RESEARCH ARTICLE

Figures and figure supplements

Microsaccades as a marker not a cause for attention-related modulation

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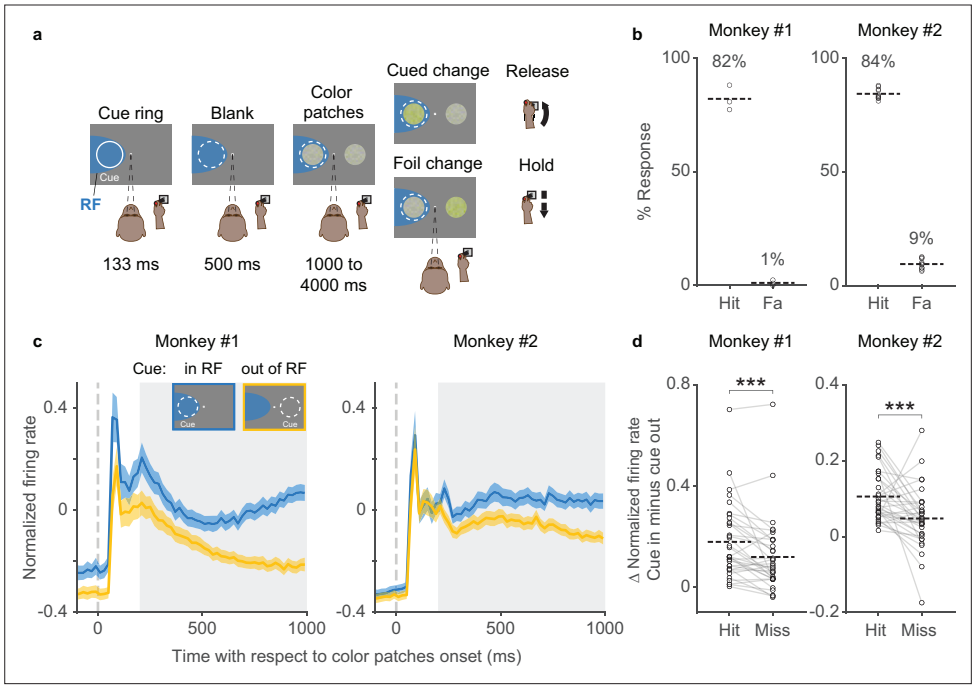

Figure 1. Behavioral performance and SC neuronal activity in a covert spatial attention task. (**a**) The monkey was required to maintain central fixation, releasing a joystick in response to a color change at the cued location and holding their response if the change occurred at the opposing foil location. The dashed white ring illustrates the cued location and the blue shaded area denotes the response field (RF) of SC neurons; neither were visible to the monkey. (**b**) Hit rates (Hit) and false-alarm rates (Fa) for monkeys 1 and 2 in each session. Each circle represents data from one behavioral session. Percentages and horizontal dashed lines denote average hit rates and false-alarm rates across sessions. (**c**) Population SC average normalized firing rates for cue-in-RF (blue) and cue-out-of-RF (yellow) conditions, aligned on the onset of the color patches. The insets illustrate the cue conditions when the SC RFs were on the left side. The gray shaded areas denote the time windows (the delay period) used for measuring the difference (Δ) in average normalized firing rates for (**d**). The difference (Δ) in average normalized firing rates between cue-in-RF and cue-out-of-RF of SC neurons in hit and miss trials. Each pair of circles connected by a gray line represents the data from one SC neuron. Horizontal dashed lines denote the averages across neurons. *** denotes $p<0.001$, Wilcoxon signed-rank test. SC, superior colliculus.

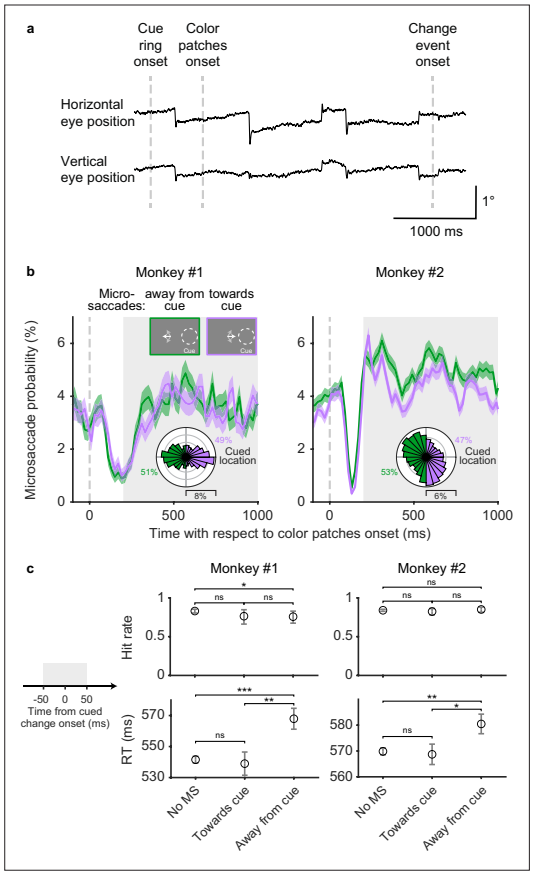

Figure 2. Microsaccades in the covert attention task. (**a**) Single-trial horizontal and vertical eye position traces. The abrupt deflections are microsaccades. (**b**) Session average probability of microsaccades toward the cued location (purple) and away from the cued location (green), aligned on color patches onset. The insets at top illustrated the microsaccade conditions when the cue was on the right side. The white arrows schematically represent microsaccades. The polar plots show the directional distribution of microsaccades during the delay period (gray shaded area), relative to the cued location. The numbers beside the polar plots denote the total proportion of microsaccades toward and away from the cued location. Error bars denote the standard error of the mean (SEM). (**c**) Average hit rates (top row) and reaction times (RT, bottom row) when there were no microsaccades, microsaccades toward the cued location, and microsaccades away from the cued location –50 to 50 ms relative to cued change onset (left schematic, gray shaded area). Error bars of hit rates denote the 95% binomial confidence interval. Error bars of reaction times denote the SEM. *** denotes p<0.001, ** denotes p<0.01, * denotes p<0.05, 'ns' denotes p>0.05, chi-square proportion test for hit rates and Wilcoxon rank-sum test for reaction times.

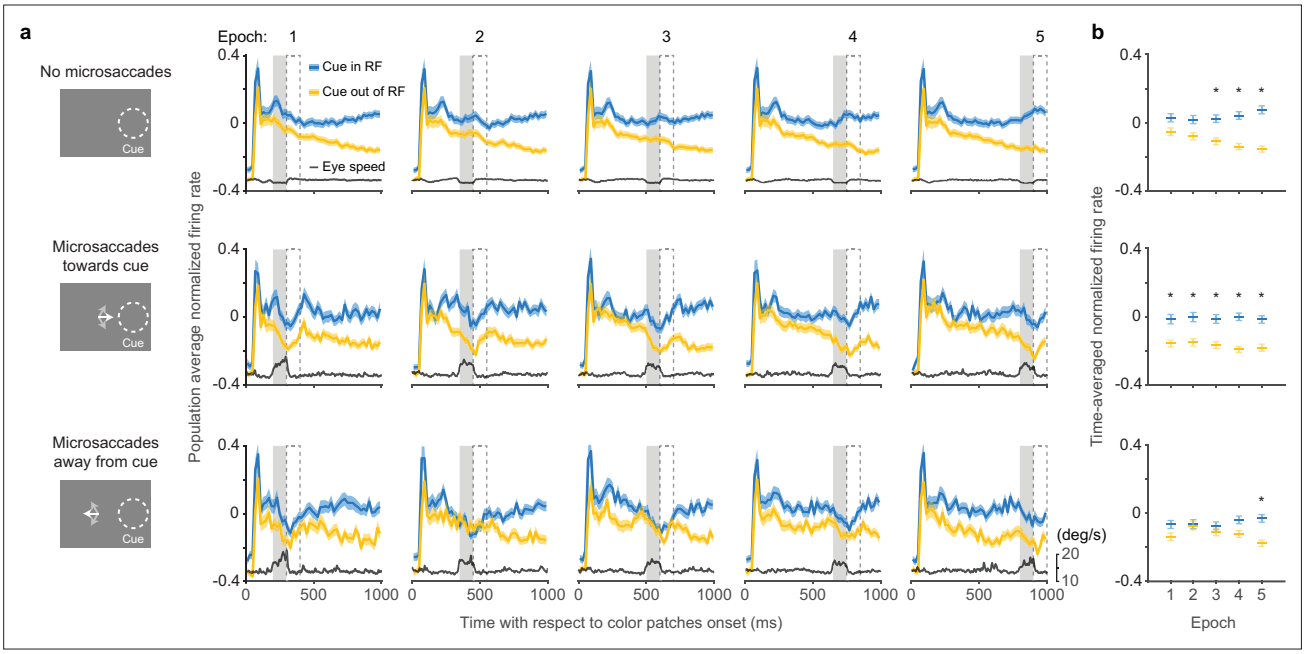

Figure 3. Effects of microsaccades on the time course of SC attention-related modulation. (**a**) Each panel depicts population average SC normalized firing rates (blue, cue-in-RF; yellow, cue-out-of-RF) and eye speed traces (black) from subsets of trials in which there were either no microsaccades (top row), microsaccades toward the cue (middle row), or microsaccades away from the cue (bottom row), within a particular 100-ms epoch indicated by the gray shaded area. The chosen time epochs were: 200–300 ms, 350–450 ms, 500–600 ms, 650–750 ms, and 800–900 ms after color patches onset. The 100-ms dashed boxes following the gray shaded areas denote the time windows used for measuring time-averaged normalized firing rates for (**b**). Time-averaged normalized firing rates as a function of epoch. The asterisks denote the epochs with significant higher activity for cue-in-RF than for cue-out-of-RF, $p<0.05$, ANOVA, Tukey-Kramer post hoc comparisons. Error bars denote SEM. SC, superior colliculus.

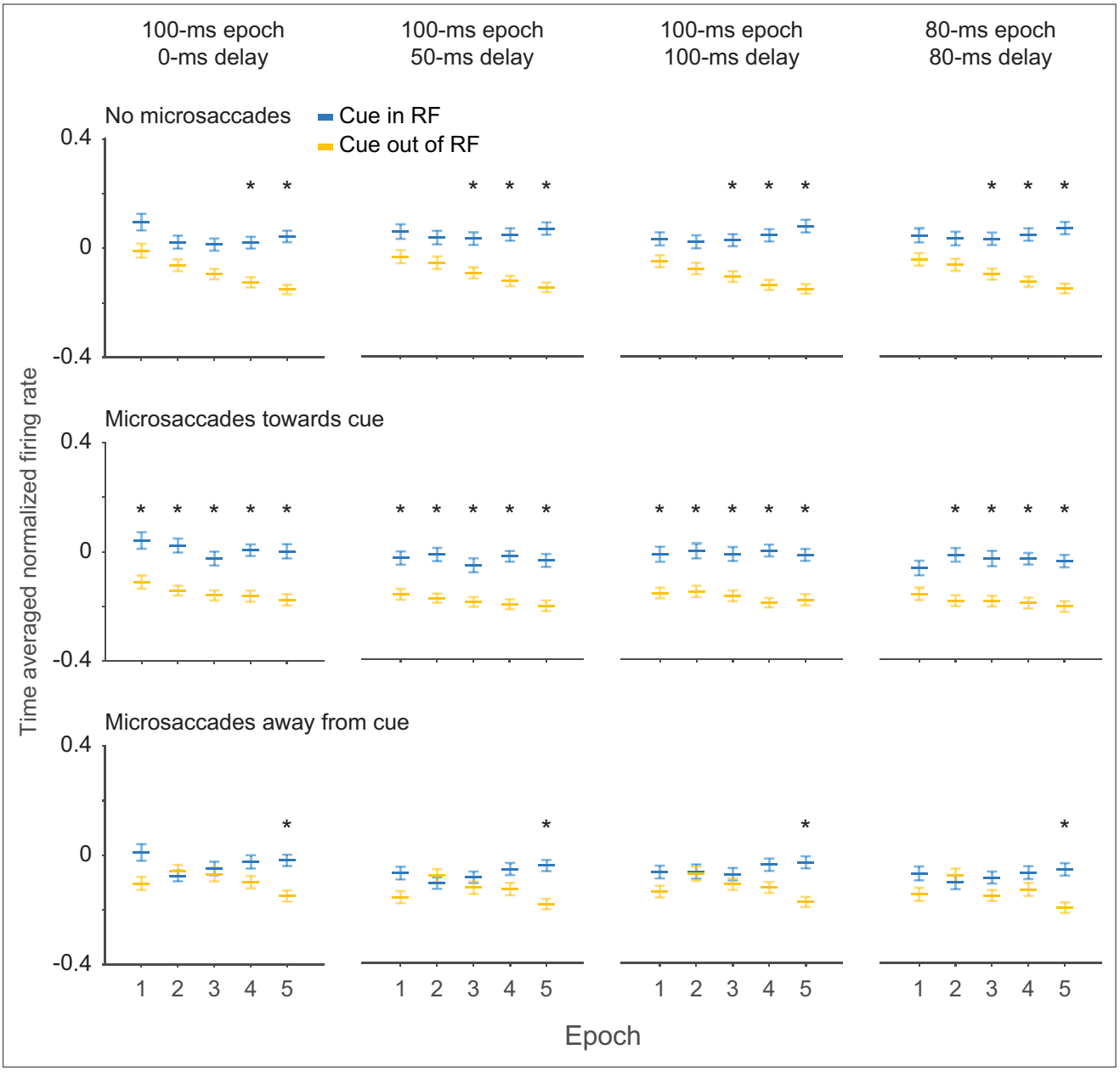

Figure 3—figure supplement 1. Effects of microsaccades on the time course of SC attention-related modulation were similar when using other measurement windows. In our main results, we used five different 100 ms epochs during the delay period to categorize trials based on the occurrence of microsaccades, and corresponding 100 ms windows (delayed by 100 ms) for each epoch to measure the average neuronal activity. Here, the plots are in similar format as ***Figure 3b*** with no microsaccades (top row), microsaccades toward the cue (middle row), and microsaccades away from the cue (bottom row) conditions. But we tested four different combinations of microsaccade epochs and measurement windows: 100 ms microsaccade epochs with 100 ms measurement windows after a 0 ms delay (first column), 100 ms epochs with 100 ms measurement windows after a 50-ms delay (second column), 100 ms epochs with 100 ms measurement windows after a 100-ms delay (third column, same as ***Figure 2b***), and 80 ms epochs with 80 ms measurement windows after an 80-ms delay (fourth column). We observed qualitatively similar results across all combinations of delay and window duration. The asterisks denote the epochs with significant higher activity for cue-in-RF than for cue-out-of-RF, $p<0.05$, ANOVA, Tukey-Kramer post hoc comparisons. Error bars denote SEM. SC, superior colliculus.

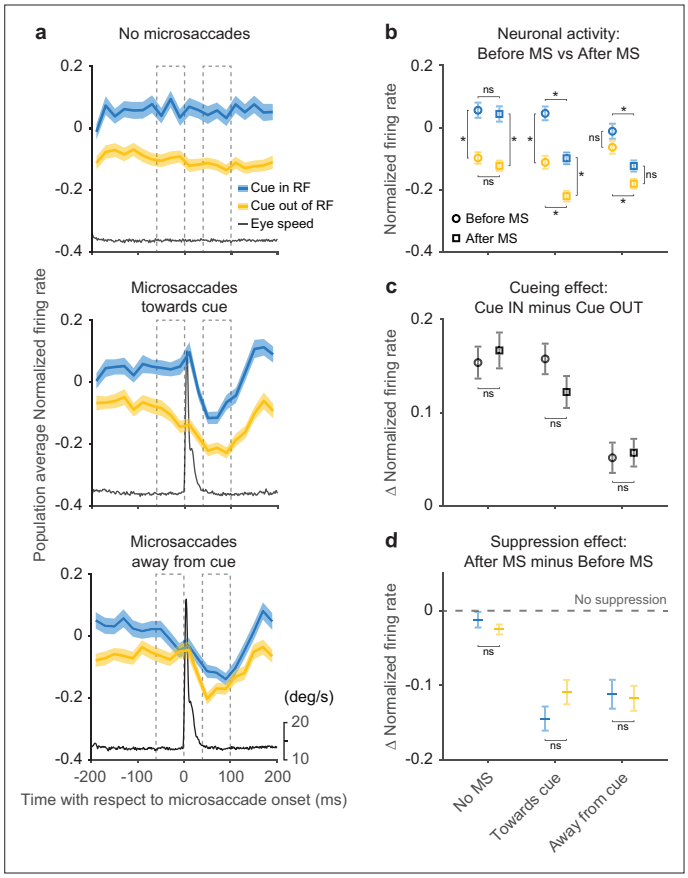

Figure 4. Peri-microsaccadic attention-related modulation. (**a**) Population average SC normalized firing rates aligned to the onset of individual microsaccades under three conditions: timing-matched no microsaccades (top), microsaccades toward cue (middle), and microsaccades away from cue (bottom). The gray line denotes the average eye speed. The dashed boxes depict the windows we used to calculate the average normalized firing rates before (−60 to 0 ms) and after (40–100 ms) microsaccades. (**b**) Average normalized firing rates before (circle) and after (square) microsaccades. (**c**) The difference (Δ) in average normalized firing rates between cue-in-RF and cue-out-of-RF during 'before microsaccade' (circle) and 'after microsaccade' (square) windows. (**d**) The difference (Δ) in average normalized firing rates between 'after' and 'before' window for cue-in-RF (blue) and cue-out-of-RF (yellow) conditions. The dashed line indicates the level of 'no suppression.' The asterisk denotes $p<0.05$ and 'ns' denotes $p>0.05$, ANOVA, Tukey-Kramer post hoc comparisons. Error bars denote SEM. SC, superior colliculus.

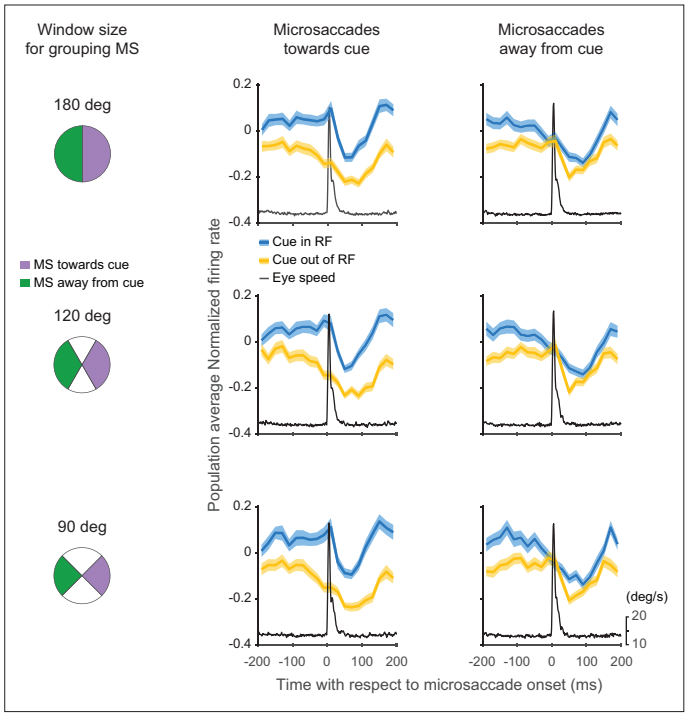

Figure 4—figure supplement 1. Peri-microsaccadic attention-related modulation was similar when using narrower windows to group microsaccades toward/away from the cued location. In our main results, microsaccades with directions ±90° (window size 180°) relative to the cued location were grouped as 'microsaccades toward the cued location,' and the other half of microsaccades were grouped as 'microsaccades away from the cued location.' Here, we tried three different window sizes to group microsaccades toward/away from the cued location: ±90° (window size 180°, top row), ±60° (window size 120°, middle row) and ±45° (window size 90°, bottom row). We observed qualitatively similar results across the three window sizes.

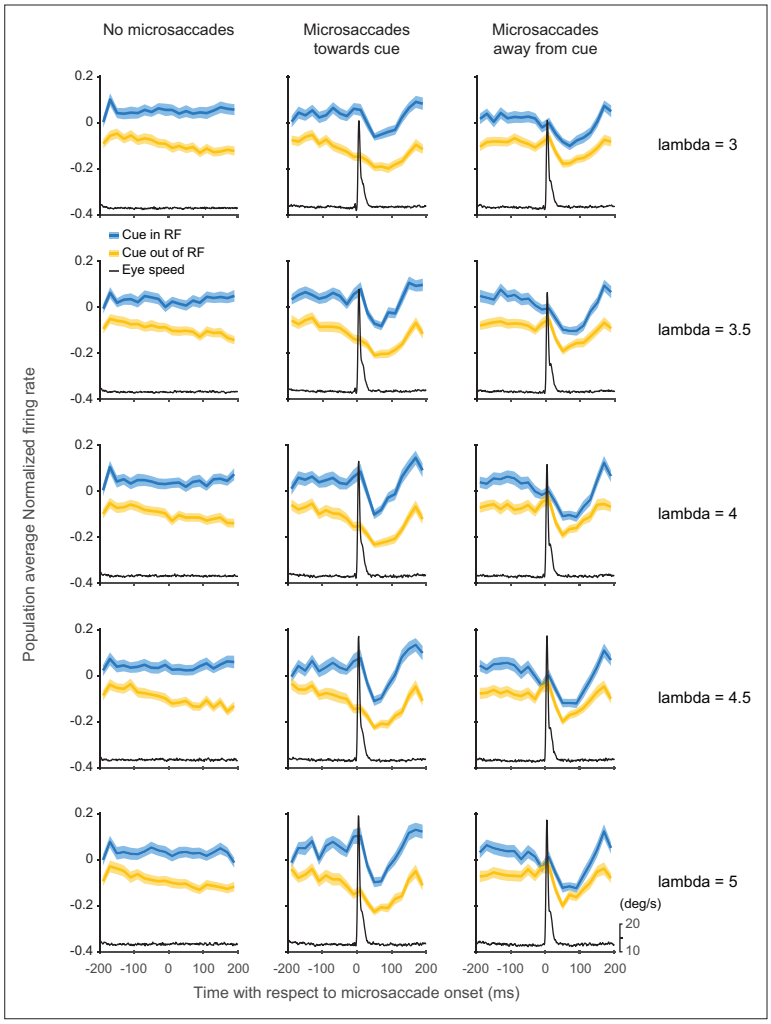

Figure 4—figure supplement 2. Peri-microsaccadic attention-related modulation was robust to the thresholds for microsaccade detection. In our main results, all the microsaccades were detected using a threshold of 4 ('lambda' value in the microsaccade detection method developed by Engbert and Kliegl) and inspected by the experimenter. Here, we repeated the microsaccade detection analyses using five different thresholds ('lambda' value: 3, 3.5, 4, 4.5, and 5), and replot population average SC normalized firing rates aligned to the onset of individual microsaccades (similar to the presentation of data in ***Figure 4***). The results from these additional analyses verify the robustness of our findings across the range of more lenient and more strict detection thresholds. SC, superior colliculus.

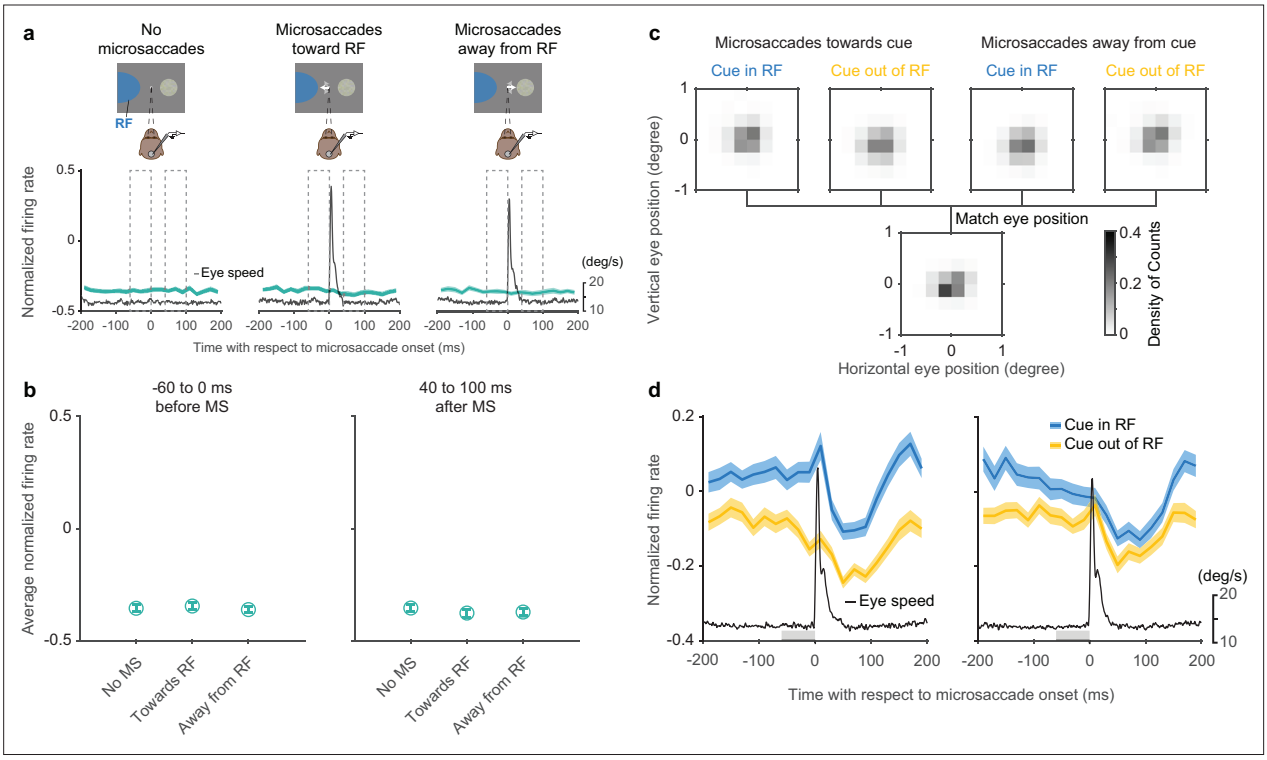

Figure 4—figure supplement 3. Peri-microsaccadic attention-related modulation was not explained by motor effects or differences in eye position. (**a**) To address whether peri-microsaccadic attention-related modulation might be related to motor effects associated with microsaccade generation, we used a subset of our data in which only a single color patch was presented, and this single patch was located in the ipsilateral visual field (schemes above data panels), so that there was no visual stimulus inside the neurons' RFs (blue shaded area) and any potential modulation would be due to motor effects, not sensory. The white arrows in the schemes denote microsaccades. SC normalized firing rates are plotted aligned on the onset of individual microsaccades (data panels) in each of three conditions: timing-matched no microsaccades (left), microsaccades toward SC RF (middle), and microsaccades away from SC RF (right). The gray line denotes the average eye speed. The dashed boxes depict the windows we used to calculate the time-averaged normalized firing rates 'before' (−60 to 0 ms) and 'after' (40–100 ms) microsaccades. (**b**) Average normalized firing rates before microsaccades (left) and after microsaccades (right). Error bars denote SEM. If our SC neurons were modulated by the motor preparation of microsaccades, we should find higher activity for microsaccades directed toward the RF compared to those directed away, or the 'no microsaccade' dataset. Instead, we found no differences in activity across any of these conditions (ANOVA, post hoc comparisons, all $p>0.05$). Thus, the peri-microsaccade attention-related modulation cannot be explained by the motor effects of microsaccades. (**c**) To address the possibility that the peri-microsaccadic attention-related modulation might be explained by systematic differences in eye position before microsaccades, we calculated the 2-D spatial distribution of eye positions (upper row) before the onset of microsaccades (calculation window indicated by the gray bars in (**b**)) across microsaccade directions (toward/away from the cue) and attention conditions (cue-in-RF/cue-out-of-RF), matched all the distributions (lower row). The gray scale bar denotes the magnitude of the proportion. (**d**) After matching eye position distributions, we recalculated the SC normalized firing rates (for cue-in or out-of-the-RF) aligned on the onset of individual microsaccades for conditions with microsaccades toward the cue (left) and microsaccades away from the cue (right). The gray trace indicates the eye speed. The light gray bars immediately above the horizontal axes depict the time windows (−60 to 0 ms) used to match eye position distributions. The results were unchanged – the attention-related modulation was present only around microsaccades toward cued location (ANOVA, post hoc comparison, $p<0.05$) but not microsaccades away from cued location (ANOVA, post hoc comparison, $p>0.05$). Therefore, the peri-microsaccade attention-related modulation cannot be explained by differences in eye position before the microsaccade. RF, response field; SC, superior colliculus.