Much higher covariation with foveation timing by superior colliculus than primary visual cortical neuronal activity

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Abstract

The superior colliculus (SC) is a potent driver for orienting movements, but it also possesses robust, short-latency visual responses. Even though such responses largely derive from direct primary visual cortical (V1) input, their relationship to V1 activity has never been analyzed with the same experimental subjects and stimuli. Here, we revisited pioneering observations that trial-by-trial variability in either the SC or V1 may predict variability in eye movement timing. We found that V1 covariation with behavioral variability was virtually non-existent in comparison to the SC, whether with respect to visual response onset latency, visual response strength, or pre-stimulus state. By far, the largest predictor of behavioral variability was visual response strength in SC visual-motor neurons. These results suggest that the SC reformats its inputs for exploitation of the SC's proximity to the motor periphery; V1 aids in jumpstarting the sensing process, but the SC much more directly supports visually-driven orienting.

Introduction

Visually-driven orienting movements are a fundamental component of active sensing in many organisms. In primates, rapid foveating saccadic eye movements are often thought of as a "visual grasp reflex" [1-4], given how effectively eccentric visual stimulus events can attract such eye movements. However, and perhaps remarkably in the case of a single visual onset in an otherwise uniform visual field, reflexive saccades towards the very same appearing stimulus can exhibit quite large variability in their reaction times [5-9]. One aspect of such variability is related to the asynchronous nature of exogenous stimuli relative to the internal oculomotor rhythm [10], and others include cognitive processes such as target selection [9,11] and decision making [9,12]. However, none of these processes can even begin before sensory detection of stimulus occurrence takes place; thus, variability in the sensing process itself can be an additional important contributor to behavioral variability.

Previous pioneering work has demonstrated that visual stimulus properties, such as intensity, are indeed reflected in both the average visual response latencies of superior colliculus (SC) neurons as well as in the subsequent average foveating eye movement times [13]. Later studies have also shown how both SC visual response strength and visual response timing can reliably predict average saccadic reaction times [14,15], and how this could also happen for different visual feature dimensions [16-18] as well as when the same responses to the same stimuli are modified by processes such as saccadic suppression [18-20]. In the latter case, this indicates that trial-by-trial variability in SC visual responses (to the same stimulus) can correlate with trial-by-trial saccadic reaction time variability, which has also been demonstrated [14].

All of the above results support the view that SC visual responses are integral to saccade generation [21], but they also raise a fundamental question: what is special about these visual responses? For example, in equally important pioneering work, this time in the primary visual cortex (V1) [22], it was shown that variability in V1 visual response timing to repeated presentations of the same visual stimulus also correlates well with the timing of saccadic eye movements. Given that the majority of visual inputs to the SC anatomically derive from a direct connection originating in V1 [23-29], does this mean that the SC's contributions to behavioral variability are merely inherited from V1? Answering this important question requires measuring SC and V1 neuronal variability in the very same experimental subjects and with the very same stimuli and behaviors, which we did here. We measured trial-by-trial variability in reflexive saccade timing, and we related it to trial-by-trial variability in visual response onset latency, visual response strength, and pre-stimulus neuronal activity. Visual response onset latency allowed us to assess the importance of a temporal code in dictating the timing of saccadic eye movements [22], whereas visual response strength revealed the importance of sensory evidence integration.

Even though the primate SC receives the majority of its inputs from V1 [23,24,26-28,30], we found a striking difference in how its visual responses correlated to foveation timing, with the V1 effects being effectively non-existent when compared to the SC. We also found that a rate, rather than temporal, code dominated the relationship between trial-by-trial SC visual response variability and saccadic reaction times. Relying on such a rate code, necessitating

temporal integration, can reduce sensitivity to noise, which can erroneously trigger movements through the SC's strong excitatory downstream projections.

Results

We collected neuronal activity from the SC and V1 while rhesus macaque monkeys performed a reflexive gaze orienting behavior to the onset of an eccentric visual stimulus in the recorded neurons' response fields (RF's). The stimulus appeared simultaneously with the offset of the initial fixation target, and it consisted of a luminance disc of 0.51 deg radius; across trials, the stimulus could have different levels of Weber contrast or luminance polarity (bright or dark) relative to the surrounding gray background (Methods) [17]. We focused on trial-by-trial variability in the timing of the foveating saccadic eye movements, and we asked what aspects of neuronal activity in the two inter-connected brain areas could better predict such variability, and to what extent.

Superior colliculus visual response latencies are better predictors of foveation timing than primary visual cortical visual response latencies

Figure 1A (top) shows the activity of an example SC neuron when the appearing disc was darker than the surrounding background and had a 50% Weber contrast. The top panel shows spike rasters, indicating that the neuron emitted a robust visual response shortly after stimulus onset. The neuron also emitted a subsequent saccade-related motor burst. To estimate the neuron's visual response onset latency from individual trials, we convolved the within-trial spike times with an asymmetric firing rate kernel that avoided the blurring of response onset estimates backwards in time (Methods; Fig. S1) [22]. We then used the individual-trial firing rate estimates to compare post- to pre-stimulus activity and infer the visual response onset time (Methods) [22]. For the shown example neuron, the estimated onsets of visual responses on individual trials are shown by the brown symbols. We also detected the onsets of foveating saccades (magenta symbols in the figure; Methods), and we sorted trials according to these saccades' times. This example neuron's visual response onset was delayed on the trials in which the subsequent saccade was late (larger trial numbers in the top panel of Fig. 1A), and this effect can also be seen in the bottom left panel of Fig. 1A. In this panel, we plotted visual response onset latency (the brown symbols in the top panel) against saccadic reaction time (the magenta symbols). The Spearman correlation between trial-by-trial visual response onset latency and saccadic reaction time was 0.2053. In contrast, for an example V1 neuron (Fig. 1B), there was no positive correlation between trial-by-trial visual response timing and trial-by-trial saccade timing, unlike in previous work [22]; the Spearman correlation in this case was -0.2361. Similar conclusions could also be reached from two additional example neurons, one from the SC and one from V1 (Fig. 1C, D; 0.2221 versus 0.1124 Spearman correlations, respectively, indicating better covariation with behavior in the SC than in the V1 neuron). Thus, at least for these four example neurons, there was a substantially clearer positive correlation between visual response onset latency and saccadic reaction time in the SC than in V1.

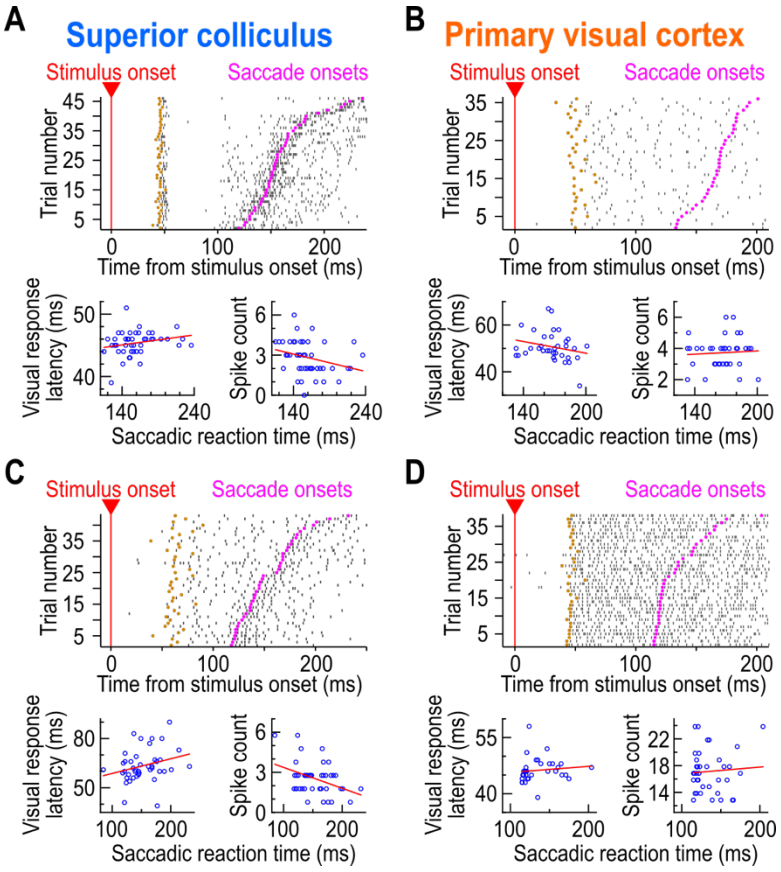

Figure 1 Qualitatively similar, but quantitatively different, visual responses in the superior colliculus (SC) and primary visual cortex (V1). (A) The top panel shows neuronal activity of an example SC neuron when the monkey reflexively foveated a suddenly appearing eccentric stimulus (50% dark contrast; Methods). Trials are sorted by the time of stimulus foveation (magenta; saccade onsets). The neuron emitted a short-latency visual burst shortly after stimulus onset in its response field (RF); brown indicates the estimated visual response onset time on each trial (Methods). On the slow trials, there were later visual response onsets (compare the timing of the brown symbols across trials); additionally, the visual responses were weaker (there were fewer spike counts in the visual bursts for the slow trials). The bottom panels relate the visual response latency (left) and spike count in the visual burst interval (right) to the time of foveation by saccades. Red lines show trend lines (least-squares fits): there were both later and weaker visual bursts in the slow trials. **(B)** For an example V1 neuron, this relationship was not present. Note how the SC neuron (**A**) also exhibited a saccade-related burst at eye movement onset, whereas the V1 neuron did not. **(C, D)** An additional pair of example neurons from the two brain areas. Once again, visual responses in the SC neuron were later and weaker for slow than fast saccadic reaction time trials, but this was not systematically the case for the V1 neuron.

Across neurons in either the SC or V1, we first measured, for each neuron, the Spearman correlation between trial-by-trial visual response onset latency and trial-by-trial saccadic reaction time. We then obtained summary statistics across the population. In the SC, there was a very clear positive correlation for all tested contrasts, consistent with earlier reports [14,15]. For example, for the dark contrasts shown in Fig. 2A, the average correlation coefficient was more than 0.1 for 10-20% contrast levels, and more than 0.06 for the higher contrasts

(Fig. 2A; results for bright contrasts were qualitatively similar and will be further explicitly discussed below). Critically, these effects were greater than approximately 2.5-6 times the effects observed in V1 for the very same visual stimuli (Fig. 2A). That is, SC visual response onset latencies were a significantly better predictor of saccade timing than V1 visual response onset latencies.

Statistically, we performed a Mann-Whitney U test comparing the SC and V1 correlation values across all contrasts (Methods). Such correlations were significantly higher in the SC than in V1 ($U=664365$, $z=5.69$, $p<0.0001$, $n_{SC}=689$, $n_{V1}=1067$, $median_{SC}=0.0808$, $median_{V1}=0.0225$). Moreover, at each contrast level individually, the SC correlation values were again higher than the V1 correlation values after Bonferroni correction (Mann-Whitney U tests; $p<0.01$ in each comparison). Therefore, we statistically confirmed the existence of a substantially stronger covariation with behavioral variability in the SC's visual response onset latencies than in V1.

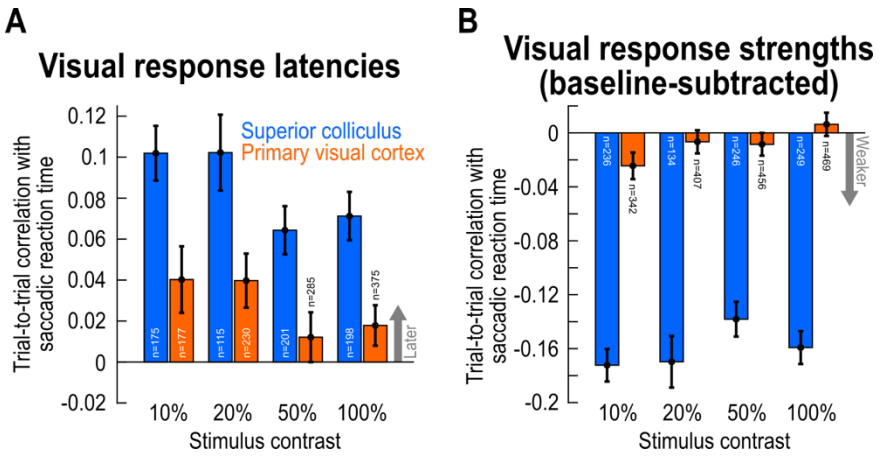

Figure 2 Much higher trial-by-trial correlations with saccade timing in the SC than in V1, for both visual response latencies as well as visual response strengths. (A) For each stimulus Weber contrast that we tested (all being darker than the gray background in this figure; Methods), we calculated the Spearman correlation (within any given neuron) between the individual trial saccadic reaction times and the individual trial neuronal visual response latencies. We then summarized such correlation values across all neurons in our population. Positive correlations indicate later visual response latencies for longer saccadic reaction times. The SC correlations were greater than approximately 2.5-6 times the V1 correlations. **(B)** We also characterized the correlations between trial-by-trial visual response strengths and trial-by-trial saccadic reaction times; the former were defined as the spike counts within a visual burst interval, after subtracting the baseline pre-stimulus activity (Methods). Negative correlations indicate weaker visual burst strengths for later saccades. The correlations in V1 were practically zero, but they were strongly negative in the SC, and even more so, in absolute value, than the visual response latency correlations in **A**. Thus, visual response strength in the SC is significantly better correlated with the timing of saccadic behavior than visual response latency; and, for both measures, the effects are much weaker, if present at all, in V1. Error bars denote SEM across neurons, and the numbers of neurons for each analysis are included in the figure. Note that in **A**, results are only shown for the neurons in which our criteria for sufficient visual response onset latency measurements were met (Methods), explaining the slightly lower neuron counts than in **B**.

We also noticed a trend for higher correlation values to behavioral variability at low contrasts, even for V1 (Fig. 2A). For example, at 10% contrast, a Wilcoxon Signed-Rank test against zero revealed significantly positive correlations in the V1 population after Bonferroni correction (W=9792, z=2.81, p=0.0050, n=177). This was also the case at 20% contrast (W=16136, z=2.82, p=0.0047, n=230). These observations might explain why some significantly positive correlations between V1 visual response onset latencies and saccadic reaction times were previously reported [22]; in that study, generally low contrasts were investigated [22].

Superior colliculus visual response strengths are the best predictors of foveation timing

Our results above suggest that the time of first stimulus detection is positively correlated with trial-by-trial saccadic reaction time variability in the SC, and that this effect is much weaker in V1. However, perceptual decisions aiding in foveation involve not only jumpstarting the sensing process, via the timing of the first spike in a sensory response (Fig. 2A), but they also require sensory evidence integration [17,31-34]. For example, and particularly for weak stimuli, the onset a neuronal visual response to a stimulus may itself be difficult to assess by recipient neurons. Moreover, given how even individual action potentials by individual SC neurons can very easily modify or even trigger new eye movements [10,35,36], it might make much more sense for the SC, in particular, to favor sensory evidence integration (a rate code) over a temporal code (visual response onset latency); this way, the final oculomotor behavior may be much more immune to potential spiking variability in SC neuronal activity than otherwise. To test this, we investigated how visual response strength (the number of stimulus-evoked spikes emitted by an individual neuron during a so-called visual response epoch; Methods) could predict saccadic reaction time variability in both the SC and V1.

Visual response strength overwhelmingly dominated the predictive powers of visual responses in the SC about saccade timing variability, and the large dissonance from V1 effects that we observed in Fig. 2A was also rendered much more substantial. Consider, first, the same example SC neuron of Fig. 1A. Visually-evoked spikes were clearly fewer on the slow reaction time trials (top panel), and this observation itself was likely the primary reason for why the visual response onset latencies were later on such trials. Quantitatively, the Spearman correlation between trial-by-trial spike count in the visual burst epoch (Methods) and trial-by-trial saccadic reaction time for this example neuron was -0.38972 (Fig. 1A; bottom right panel). This suggests that weaker SC visual bursts were associated with later saccades, again as might be expected based on across-trial average measures [14,16,18,19,37,38]. Importantly, this trial-by-trial correlation was quantitatively larger, in absolute value, than the correlation between trial-by-trial visual response onset latency and trial-by-trial saccadic reaction time in the same neuron. It was also quantitatively larger than the correlation seen in the two example V1 neurons of Fig. 1B, D (bottom right; 0.097541 and -0.023898 Spearman correlations between visual response strength and saccadic reaction time, respectively). These observations also held for the second example SC neuron of Fig. 1C (-0.27387 Spearman correlation in this case). Therefore, visual response strength in the SC

was the best predictor of saccadic reaction time variability, at least in the four example neurons of Fig. 1.

Across the population, these effects were very robust (Fig. 2B). In the SC, the absolute values of the correlation coefficients in Fig. 2B were significantly larger than those associated with visual response onset latencies in Fig. 2A (U=481876, z=-6.12, p<0.0001, $n_{\text{visual response latencies}}$=689, $n_{\text{visual response strengths}}$=865, $\text{median}_{\text{visual response latencies}}$=0.0808, $\text{median}_{\text{visual response strengths}}$=0.1557; Mann-Whitney U test comparing the absolute value of correlations for each correlation type – response onset latency versus response strength – across stimulus contrasts), and these effects also held at each contrast level individually after Bonferroni correction (except for 50% contrasts). Moreover, comparing the SC to V1 for visual response strength effects (Fig. 2B) revealed an essentially complete absence of correlations between trial-by-trial visual response strength and trial-by-trial saccadic reaction time in V1; in the SC, there were clear negative correlations throughout all contrasts. We confirmed these observations statistically. Across all contrasts, V1 correlations were not significantly different from zero (W=677333, z=-1.11, p=0.2656, n=1672, Wilcoxon Signed-Rank test against zero). Moreover, at each contrast level individually, there were again no significant differences in the V1 correlations from zero after Bonferroni correction (10% contrast: W=25555, z=-1.98, p=0.0481, n=341; 20% contrast: W=40077, z=-0.52, p=0.6021, n=406; 50% contrast: W=50146, z=-0.69, p=0.4881, n=456; 100% contrast: W=57125, z=0.69, p=0.4921, n=469). Finally, a Mann-Whitney U test comparing the SC to V1 correlations revealed stronger SC effects across all contrast levels (U=785796, z=-17.86, p<0.0001, n_{SC}=865, n_{V1}=1674, median_{SC}=0.0493, median_{V1}=0.0225), and all individual comparisons within each contrast level were also significant after Bonferroni correction (p<0.0001).

Therefore, even though correlations to trial-by-trial saccade timing have been previously reported in the variability of V1 neurons (particularly in terms of visual response onset latency) [22], such correlations very strongly paled in comparison to those that we observed in the SC (Fig. 2A), and even more so when considering visual response strength (Fig. 2B). As we show next, this difference between the SC and V1 was amplified even further when considering the visual responses of one specific functional class of visually-responsive SC neurons.

Greatest covariation with behavior in superior colliculus visual-motor neurons

The SC has two main functional classes of visually-responsive neurons: visual-only and visual-motor [26,39,40]. The former appear like V1 neurons in the sense that they do not emit a saccade-related motor burst at the time of eye movement triggering, whereas the latter emit both visual and motor bursts (like in the example neurons of Fig. 1A, C). The visual neurons also receive the bulk of direct V1 inputs [23-28]. We found that correlations to behavioral variability in saccade times were always the highest in the visual responses of the SC's visual-motor neurons, maximally differentiating these neurons from V1 neurons. This can be seen in Fig. 3A, B, in which we split the SC population of Fig. 2 according to the functional class of the encountered neurons (Methods). For both visual response onset latency (Fig. 3A) and visual response strength (Fig. 3B), visual-motor neurons clearly had the biggest effects. For example, at 50% and 100% (dark) contrasts, the visual response onset

latency of visual SC neurons was barely correlated with trial-by-trial saccadic reaction time (50%: W=2987, z=1.39, p=0.1633, n=101; 100%: W=2639, z=1.33, p=0.1827, n=95; Wilcoxon Signed-Rank tests against zero, with Bonferroni correction), but the correlations were approximately three times larger for the visual-motor neurons (Fig. 3A; at each contrast level, correlations for visual-motor neurons were always significantly different from zero after Bonferroni correction, Wilcoxon Signed-Rank tests, p<0.0002). Across all contrasts, a Mann-Whitney U test revealed significantly larger correlations for visual-motor than visual SC neurons (U=108665, z=-4.06, p<0.0001, n_{visual}=347, $n_{visual\ motor}$=339, $median_{visual}$=0.0493, $median_{Visual\ motor}$=0.1024), with significant individual-contrast effects being also clear at 50% and 100% contrasts, after Bonferroni correction.

Similarly, the correlations between trial-by-trial visual response strength and trial-by-trial saccadic reaction time were up to at least three times larger in visual-motor than visual SC neurons (Fig. 3B; U=205046, z=8.00, p<0.0001, n_{visual}=414, $n_{visual\ motor}$=437, $median_{visual}$=-0.0958, $median_{Visual\ motor}$=-0.2044; at each contrast individually, only 20% did not show a significant difference after Bonferroni correction). Thus, modulations in the visual responses of SC visual-motor neurons were the best predictors of subsequent behavioral variability [18].

Most critically, when comparing these results to those of V1 from Fig. 2, we confirmed how much stronger the difference between the SC and V1 became (Fig. 3C, D). In fact, even though the SC's visual neurons were the worst of the two functional SC neuronal types in the current analyses, they were still the better predictor of behavioral variability than V1 neurons (despite being the biggest recipients of direct V1 inputs [24,26,28,41]). This conclusion can be inferred from Fig. 3C, D; the horizontal purple lines indicate the mean correlation values across the population of the SC's visual neurons, and they were still quantitatively higher (in absolute value) than the V1 correlations. Statistically, the correlations between visual response onset latencies and saccadic reaction times (Fig. 3C) were higher in the SC visual neurons (horizontal purple lines) than in V1 neurons across stimulus contrasts (U=260456, z=2.26, p=0.0236, n_{SC}=347, n_{V1}=1067, $median_{SC}$=0.0493, $median_{V1}$=0.0225; Mann-Whitney U test), and the correlations between visual response strengths and eye movement variability (Fig. 3D) were also higher in absolute value than in V1 (U=333007, z=-9.05, p<0.0001, n_{SC}=414, n_{V1}=1674, $median_{SC}$=-0.0958, $median_{V1}$=-0.0019; Mann-Whitney U test). When testing at each individual contrast level, the correlations were again stronger in SC visual neurons than in V1 neurons in terms of visual response strength (after Bonferroni correction; p<0.003, but this was not the case for visual response onset latency. Thus, even though the SC's visual neurons receive more of the direct V1 inputs than visual-motor neurons, they were still functionally distinct from V1 neurons. This is consistent with the idea that visual responses in the SC are significantly reformatted versions of the inputs that they receive from the cortex and elsewhere [23].

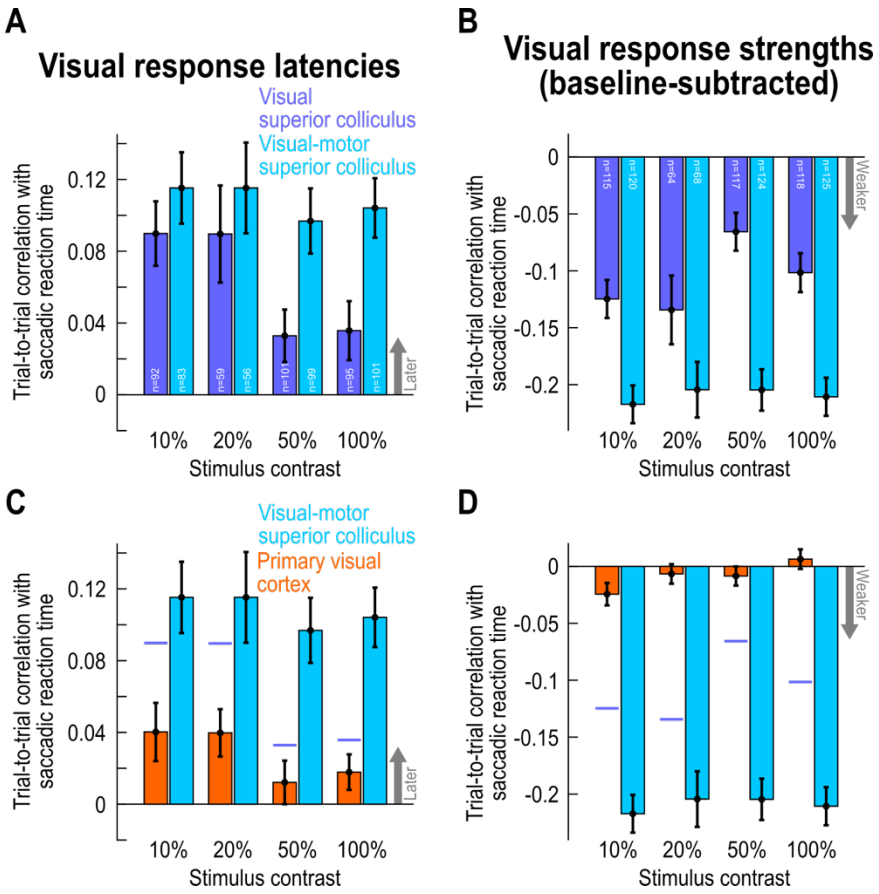

Figure 3 Strongest correlations with trial-by-trial eye movement timing in the visual responses of SC visual-motor neurons. (A, B) Same analyses as in Fig. 2 for the SC neuronal population, but now after separating the neurons according to whether they were visual-only or visual-motor (Methods). Visual-only neurons did not emit a saccade-related burst at saccade onset, whereas visual-motor neurons (as in the examples of Fig. 1A, C) emitted both a visual burst as well as a saccade-related one. For both visual response latencies (**A**) and visual response strengths (**B**), visual-motor neurons were better correlated with trial-by-trial saccade timing variability than visual neurons. Moreover, the effect was much clearer for visual response strengths than visual response onset latencies. For example, correlations to saccade timing in the visual response strengths of visual-motor neurons were approximately twice as high as those of visual neurons (**B**). **(C, D)** Including the V1 data into the analyses for comparison revealed how much stronger the covariations in visual response properties were with saccade timing in the SC than in V1. The V1 data are the same as those in Fig. 2, and the SC data are the same as in **A**, **B**. The solid horizontal lines indicate the average correlations of the SC visual neurons from **A**, **B**, and the cyan bars show the results for visual-motor neurons. Error bars denote SEM across neurons, and the numbers of neurons in each analysis are indicated in the figure (bars with missing numbers are replicates of other figures in which the numbers were given).

Superior colliculus pre-stimulus activity is lower for later saccades

The activity of SC neurons can additionally reflect a readiness to move the eyes even before stimulus onset [38,42,43]. Therefore, and for completeness, we next measured pre-stimulus (baseline) activity and checked how well it predicted trial-by-trial variability in saccade timing. For V1, there were no consistent effects (Fig. 4A), in line with all of our results above. Specifically, a Wilcoxon Signed-Rank test revealed no difference from zero across contrasts (W=371064, z=-0.79, p=0.4276, n=1234), although for only the 20% contrast individually,

there was a significant positive correlation after Bonferroni correction (W=27104, z=3.48, p=0.0005, n=296). On the other hand, and in agreement with the prior SC literature on motor readiness [38,43], SC pre-stimulus activity was systematically weaker for slower saccadic reaction times (Fig. 4A; W=91065, z=-9.51, p<0.0001, n=775; Wilcoxon Signed-Rank test against zero across contrasts; comparisons to zero at each contrast level individually were also significant after Bonferroni correction, with p<0.0001). Moreover, the difference between the SC and V1 effects was statistically significant (U=954256, z=-8.28, p<0.0001, n_{SC}=865, n_{V1}=1674, $median_{SC}$=-0.0421, $median_{V1}$=0; Mann-Whitney U test comparing SC to V1 across contrasts; comparisons across the brain areas for each contrast level individually were also significant after Bonferroni correction, with p<0.0021). Therefore, we identified another substantial difference between the SC and V1, this time with respect to pre-stimulus state.

We likewise repeated the same analysis of Fig. 4A, but now separately for either visual or visual-motor SC neurons. There were similar relationships between pre-stimulus SC state and saccadic reaction time for both types of neurons (Fig. 4B). Specifically, was no effect of neuron type (U=177800, z=0.40, p=0.6886, n_{visual}=414, $n_{visual\ motor}$=437, $median_{visual}$=-0.0435, $median_{visual\ motor}$=-0.0343; Mann-Whitney U test across contrasts; comparisons at each contrast level individually also revealed no significant differences after Bonferroni correction). Thus, pre-stimulus SC state was, in general, an important determinant of saccade timing variability.

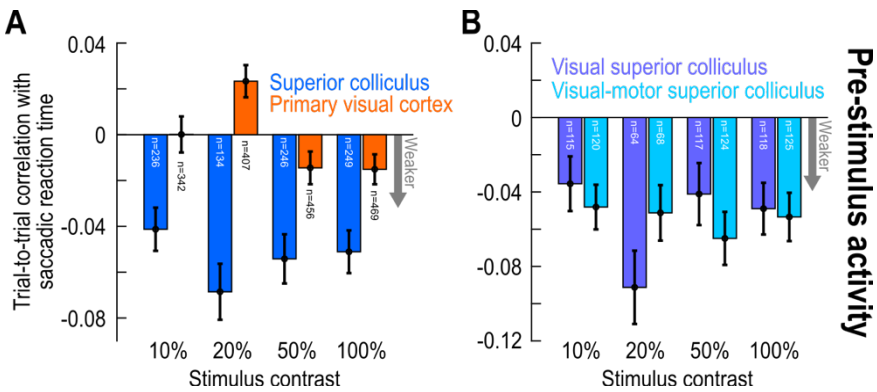

Figure 4 An additional correlation with saccade timing in the SC's pre-stimulus (baseline) neuronal activity. (A) Similar to the analyses in Fig. 2. However, here, we measured the correlation between trial-by-trial pre-stimulus (baseline) activity (Methods) and trial-by-trial saccade timing. In the SC, there was a clear negative correlation at all tested contrasts: higher pre-stimulus activity was associated with faster saccadic reaction times. This was not the case in V1. **(B)** This effect was similar regardless of whether the SC neurons were visual-only or visual-motor. Thus, in addition to visual response latency and strength in the SC (Figs. 1-3), the pre-stimulus state of the SC can much more strongly influence saccadic reaction times than the V1 pre-stimulus state. Error bars denote SEM, and the numbers of neurons in each analysis are indicated in the figure.

Taken together, our results so far reveal a striking difference in the relationship between SC and V1 neuronal activity to trial-by-trial variability in saccadic reaction times, with our take-home message being: there is a much higher covariation with foveation timing by SC than V1 activity. Figure 5 succinctly summarizes this take-home message. Here, we normalized each neuron's firing rate curve before averaging across neurons (Methods), and we compared the fastest and slowest third of all trials (in terms of saccadic reaction time) within a given session. In the SC (Fig. 5A), faster saccadic reaction time trials were associated with: (1) higher pre-stimulus (baseline) activity; (2) earlier visual response onset latency; (3) and higher visual response strength. Moreover, note that the visual response strength effect was not simply inherited from the pre-stimulus (baseline) effect; in fact, in all of our visual response strength analyses above and in the remainder of this study, we always measured the baseline-subtracted visual response strength (Methods) in order to exclude the impact of pre-stimulus activity. As for V1, neuronal activity did not differ on the fast and slow trials at all (Fig. 5B), consistent with the results of Figs. 1-4, but inconsistent with previous results on visual response onset latency [22]. In what follows, we describe our investigations of whether and how stimulus luminance polarity (bright versus dark) or V1 neuronal classes might have qualified (at least ever so slightly) the above conclusions.

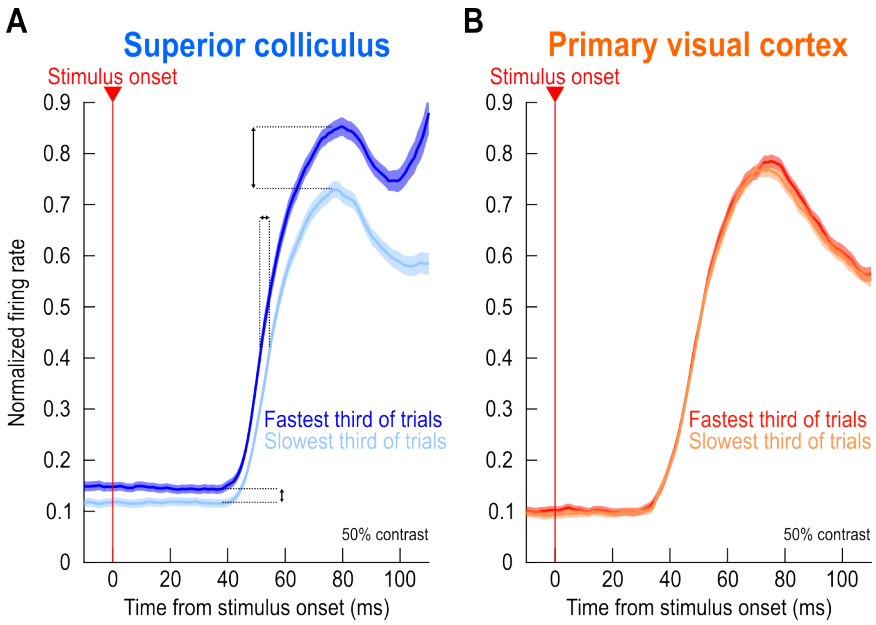

Figure 5 Summary of the differences between the SC and V1 with respect to the timing of foveating saccadic eye movements. (A) For an example stimulus level (50% dark contrast), we normalized each neuron's trial-by-trial activity to the peak of its average visual response across trials (Methods). We then measured neuronal activity on either the fastest or slowest third of all trials for the neuron. Across neurons in our entire SC populations, trials with the fastest saccadic reaction times were consistently the ones with elevated pre-stimulus (baseline activity), early visual latencies, and strong visual burst amplitudes. **(B)** On the other hand, V1 activity did not predict saccadic reaction times. Error bars denote SEM across neurons, and the numbers of neurons are the same as those that went into the visual response strength analyses of Fig. 2.

Modestly clearer covariation with behavior for bright stimuli in the primary visual cortex but not superior colliculus

Besides presenting negative luminance polarity stimuli (Figs. 1-5), we also included similar Weber contrast levels, but with stimuli that were now brighter than the surrounding background (Methods) [17].

For the SC, there were no differences in correlation amplitudes between visual response onset latency and trial-by-trial variability in saccadic reaction times (Fig. 6A; U=468013, z=0.46, p=0.6460, $n_{brights}$=659, n_{darks}=689, $median_{brights}$=0.0796, $median_{darks}$=0.0808; Mann-Whitney U test across contrasts; comparisons within each contrast level individually were also not significant after Bonferroni correction). In terms of visual response strengths (Fig. 6B), there were slightly higher correlations for dark than bright contrasts (U=468013, z=-2.00, p=0.0458, $n_{brights}$=854, n_{darks}=865, $median_{brights}$=-0.1346, $median_{darks}$=-0.1557; Mann-Whitney U test across contrast levels), but none of the comparisons within each contrast level were individually significant after Bonferroni correction.

In contrast, pre-stimulus SC state did substantially differ between bright and dark stimuli (Fig. 6C). For bright stimuli, variability in pre-stimulus SC state was less related to variability in trial-by-trial saccadic reaction times than for dark stimuli, and this effect was significant (U=701034, z=-4.17, p<0.0001, $n_{brights}$=854, n_{darks}=865, $median_{brights}$=-0.0050, $median_{darks}$=-0.0421; Mann-Whitney U test comparing brights and darks across contrasts). At the individual contrast levels, the statistical comparisons between bright and dark stimuli were significant after Bonferroni correction at 20% and 50% contrasts (p<0.012). These observations add to evidence that the recruitment of SC visual bursts can be different between bright and dark stimuli [17,44] (also see Fig. 7 below for further evidence). Indeed, when we measured the correlation between trial-by-trial pre-stimulus state and the trial-by-trial visual response onset latency of SC neurons, there was no difference between dark and bright stimuli (Fig. S2A; U=418557, z=0.14, p=0.8885, $n_{brights}$=634, n_{darks}=650, $median_{brights}$=-0.1148, $median_{darks}$=-0.1059; Mann-Whitney U test comparing brights and darks across contrasts). That is, for both types of stimuli, weaker pre-stimulus SC activity was associated with later visual response onset latencies, and by a similar amount whether the appearing stimuli were bright or dark. Thus, the negative correlations to behavioral variability in Fig. 6C were not directly explained by variability in visual response onset latency in the SC, and likely reflected intrinsically different mechanisms for generating visual responses to bright stimuli [17].

In terms of SC neuron type, the pre-stimulus effect seen in Fig. 6C was slightly clearer in visual than visual-motor SC neurons (Fig. S3). Thus, just like in Fig. 3, there were still potential functional differences between SC visual and visual-motor neurons when exploring effects of stimulus luminance polarity.

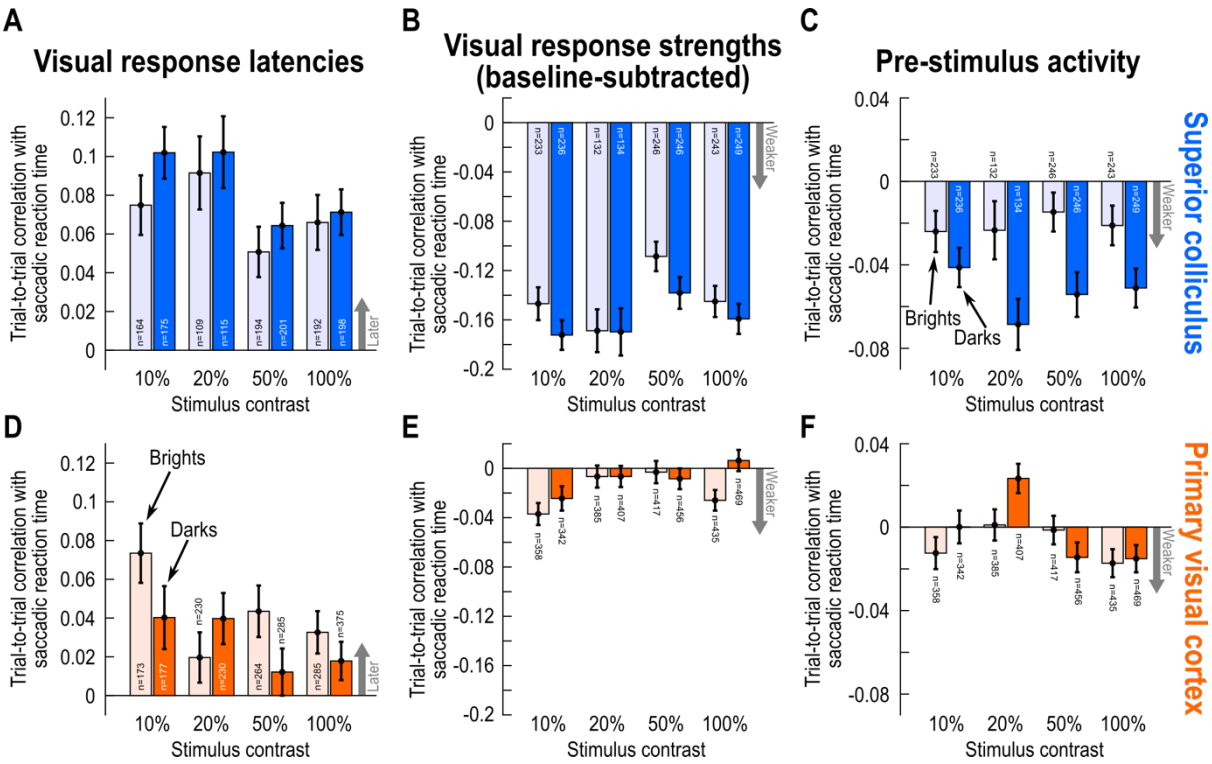

Figure 6 Weaker effects for bright contrasts in the SC, but stronger visual response latency correlations with saccade timing for bright contrasts in V1. (A-C) Same analyses as in Figs. 2, 4A for the SC neurons in our population. The saturated colors show correlations with trial-by-trial saccade timing (of the relevant neuronal parameter in each panel) for the dark contrast stimuli in our experiments (same as in Figs. 2, 4A). The faint colors show results for bright contrasts (stimulus onsets brighter than the surrounding gray background; Methods). There were generally weaker correlations for the bright contrasts, especially in pre-stimulus (baseline) activity (**C**). **(D-F)** Similar analyses for V1. In this case, only for visual response timing (D) was there a higher correlation with trial-by-trial saccadic reaction time for bright than dark contrasts. Thus, in V1, only visual response latency could, in general, show a mild correlation with saccade timing. Error bars denote SEM, and the numbers of neurons in each analysis are shown in the figure.

Interestingly, V1 showed directly opposite effects from the SC, especially in terms of visual response onset latency and visual response strength. For example, for visual response onset latency, even though there was no statistically significant difference in the correlations to behavioral variability between bright and dark stimuli (Fig. 6D; U=1058746, z=-1.45, p=0.1479, $n_{brights}$=952, n_{darks}=1067, $median_{brights}$=0.0356, $median_{darks}$=0.0225; Mann-Whitney U test across contrasts), the correlation values for bright stimuli were significantly higher than zero across contrasts (W=273618, z=5.58, p<0.0001, n=951; Wilcoxon Signed-Rank test against zero, across contrasts). Moreover, comparisons to zero at each individual contrast level were statistically significant after Bonferroni correction (adjusted α=0.005), except for 20% (W=14193, z=1.02, p=0.3069, n=229; Wilcoxon Signed-Rank test against zero). This is different from the results with dark contrasts already presented in Fig. 2A above, in which only the lowest two contrast levels had individually statistically significant positive correlations to behavioral variability. Given the prior literature, these observations are interesting because they can help (at least partially) reconcile the apparent contradiction between our results in Figs. 1-5 above with previous V1 findings [22]. Specifically, in the earlier work, Gabor gratings (having positive luminance polarity image patches within them) were

used, and this could explain why these authors found positive correlations in their experiments: it could be that their results were dictated by the positive luminance polarity image components of their saccade targets.

In terms of visual response strengths (Fig. 6E), there were again larger absolute values of correlations to behavioral variability for brights than darks in V1 (U=2795514, z=2.17, p=0.0300, $n_{brights}$=1595, n_{darks}=1674, $median_{brights}$=-0.0168, $median_{darks}$=-0.0019; Mann-Whitney U test across contrasts), but for the individual contrast levels, only 100% contrasts revealed a difference between bright and dark stimuli after Bonferroni correction (p=0.0104; Mann-Whitney U test). This again suggests moderately clearer covariation with behavioral variability in V1 neurons when the visual stimuli involved were brighter than the background.

Finally, for pre-stimulus (baseline) activity (Figs. 6F, S2B), there were no differences in correlation values between bright and dark stimuli in V1 (U=2765918, z=1.08, p=0.2791, $n_{brights}$=1595, n_{darks}=1674, $median_{brights}$=0, $median_{darks}$=0). Thus, it was only visual response onset latency and strength that were slightly better correlated to behavioral variability for bright than dark stimuli in V1.

Average visual response onset latencies are similarly different between brights and darks in the superior colliculus and primary visual cortex

As alluded to above, quantitative differences between darks and brights (like the ones seen in Fig. 6) suggest that the recruitment of visual responses, in both the SC [17,44] and V1 [45-47], can be fundamentally different between bright and dark stimuli. Consistent with this, across-trial average visual response onset latencies in our current data set were always longer for bright than dark stimuli, and at all tested contrasts (Fig. 7). These analyses are important to document here because they compare, for the first time, the visual response latencies from both brain areas in the very same animals and with the very same stimuli. Interestingly, the contrast-dependent difference between bright and dark average visual response onset latencies (Fig. 7B; top right panel) is similar between the SC and V1, and the SC data quantitatively replicate our previous observations [17]. Additionally, Fig. 7 shows that SC visual responses were always 5-6 ms later than V1 visual responses, on average (Fig. 7B; bottom right panel).

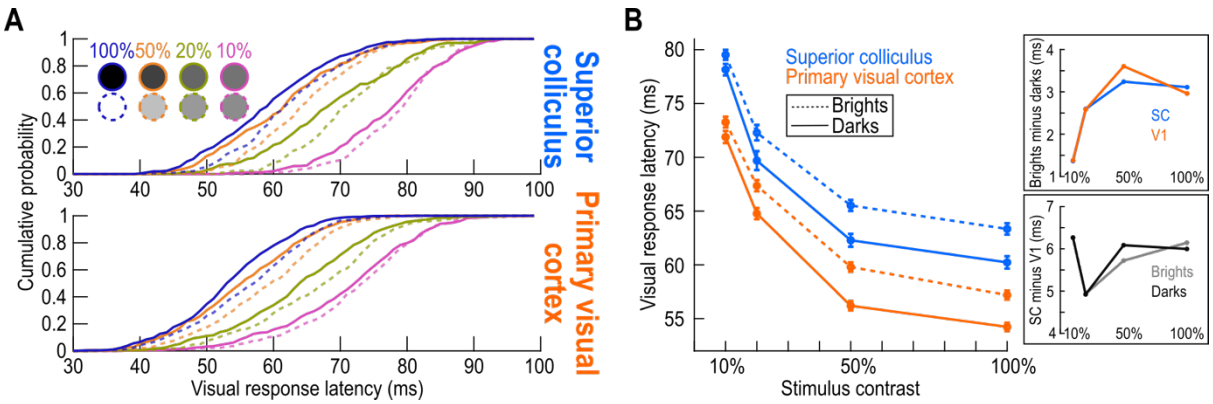

Figure 7 Visual responses are generally later for bright than dark contrasts in both the SC and V1. (A) Cumulative distributions of the average visual response latency (across trials) of each neuron in our SC (top) and V1 (bottom) populations. Low contrasts were associated with generally longer average visual response latencies in both areas than high contrasts, as expected. Additionally, at each contrast level, dark stimuli had earlier visual response latencies. **(B)** Summary statistics of the results in **A**. V1 visual responses were earlier than SC visual responses (by 5-6 ms; see bottom right inset); moreover, in both areas, bright contrasts had later visual responses than dark contrasts (by 1-3 ms; see top right inset). The contrast-dependence of bright and dark latency differences in the SC (top right inset) quantitatively and qualitatively replicates our earlier results (primarily from a gaze fixation task) [17]. Error bars in the main panel of B denote SEM across neurons, and the numbers of neurons included at each contrast level are the same as those for the visual response strength analyses of Fig. 2.

Lack of difference across cortical layers and putative cortical cell types

Because of the striking difference in how SC and V1 visual neuronal activity variability related to behavior (Figs. 1-6), we next wondered whether individual subsets of V1 neurons might have shown substantially larger effects than in our summary analyses.

We first considered cortical depth. Using current source density (CSD) analysis [48], we estimated the input layers of V1 from each session, and we then classified our V1 neurons as being either in the superficial or deep cortical layers (Methods). For both visual response onset latencies and visual response strengths, the V1 correlations to behavioral variability were similar across cortical depths (Fig. S4). We did not perform similar analyses for V1 pre-stimulus state, since V1 baseline activity was anyway already much weaker than SC pre-stimulus activity (Fig. S5A), and also because all of our other analyses did not show effects with V1 pre-stimulus state. Moreover, pre-stimulus (baseline) V1 activity was similar for the deep and superficial cortical layers (Fig. S5B).

Finally, for a subset of our V1 neurons, we additionally had suitable spike waveforms, allowing us to classify the neurons by their waveform widths (Methods). We used a similar approach to earlier studies, which classified putative excitatory and inhibitory early visual cortical neurons based on their spike waveform widths [49] (Methods). Once again, there were no differences between putative excitatory (broad-spiking) and putative inhibitory (narrow-spiking) V1 neurons (Fig. S6).

Thus, the large difference that we observed between the SC and V1 in our data set (e.g. Fig. 5) was a general property of the two brain areas, and did not depend on specific cortical subunits.

Discussion

We were motivated by the idea that multiple brain areas can exhibit qualitatively similar short-latency visual responses to appearing stimuli, raising the question of why such apparent parallelism is necessary at all. This question is even more pressing when two brain areas, like the SC and V1, are directly connected to each other, and also when previous work [14,22] has shown that both such areas might be relevant for behavioral variability. By studying both areas within the same animals and with the same visual stimuli, we could identify important functional differences between their visual responses.

On average, neurons in V1 detect new visual stimuli 5-6 ms earlier than SC neurons (Fig. 7), but SC neurons much more directly, and much more effectively, drive behavior (Figs. 1-6). This is consistent with the proximity of the SC to the motor output [50], and it is also consistent with the role of the SC in salience representation [51-54]. Thus, V1 senses the environment, but the SC evaluates and acts on it.

It is interesting to contemplate here about how our results relate to neuronal variability in general, both cortically and sub-cortically. Specifically, because saccadic reaction time variability is generally quite high (Figs. 1, S7), does the higher correlation that we observed in the SC to such variability automatically imply that SC visual responses are intrinsically much less reliable than V1 visual responses? In our data, this was decidedly not the case. For example, for each neuron in either V1 or the SC, we measured the standard deviation across trials of visual response onset latency (Fig. 8A, B) or visual response strength (Fig. 8C, D) within any given session. Across the population, and perhaps unexpectedly, we found systematically higher variability across trials in V1 than in the SC. Thus, SC visual responses were actually more reliable than V1 visual responses. This is somewhat surprising given prior suggestions that V1 visual responses are highly reliable [55], and it suggests that there are other sources of variability in V1 beyond those that ultimately get reflected in the final eye movement behavioral variability. These other sources are unlikely to include changes in stimulus position in the V1 RF's from trial to trial, because we used a relatively big stimulus for V1 RF sizes, and also because we did not see much evidence of strong modulations due to, say, microsaccades in V1 (also see below). It would be interesting in the future to understand the functional advantages of such high response reliability that we observed in the SC in Fig. 8.

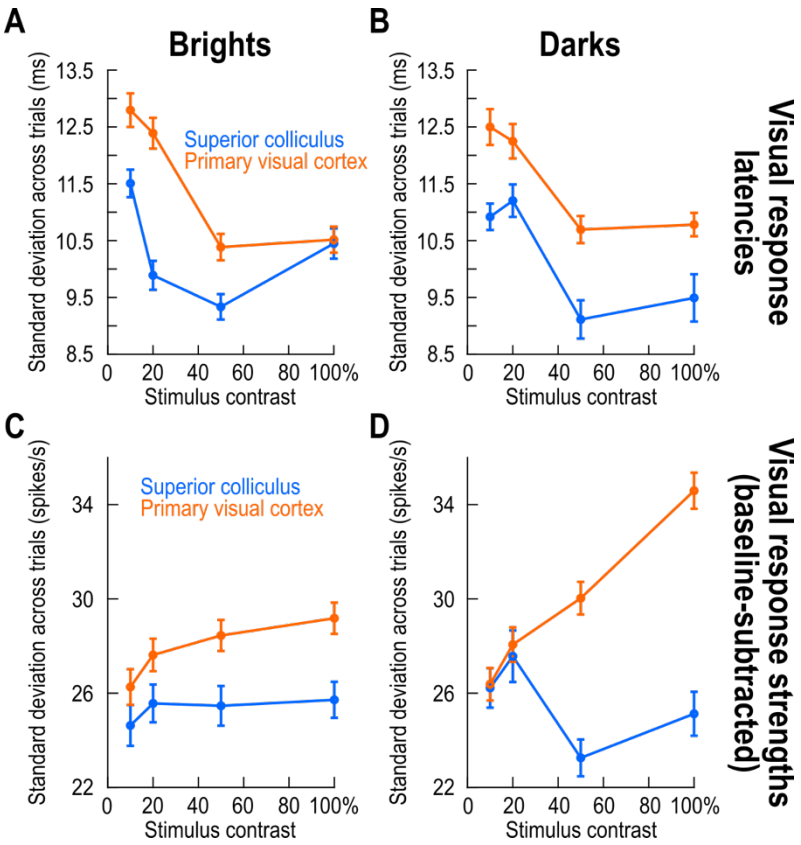

Figure 8 Higher response reliability in SC than V1 visual responses, across contrasts and luminance polarities. (A, B) For each neuron in either the SC or V1 (different colors), we measured the standard deviation of visual response onset latencies across trials within a given session. Then, we summarized this measure across all neurons in the population, by plotting the mean and SEM ranges. For both positive (**A**) and negative (**B**) luminance polarity stimuli, SC visual responses were actually more reliable than V1 visual responses across trial repetitions. Thus, given that V1 correlations to behavioral variability were very low, and given that behavioral variability was itself very high (Fig. S7), V1 visual responses have other sources of variability in them, which are not ultimately reflected in the behavioral eye movement output variability. **(C, D)** Same as **A**, **B** but for baseline-subtracted visual response strength variability. Dark stimuli had a monotonic increase in V1 variability with increasing contrast (in terms of visual response strength), which suggests that contrast sensitivity in V1 is different between bright and dark stimuli. Error bars indicate SEM across neurons.

Our results also allow making concrete predictions about further differences between SC and V1 visual responses. For example, a well-established reason for variability in SC visual responses, and subsequent saccadic behavior, is the occurrence of peri-stimulus microsaccades. Such microsaccades significantly alter SC visual sensitivity [18,19,44,56], and with direct consequences on behavioral timing variability [18,19,57]. The fact that we observed weaker V1 than SC trial-by-trial covariation with subsequent behavioral timing suggests that peri-stimulus microsaccades might have weaker influences on V1 than on the SC. This is a fundamentally important prediction because it can allow identifying the brain loci for robust perceptual and cognitive phenomena, such as saccadic suppression of perceptual sensitivity [58,59] or covert visual attentional performance [60,61], which are known to be strongly influenced by microsaccades. In fact, the modest positive correlations to behavioral variability that we observed in V1 for bright stimuli (Fig. 6D) can even allow predicting a dichotomous influence

of saccadic suppression (due to peri-stimulus microsaccades) on either bright or dark visual stimuli in V1; it could be the case that dark contrasts are immune to saccadic suppression in V1 but not in the SC, consistent with existing preliminary evidence [62]. This would be very interesting to further investigate, especially because in other species, there seems to indeed be a dependence of saccadic suppression on stimulus luminance polarity and behavioral context [63,64].

Our observations also confirm assertions that visual responses in the SC are not merely a copy of V1 visual responses, but are instead reformatted versions that are better suited for the SC's functional roles [23]. In other words, assuming that the SC and V1 have distinct functional roles in perception, cognition, and behavior, which is a fair assumption, then their individual visual response properties should support their respective individual roles. Indeed, SC neurons prefer low spatial frequencies [16], whereas V1 neurons are more band-pass at similar retinotopic eccentricities [65,66], and this is directly relevant for saccade timing [16,18]. Similarly, SC neurons prefer (in visual sensitivity, resolution, and potential over-representation) the upper visual field [67,68], whereas V1 might even over-represent at least parts of the lower visual field, such as the lower vertical meridian [69-71], and this is again relevant for the use of eye movements to achieve remote sensing [67,72]. Finally, SC neurons do not directly copy the preference of V1 neurons for dark contrasts [17], and exhibit a dissociation between response strength and response timing that likely matters for how quickly the SC can trigger eye movements [16,17]. Our present results add that response variability in both brain areas is also very different, but in a perfectly meaningful way when it comes to how visual responses in each of the areas may be subsequently used by downstream neurons.

Finally, we have to emphasize that we cannot quantitatively compare our correlation values in the SC with previous SC measures of correlations between visual responses and foveation timing [14,15]. This is because we used an immediate, reflexive orienting task in our experiments, whereas this prior work used either a delayed or gap saccade task. In the former, the animal sees the eccentric target for a long time and has to withhold orienting towards it until instructed to do so, and in the latter, the initial fixation target is extinguished before the appearance of the eccentric target; this dramatically increases motor preparation especially because target location is usually known a priori. Thus, a direct quantitative comparison is not possible, and that is why we elected to compare our two brain areas directly with the very same task in the same animals. Interestingly, and on a related note, it might additionally be wondered what the removal of the foveated fixation target (together with the eccentric target appearance) does to behavioral variability in our task. This is an ongoing question, but our hypothesis is that foveation timing variability is much more robustly related to visual responses in the SC, than to the effects of foveal target removal at the initial fixation position.

Competing interests statement

The authors declare no competing interests.

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Author contributions

YY, TZ, MPB, TM, ZMH collected the data. CT, YY, TZ, MPB, TM, ZMH analyzed behavioral and neuronal data. SP, TM, ZMH processed V1 spike waveform shapes. All authors wrote the manuscript.

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Methods

Experimental animals and ethical approvals

We collected data from SC and V1 neurons in a total of three rhesus macaque monkeys (A, F, and M). A subset of the SC data (from monkeys A and M) were re-analyzed from a previous study [17] (particularly, the reflexive saccade task in that study). Additional SC data were then collected anew, for the purposes of the current project, from monkeys A and F. Data from V1 were also collected anew for the current project from monkeys A and F.

The monkeys were all adult (A: 10-13 years, F=14 years, and M=9 years) and male, and they were all previously prepared for behavioral and neurophysiological experiments. Briefly, each animal underwent surgeries for head-holder implants, chamber implants (and craniotomies), and scleral search coils. The recording chambers were placed centered on the midline of the skull and tilted backward from vertical by an angle of 38 deg. The chamber centers were aimed, based on structural MRI images, to allow electrodes a straight path towards the SC. In monkeys A and F, we placed the midline chambers (along the anterior-posterior dimension) in such a way to allow targeting dorsal V1 from the posterior halves of these chambers and the whole SC from the anterior halves. For both the SC and V1, we could target both the right and left hemisphere in each animal using the same midline chamber. The scleral search coils allowed us to measure eye movements with very high precision using the magnetic induction technique [73,74].

All experiments were approved by the local governmental authorities of the city of Tübingen (Regierungspräsidium Tübingen), under experimental license approval number CIN 04/19G.

Laboratory setup

We used the same experimental setup as that described previously [17,75,76]. Briefly, we employed a custom-built modification to PLDAPS [77], which interfaced to the PsychToolbox [78-80] for display control. Data were stored using the OmniPlex system from Plexon, and the display refresh rate was 85 Hz. The display monitor itself was a CRT display, which was calibrated for luminance and linearized.

In all experiments, the monkeys sat in a dark room, and the CRT display in front of them had a gray background (26.11 cd/m^2). White stimuli (like the fixation spot; see below) had a luminance of 79.9 cd/m^2. Also, the distance to the display was 72 cm, giving a display pixel resolution ~34 pixels/deg.

For the newly recorded data, we used linear multi-electrode arrays (V-Probes from Plexon). We typically used 16-, 20-, or 24-channel arrays in the SC and 16- or 24-channel arrays in V1. Inter-electrode spacing was 50 μm, except for 2 V1 sessions in which it was 100 μm.

Experimental procedures

The behavioral task for the monkeys consisted of an immediate visually-guided saccade task. Each trial started with the appearance of a small white fixation spot (~10.8 x ~10.8 min arc dimensions). After approximately 600-1500 ms from fixing gaze on the spot, the spot disappeared and a simultaneous eccentric stimulus (disc of 0.51 deg radius) appeared at a single location across trials per session. The eccentric stimulus was a uniform circular disc of 10%, 20%, 50%, or 100% Weber contrast relative to the gray background (Weber contrast being defined as the absolute difference in luminance between stimulus and background, divided by background luminance). For monkeys M and A, we sometimes removed the 20% contrast condition, just to reduce the overall number of trials (other tasks had to be performed within the same sessions, requiring some tradeoffs). The stimulus could either be brighter (positive luminance polarity) or darker (negative luminance polarity) than the background, and it was placed at the optimum response field (RF) location of the recorded neurons within a given session. The monkeys' task was to initiate a foveating eye movement towards the disc as quickly as possible (with a grace period of 500 ms, but their reaction times were typically significantly faster; see Fig. 1 for examples). With their gaze now at the eccentric disc, they were required to maintain fixation on it for another 500 ms before receiving juice or water reward. We typically collected 35-45 trials per condition per session. Figure S8 shows the range of target locations used across all sessions in our experiments.

The above task was always run with other behavioral tasks during the same session. These other tasks were collected for purposes of other scientific questions, but some of them helped us decide on where to place the eccentric stimulus in a session. Specifically, we ran RF mapping tasks, typically with a delayed, visually- or memory-guided saccade task [17,75,81] allowing us to map both visual as well as (if present) motor RF's.

We included a total of 29 SC sessions and 19 V1 sessions in the current study.

Data analysis

We detected all microsaccades and saccades using our previously established methods [82,83]. We included all trials in which the monkeys were rewarded, regardless of peri-stimulus microsaccades, because we wanted as much variability in visual responses as possible, and we knew that peri-stimulus microsaccades can modulate visual responses for the same stimuli [18,19,44,56].

For neuronal analyses, we sorted single units offline after the experiments using KiloSort [84]. We then excluded units that did not exhibit visual bursts. In total, our database consisted of 254 SC neurons and 489 V1 neurons.

We detected trial-wise visual response onset latency using a method similar to that used previously [22]. Specifically, we convolved spike times with an asymmetric kernel that did not blur firing rates backwards in time (Fig. S1A). Then, we found the peak firing rate within a trial by searching within a window shortly after stimulus onset (SC two lowest contrasts: 40-110 ms; SC two highest contrasts: 40-100 ms; V1 two lowest contrasts: 30-105 ms; V1 two

highest contrasts: 30-95 ms; Fig. S1B). Then, we moved back in time until the trial-wise firing rate dropped below a threshold for at least 5 ms. This threshold was defined based on pre-stimulus activity. In particular, the threshold was defined as 2 standard deviations above the mean pre-stimulus activity across trials (Fig. S1B). The timing of the last spike before crossing this threshold was defined as the trial's visual response onset latency. In all analyses of trial-by-trial visual response onset latency, we only included a neuron within a given analysis (e.g. 10% dark contrast stimuli) if we could estimate visual response onset latency on at least 60% of all trials within the neuron's session. This is why neuron numbers reported in Results had different values for different contrasts. For example, neurons with very weak visual responses for low contrast stimuli may not have had sufficient trials with a visual response to warrant inclusion in visual response onset latency analyses.

For visual response strength analyses, we simply counted the number of spikes (per trial) occurring within a short, stimulus-aligned visual response epoch, and we then subtracted the mean number of spikes occurring in a 50-ms baseline epoch across trials (pre-stimulus and ending at stimulus onset). The visual response epoch was area- and stimulus-dependent. Specifically, we typically found that V1 visual bursts were longer lasting than SC visual bursts. Therefore, for V1, our spike count analysis involved counting spikes in an 85 ms time window. Because visual bursts were also contrast-dependent (Fig. 7), the 85 ms window started at 35 ms for the two lowest contrasts (35-120 ms) and at 30 ms for the two highest contrasts (30-115 ms). For the SC, our visual burst epoch was always 60 ms long, and it started at 50 ms for the two lowest contrasts (50-110 ms) and 40 ms for the two highest contrasts (40-100 ms). All of our visual response strength analyses were baseline-subtracted. That is, we always counted spikes in the visual response epoch and subtracted, from the same trial, the average (across trials) number of spikes in a pre-stimulus epoch ending at eccentric stimulus onset.

For pre-stimulus state, we counted spikes, on each trial, within the final 50 ms before stimulus onset, and we then correlated this count to trial-by-trial saccadic reaction time variability. When a neuron had no pre-stimulus activity (primarily in V1), we designated the neuron as having zero correlation to behavioral variability. For some analyses (e.g. Fig. S5), we estimated baseline firing rate. This was done by convolving our discrete spike times with the asymmetric kernel of Fig. S1A.

Once the neuronal parameters were collected, we took the array of trial-by-trial measures and obtained a Spearman correlation with the trial-by-trial saccadic reaction times. In some analyses (e.g. Fig. S2), we obtained the Spearman correlation between two neuronal measures and not between a neuronal measure and behavior. Specifically, we correlated pre-stimulus activity to visual response onset latency. For Fig. 1, the red lines in the bottom left and right panels of each example neuron were simple least-squares fitted lines, just for visualization. All analyses were performed on Spearman correlations.

We also sometimes assessed variability independently of correlating to behavior (e.g. Fig. 8). To do so, for each neuron, we measured the standard deviation of a neuronal parameter (e.g. visual response onset latency) across trials within a session. Then, across all neurons, we took the mean and SEM of these standard deviation measures. Thus, the population summaries were robust population estimates of the across-trial standard deviation of a

given neuronal parameter. We also did the same to estimate the standard deviation of saccadic reaction time (Fig. S7).

For some analyses, we estimated the cortical depth of V1 neurons. To do so, in each session, we performed current source density (CSD) analysis [48], for which we extracted the local field potentials from each electrode channel. These were obtained as described recently [68,75]. Specifically, we filtered (using a zero-phase-lag filter) the wideband electrode signals to the low frequency band of 0.7-6KHz, and then further low-pass filtered for subsequent analyses [68]. We then aligned LFP responses (for the highest contrast dark stimulus) to stimulus onset, and took a second spatial derivative across depth, using the method of [85]. This procedure yielded a CSD profile reflecting the distribution of current sources and sinks across cortical depths. The input layer was defined as the channel exhibiting the strongest current sink (i.e., the most negative CSD value) averaged across the period of 0-100 ms after stimulus onset. Then, after knowing the input channel, we used it and one channel above it or below it (for 50 μm electrode spacings) as the separator between superficial and deep V1 layers. That is, channels above the identified input channel and the one above it were classified as superficial V1 layers; channels below the identified input channel and the one below it were classified as deep V1 layers. For linear electrode arrays with the bigger electrode spacing than 50 μm, we took only the identified channel as the separator between deep and superficial V1. These choices were informed by estimates of cortical thickness in rhesus macaque monkeys [86]. We had few neurons in the identified input channels (because the channels were the minority in our electrode arrays), so we restricted our analyses to comparisons between deep and superficial cortex. This is justified given how these different cortical sub-units might differentially interact with the SC [87].

Also in V1, we analyzed spike waveform shape, to consider whether narrow-spiking or wide-spiking neurons behaved differently from each other. We had a detailed preprocessing pipeline for selecting waveforms. Before estimating the mean waveform shape of each neuron, we first stringently tested whether or not the units were “good”: for each neuron, we took 2000 samples of spike waveforms at the best channel on the probe for that spike, and quantified the distributions of its amplitudes in the pre-spike and spike intervals respectively. Next, we calculated the area under the receiver operating characteristic curve (AUROC) between these distributions to quantify the quality of the spike — the threshold for a “good” neuron was set at an AUROC of at least 0.95. We then ensured that the extracted waveforms for any given neuron did not include accidental noise and were not contaminated by waveforms distorted due to overlaps with waveforms from other neurons. Such contamination would be identifiable as a multimodal amplitude distribution in the spike interval. For such multimodal units, we only accepted waveforms from the largest mode, and finally, summarized it in the mean waveform for that neuron. The waveforms were upsampled from a sampling period of 25 μs to 5 μs using cubic interpolation. After normalizing the waveform amplitudes, we measured the trough-to-peak time interval to assess spike waveform width. Then, we used the width threshold of earlier work [49] to define waveforms with a trough-to-peak duration of < 350 μs as being putative interneurons, and waveforms with a longer duration as being putative pyramidal neurons. We confirmed that this width threshold was a suitable separator between waveform classes by plotting global histograms of waveform durations in our database, and observing bimodality. In total, we could perform the spike waveform analysis on 198 of our V1 neurons.

For statistics, we found that our correlation values were generally not normally distributed, by using a Kolmogorov-Smirnov test. Therefore, we employed non-parametric statistical tests throughout our analyses. We had two classes of analyses. In the first, we performed a Mann-Whitney U test comparing correlation values across the conditions of interest. For example, in an analysis like that in Fig. 2A, we tested whether the correlations in the SC were statistically different from those in V1, and we pooled across all contrasts shown in the figure. Then, we compared individual conditions separately (e.g. each contrast level in Fig. 2A). Here, we again used a Mann-Whitney U test but applied Bonferroni correction given the multiple different tested contrasts. In a second class of analyses, we compared correlations to zero. In this case, we used a Wilcoxon Signed-Rank test, and we again applied Bonferroni correction when looking at each contrast level individually. Note that for pre-stimulus activity, and as stated above, when a neuron had no such activity at all (primarily in V1), we designated the neuron as having zero correlation to behavioral variability. As the Wilcoxon Signed-Rank test with reduced sample procedure disregards all samples that equal zero, we reported only the numbers of neurons that excluded this situation in any associated single sample statistical test. Thus, the numbers of neurons indicated in all figures related to pre-stimulus activity are not always the same as the numbers of neurons reported in the associated statistical tests.

Supplementary figures

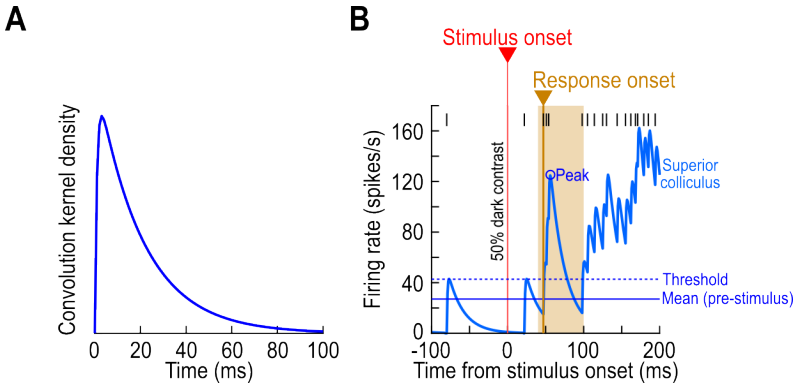

Figure S1 Estimating trial-wise visual response onset latency from a neuron's spike times. (A) The convolution kernel that we employed in order to estimate firing rates from individual-trial spike times. Each spike in a trial was convolved with this kernel density function. Note that this is the same kernel function that was used previously [22] in a similar context, and it avoids blurring the estimated firing rates backwards in time. **(B)** Example trial from an SC neuron illustrating our method for estimating trial-wise visual response onset latency. The black vertical tick marks in the top of the figure are the individual spike times emitted by the recorded neuron on the shown trial. The blue curve is the estimated firing rate curve of the trial, given the convolution of the spike times with the kernel in **A**. We estimated visual response onset latency by comparing to pre-stimulus activity. Specifically, after finding the peak firing rate (circle labeled "Peak") within a search window (brown background rectangle), we moved backwards in time until the firing rate dropped below a threshold. The threshold was defined based on measurements of pre-stimulus activity (Methods).

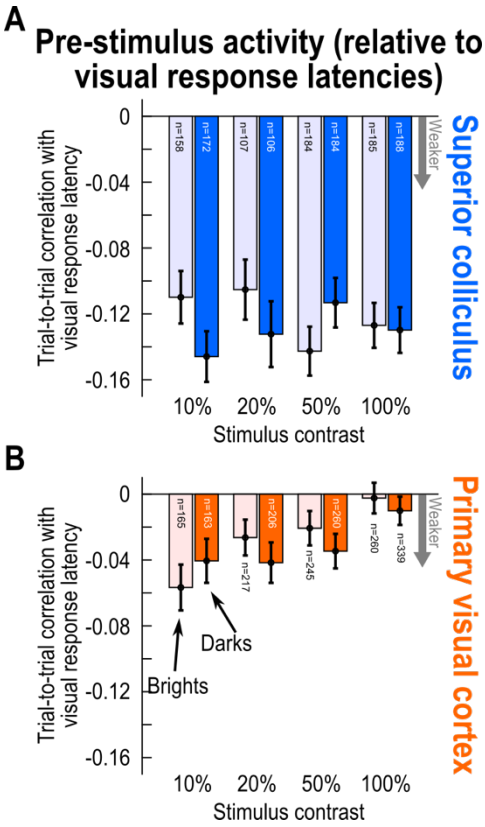

Figure S2 Relating trial-by-trial variability in pre-stimulus (baseline) activity to trial-by-trial variability in visual response latency, in both the SC and V1. (A) For each SC neuron and stimulus condition, we measured trial-by-trial pre-stimulus activity and correlated it with trial-by-trial visual response onset latency. Negative correlations mean weaker pre-stimulus activity for later stimulus-evoked visual response onset latency. The results indicate that visual response onset latencies were a function of pre-stimulus SC state, and not completely independent of it. More importantly, there was no difference in the effects between dark and bright contrasts (U=418557, z=0.14, p=0.8885, $n_{brights}$=1067, n_{darks}=689; Mann-Whitney U test comparing darks and brights across contrasts). This suggests that the weaker correlations to behavior (rather than visual response onset latency) in pre-stimulus activity for bright contrasts (Fig. 6C) was mediated by a mechanism other than a link between pre-stimulus activity and visual response latency. Potentially, this other mechanism could include visual response strength correlations (Figs. 6B, S3) because our visual response strength analyses were always independent of pre-stimulus activity (Methods). **(B)** For V1, the visual response latencies for low contrast stimuli (whether bright or dark) did generally depend on pre-stimulus activity (10% brights: W=2952, z=-3.92, p<0.0001, n=146; 10% darks: W=3547, z=-2.41, p=0.0158, n=150; 20% brights: W=6876, z=-2.04, p=0.0415, n=195; 20% darks: W=5311, z=-2.61, p=0.0090, n=190; Wilcoxon signed-rank test against zero). This could partially explain why V1 visual response latencies were better correlated to saccade timing with low contrast bright stimuli (Fig. 6D). Error bars denote SEM, and the numbers of neurons included in each analysis are included in the figure.

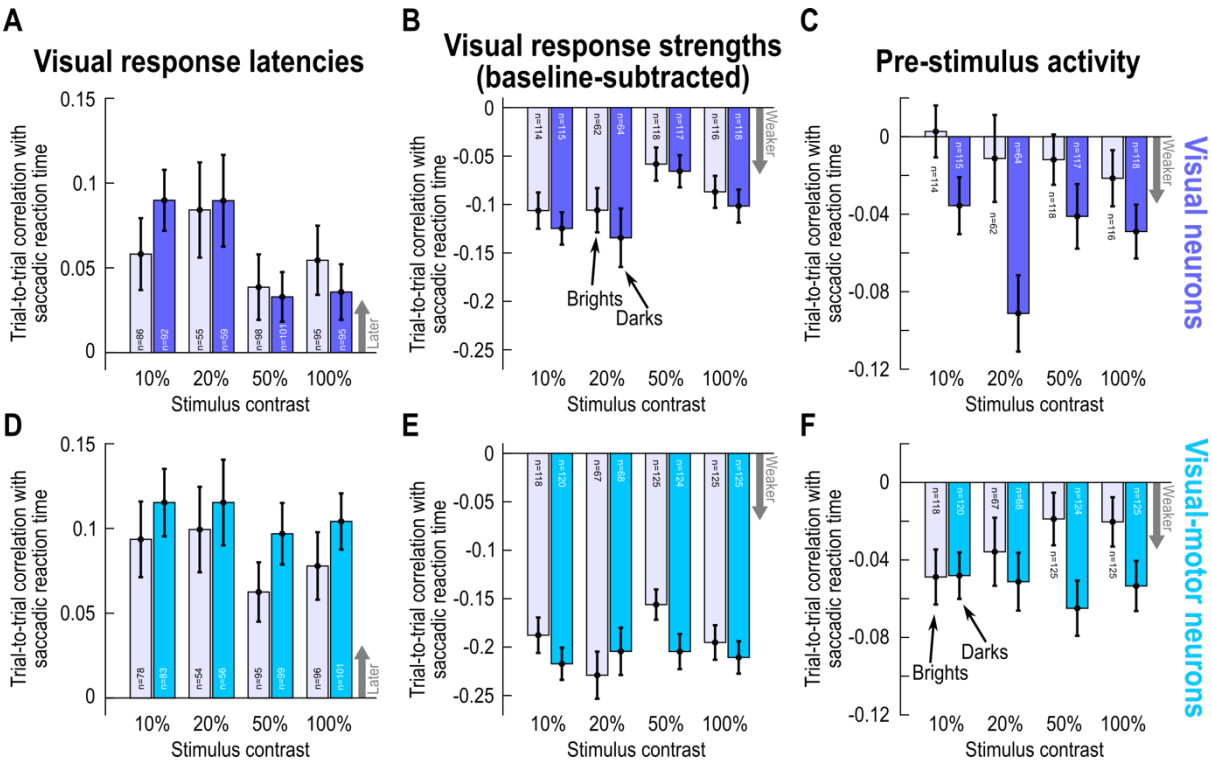

Figure S3 Similar dependencies on dark versus bright contrasts in both SC visual and visual-motor neurons. (A-C) Same analyses as in Fig. 6A-C but only for the visual-only neurons of our SC population. Saturated bars show results for dark contrasts, and faint bars show results for bright contrasts. In terms of visual response onset latency (**A**), there was no statistically significant difference between dark and bright contrasts (U=116272, z=-0.80, p=0.4234, $n_{brights}$=334, n_{darks}=347, Mann-Whitney U test comparing brights and darks across contrast levels). This was also true for visual response strength (**B**) (U=167834, z=-0.86, p=0.3894, $n_{brights}$=410, n_{darks}=414; Mann-Whitney U test comparing brights and darks across contrast levels). However, for pre-stimulus activity (**C**), there were weaker correlations with trial-by-trial saccadic reaction times in the case of bright versus dark stimuli (U=158874, z=-3.48, p=0.0005, $n_{brights}$=410, n_{darks}=414), but only 20% contrast was individually significant after Bonferroni correction. **(D-F)** Same analyses as in Fig. 6D-F but only for the visual-motor SC neurons of our population. For visual response strength (**E**) (U=183437, z=-1.97, p=0.0493, $n_{brights}$=435, n_{darks}=437) and pre-stimulus activity (**F**) (U=182032, z=-2.35, p=0.0189, $n_{brights}$=435, n_{darks}=437), there were weaker effects in the case of bright contrasts; however, none of the individual contrast comparisons reached significance after Bonferroni correction. Thus, the generally weaker effects for bright contrasts in Fig. 6 were consistent across SC neuron types.

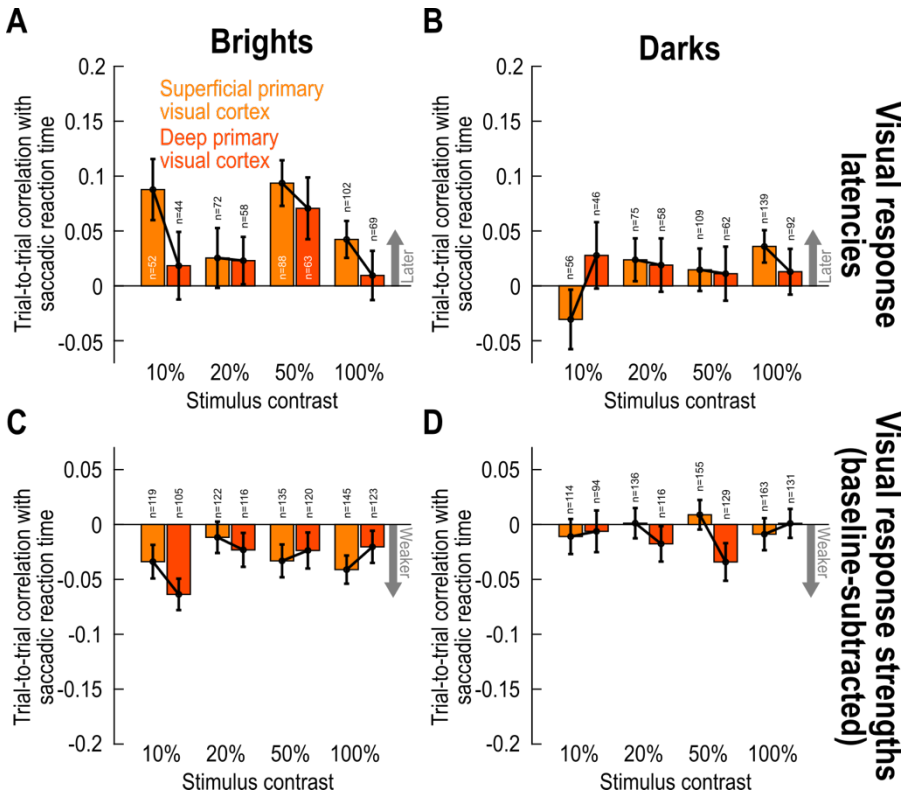

Figure S4 No consistent difference in effects between superficial and deep V1 layers. Using current source density (CSD) analysis (Methods), we classified our V1 neurons according to whether they were in putative superficial or deep V1 layers. There were no systematic differences in effects as a function of V1 depth. Only in the case of bright stimuli (**A**) was there a weak trend for larger correlations in the superficial neurons (U=89797, z=1.97, p=0.0494, $n_{superficial}$=314, n_{deep}=234; Mann-Whitney U test across contrasts). Note that we did not analyze effects related to pre-stimulus activity as a function of V1 depth because such activity was very low anyway, and also because it did not strongly depend on such depth (see Fig. S5).

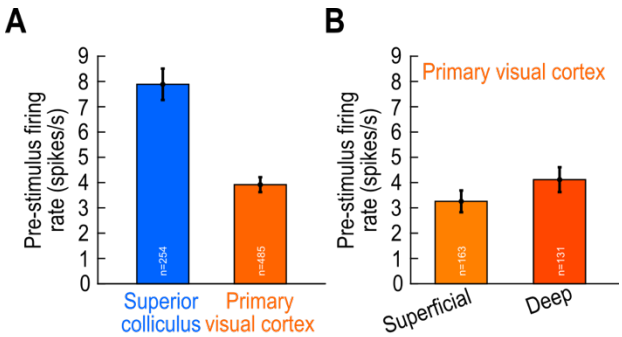

Figure S5 Weaker pre-stimulus (baseline) activity in V1 than in the SC. (A) SC neurons possessed twice the pre-stimulus (baseline) firing rate of V1 neurons. **(B)** Neurons in the superficial and deep V1 layers had similar pre-stimulus firing rates. The input layers of V1 had even weaker pre-stimulus activity (1.44 spikes/s +/- 0.395 spikes/s SEM; n=38 neurons). Error bars denote SEM, and the numbers of neurons in each analysis are shown in the figure.

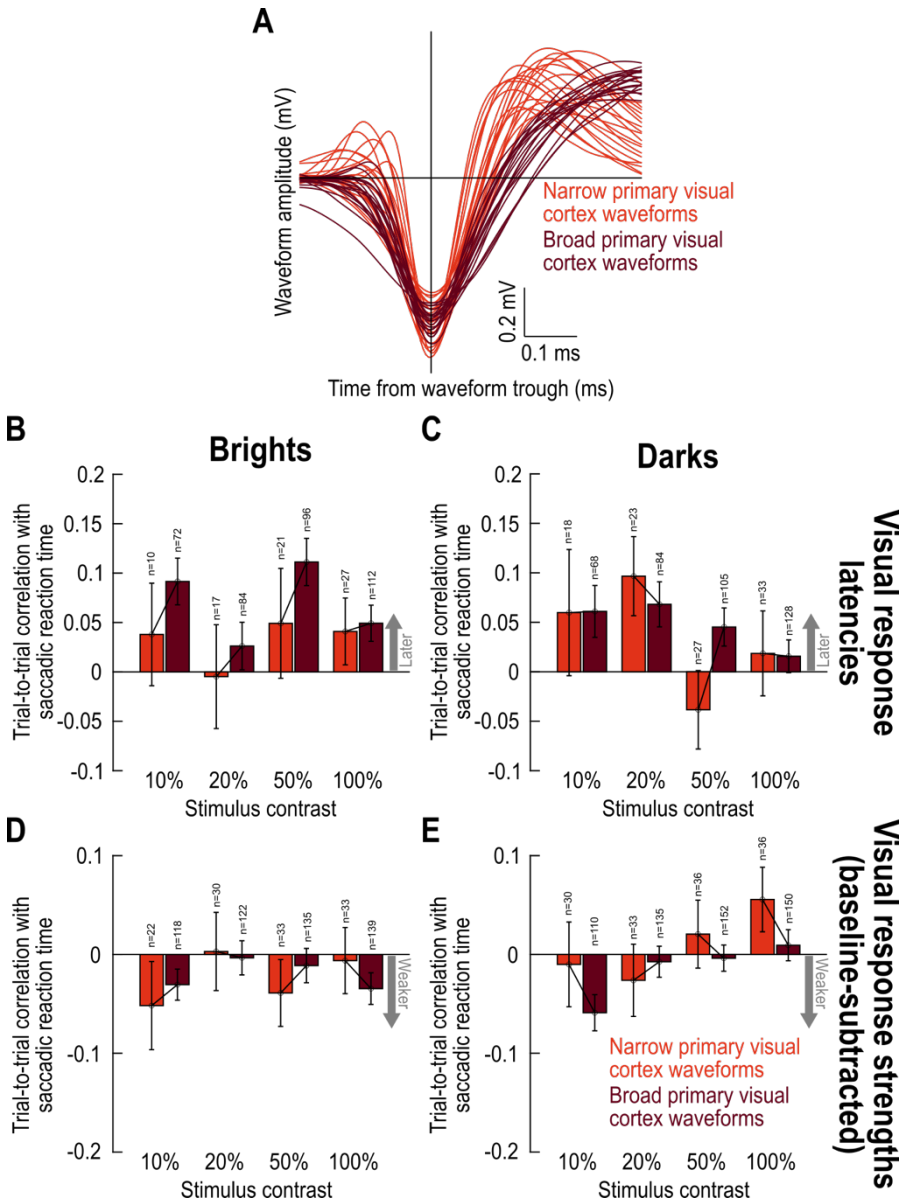

Figure S6 No consistent difference in effects between narrow- and broad-spiking V1 neurons. (**A**) Twenty randomly chosen neurons having a broad action potential waveform (dark color), and twenty having a narrow action potential waveform (lighter color; Methods). **(B-E)** None of our analyses revealed any significant differences between narrow- and wide-spiking neurons in V1, in terms of correlations to behavioral variability.

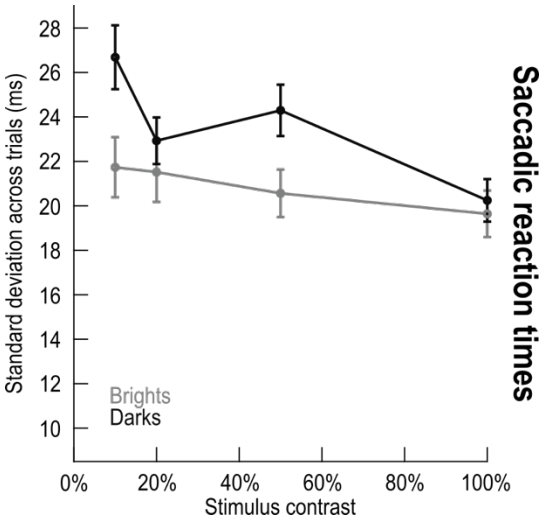

Figure S7 High variability in foveation timing. For each behavioral session, we measured the standard deviation of saccadic reaction times across trials. The shown summaries are averages and SEM ranges across sessions. In comparison to Fig. 8, the timing variability in behavior was much higher than the timing behavior of visual response onset latencies in both the SC and V1.

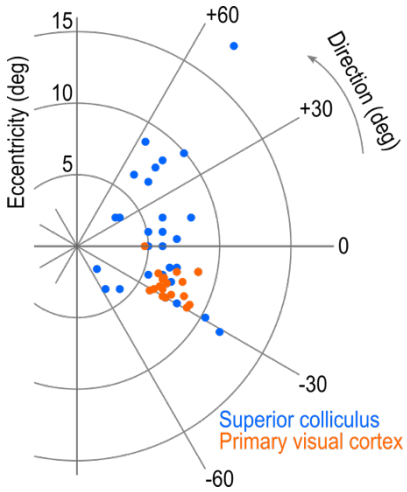

Figure S8 Sampled eccentricities and direction in SC and V1. In both brain areas, we had similar ranges of eccentricities of the saccade targets. In the SC, we sampled both upper and lower visual field stimulus locations.