trials. We collected all 80% grating trials from a sample session, and we measured average eye position 0-50 ms before grating onset. The black dots show eye position from no-microsaccade trials, and the blue dots show eye position from trials in which the stimulus appeared  $<100$  ms before a microsaccade towards the grating’s hemifield. The circled trials are trials in which eye position on microsaccade trials did not fall within the region of overlap of no-microsaccade trials. Only 3 such trials existed in this session. **(B, C)** We performed our analysis of Fig. 1B for the sample session in **A**, which was obtained from a pure visual neuron. In **B**, we performed the original analysis. In **C**, we excluded each “pre-microsaccade” trial that did not have any neighboring “no-microsaccade” trials within 1.8 min arc radius (the 3 circled trials in **A**), and we also excluded each “no-microsaccade” trial that did not have any neighboring “microsaccade” trials (within a similar radius). Thus, in panel **C**, eye position at stimulus onset was “matched” across the no-microsaccade and microsaccade trials. As can be seen, response gain enhancement was still robustly observed. Thus, the effects in Figs. 1-2 were not due to different RF

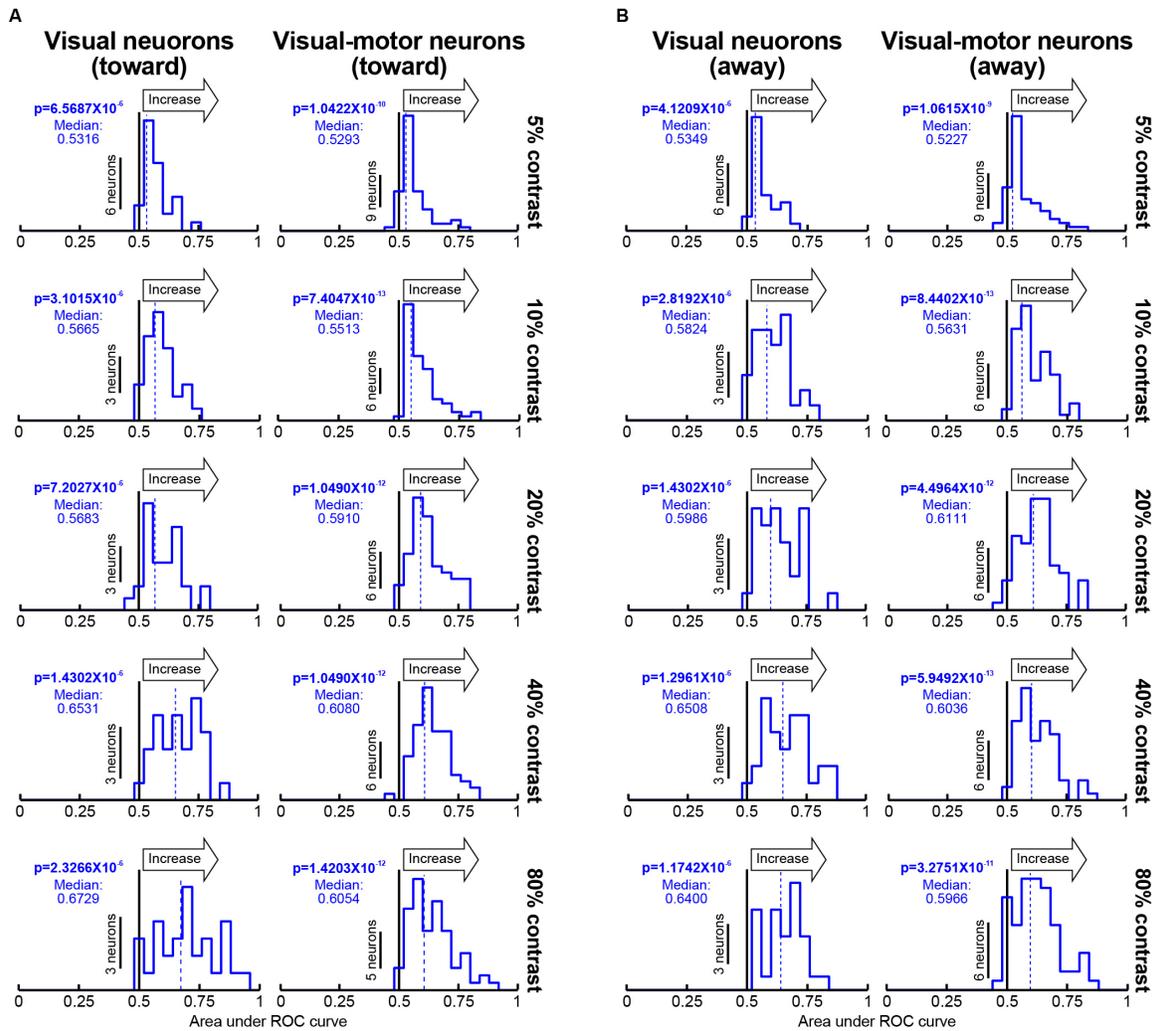
stimulus positions caused by differences in eye position at grating onset. Error bars denote s.e.m. **(D, E)** Across the population, we compared our neuronal modulation indices in “matched” eye position data sets to the original no-microsaccade baseline data from Fig. 2 (i.e. from all no-microsaccade trials before “matching”). “Matched” pre-microsaccade trials showed robust enhancement even after removing eye position outliers (blue histograms). “Matched” no-microsaccade trials were statistically indistinguishable from original no-microsaccade trials (black histograms), suggesting that our subsampling of no-microsaccade trials to obtained “matched” sets did not alter our no-microsaccade baseline reference. Thus, our effects in this paper were not due to differences in eye position between no-microsaccade and microsaccade trials. All other conventions are similar to Fig. 2. Note that we also repeated the above analyses for microsaccades away from the grating location, and also for grating onsets after microsaccades. In all cases, the conclusions presented in the main text (Figs. 2-3) were unaltered.



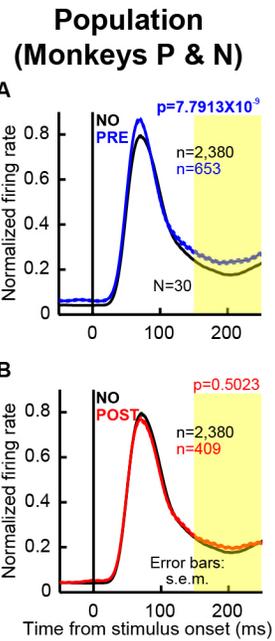
**Figure S4, Related to Figure 3** Pre-microsaccadic response gain enhancement for peripheral visual-motor SC neurons. This figure is similar to Fig. 3D. However, in this case, we only show data from visual-motor SC neurons with preferred eccentricities  $>7$  deg. Also, for simplicity, we classified microsaccade directions in this analysis as either being towards the hemifield of the RF stimulus or opposite it. As can be seen, even these eccentric visual-motor neurons showed differential modulation based on microsaccade direction, as well as pre-microsaccadic enhancement of response gain for microsaccades towards the hemifield of the stimulus. Thus, pre-microsaccadic enhancement was a robustly observed phenomenon, even in eccentric visual-motor neurons. All conventions are similar to those in Fig. 3D.



**Figure S5, Related to Figure 5** Contrast sensitivity curves like in Fig. 5 but for microsaccades opposite the hemifield of a stimulus (i.e. away from the stimulus). The figure has formatting and conventions identical to Fig. 5. Consistent with all of our earlier analyses (e.g. Fig. 3), microsaccades away from the stimulus were associated with either weaker enhancement for visual neurons or significant suppression for visual-motor neurons when the stimulus appeared before microsaccades (**A**, **B**). After microsaccades, suppression was always consistently observed (**C**, **D**).



**Figure S6, Related to Figure 6** Analyses similar to Fig. 6A, B but for all stimulus contrasts (A), and also for microsaccades opposite the hemifield of a stimulus (B). (A) Area under the ROC curve comparing activity on pre-microsaccade trials to activity on no-microsaccade trials. The left column shows pure visual SC neurons, and the right column shows visual-motor neurons. Each row shows results from a single grating contrast, and all panels show results for microsaccades towards the hemifield of the RF location. In all cases, activity on microsaccade trials was discriminable from that on no-microsaccade trials. (B) Similar analyses for microsaccades opposite the stimulus' hemifield. For these microsaccades, our earlier analyses (e.g. Fig. 3) showed that pure visual SC neurons still showed pre-microsaccadic response gain enhancement (albeit weaker), whereas visual-motor neurons showed pre-microsaccadic response gain suppression. Consistent with these results, this figure shows that whenever firing rates were either enhanced or suppressed, our ROC analyses revealed above-chance discriminability of neuronal activity between microsaccade and no-microsaccade trials. All conventions are similar to Fig. 6A, B.



**Figure S7, Related to Figure 7** Normalized population firing rate curves from the data in Fig. 7. For all neurons with a sustained response on no-microsaccade trials (Fig. 7, Experimental Procedures), we normalized all firing rates to each neuron's peak firing rate on no-microsaccade trials. We then pooled all data to obtain a single normalized population firing rate curve. We obtained such a curve for no-microsaccade trials (black) and also for trials with a stimulus appearing before (blue) or after a microsaccade (red). In this figure, we show data from the highest contrast grating. This figure shows similar results to those in Fig. 7A, C. The p-value is for the shaded analysis interval, and error bars denote s.e.m.