Chapter 2: Suppressive lateral interactions at parafoveal representations in primary visual cortex

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Summary

The perceptual salience of image elements is influenced by the presence of other elements in their vicinity. The perceptual effect of image elements on an adjacent target element depends on their relative orientation. Collinear flanking elements usually improve sensitivity for the target element while orthogonal elements have a weaker effect. It is believed that the collinear flankers exert these effects through lateral interactions between neurons in the primary visual cortex (area V1), but the precise mechanisms underlying these contextual interactions remain unknown. Here we directly examined this question by recording the effects of flankers on the responses of V1 neurons at parafoveal representations while monkeys performed a fixation task or a contrast detection task. We found, unexpectedly, that collinear flankers reduce the monkeys’ perceptual sensitivity for a central target element. This behavioural effect was explained by a flanker induced increase in the activity of V1 neurons in the absence of the central target stimulus, which reduced the amplitude of the target response. Our results indicate that the dominant effect of collinear flankers in parafoveal vision is suppression and suggest that these suppressive effects are caused by a decrease in the dynamic range of neurons coding the central target.
Introduction

Vision starts with a fragmentation of the visual image. Neurons at early levels of the visual system process only the tiny fraction of the image that falls into their small receptive fields and distant parts of an object are represented by different cells. The properties of these early visual representations are relatively well understood at the neurophysiological level where neurons act as spatial filters and the filter properties have also been described at the psychophysical level (Heeger, 1992; Carandini and Heeger, 1994). However, the main task of the visual system is not to detect localized image elements but rather to analyze the shape of spatially extended objects that evoke responses in a large population of neurons. Most theories of visual perception hold that the low-level neurons that collectively represent a shape interact with each other so that elements of a single object are grouped in perception and segregated from image elements that belong to other objects and the background (Grossberg and Mingolla, 1985; Roelfsema, 2006). The required interactions between neurons representing different spatial locations are less well understood.

It is possible to measure the interactions between the representations of image elements with psychophysical techniques, because the perception of an image element is strongly influenced by the spatial context in which it is embedded (Gilbert, 1998; Albright and Stoner, 2002). Although the complexity of possible contextual interactions increases with the number of image elements that are taken into consideration, many important insights have been obtained with a minimalistic approach that investigates the interaction between a target element and only one or two elements in its direct vicinity (Polat and Sagi, 1993; Polat and Sagi, 1994; Morgan and Dresp, 1995; Kapadia et al., 1995; Wehrhahn and Dresp, 1998; Chen and Tyler, 2002; reviewed in Series et al., 2003). The typical experiment measures the contrast threshold of subjects who have to detect a central oriented target element (Gabor element) in the presence or absence of flanking image elements. The previous studies demonstrated that flankers can either decrease or increase the contrast sensitivity and that the type of the interaction depends on the flankers’ orientation, distance and contrast (Polat and Sagi, 1993; Polat and Sagi, 1994).

Facilitatory effects have been mainly observed for foveally presented, low-contrast Gabor targets flanked by collinear Gabor elements. Collinear flankers elements induce strongest facilitation when they are at a distance of 2-4 times the wavelength, λ, of the Gabor element (Polat and Sagi, 1993; Polat and Sagi, 1994; Morgan and Dresp, 1995; Williams and Hess, 1998; Chen and Tyler, 2002) and the facilitation is also evident in the EEG as a supra-
additive effect of flanker and target on event-related potentials (Polat and Norcia, 1996). In contrast, suppression occurs for flankers closer to the target, flankers that are not collinear and also for high-contrast target stimuli (Adini and Sagi, 2001; Chen and Tyler, 2002). Importantly, the effects of flankers also depend on the eccentricity of the stimulus. Studies that revealed facilitatory flanker effects usually measured contrast sensitivity at the fovea, while the flankers tend to suppress contrast sensitivity at more peripheral locations in the visual field (Williams and Hess, 1998; Zenger-Landolt and Koch, 2001; Shani and Sagi, 2005).

The neuronal mechanisms underlying these lateral interactions may reside, in part, at the level of the primary visual cortex (area V1). While neurons in area V1 respond to narrow-band orientation and spatial frequency stimuli presented in their classical receptive fields (CRF) (Hubel and Wiesel, 1968), concurrent stimulation of regions beyond neurons’ CRFs can enhance or decrease the neuronal responses to the CRF stimulus (Hubel and Wiesel, 1968; Jones, 1970; Maffei and Fiorentini, 1976; Nelson and Frost, 1978; Knierim and van Essen, 1992; Zipser et al., 1996; Levitt and Lund, 1997; Sengpiel et al., 1998; Cavanaugh et al., 2002). These modulatory effects are thought to originate from long-range horizontal connections within V1 (Gilbert et al., 1996; Stettler et al., 2002) and from feedback connections from higher visual areas that can supply information from outside a neuron’s CRF (Hupe et al., 1998; Lamme and Roelfsema, 2000; Angelucci et al., 2002; Angelucci and Bullier, 2003). Collinear flankers placed outside neurons’ CRFs modulate the responses of neurons to a central target in a manner that depends on contrast, orientation and spatial separation.

Importantly, the pattern of facilitation and suppression in area V1 is largely in accordance with the effects of flankers on contrast detection in human perception. Collinear flankers increase the neuronal responses in the primary visual cortex evoked by a central target (Kapadia et al., 1995; Mizobe et al., 2001; Crook et al., 2002), while orthogonal flankers exert a weaker effect or even suppress neuronal activity (Kapadia et al., 1995; Das and Gilbert, 1999). The facilitatory effect of flankers is most pronounced when the central target is of low contrast, while the flankers tend to inhibit the neuronal responses for targets with higher contrast (Polat et al., 1998; Kapadia et al., 2000). At first sight, these results suggest that the increase in neuronal activity evoked by a central target in the presence of collinear flankers can account for the increase in contrast sensitivity in human observers. However, the relationship between the psychophysics and neurophysiology of these flanker effects may be more complex than was initially thought.
Later neurophysiological studies in cat primary visual cortex demonstrated that collinear flankers do not invariably increase the neuronal responses evoked by a central target, but that the effects are mixed. Chen et al. (2001) demonstrated that 38% of neurons increase their response in the presence of flankers if the central stimulus has low contrast and decrease their response if the central stimulus has a higher contrast, in accordance with human psychophysics. However, they also found neurons that increased (29% of cells) or decreased (10%) their response independently of contrast. Remarkably, they even found cells where flankers decreased the neuronal responses at low contrast and increased them at high contrast (8% of cells). Other studies observed a comparable heterogeneity of the effects of flankers on neuronal responses in area V1 (Polat et al., 1998; Ito and Gilbert, 1999), and a recent study demonstrated that flankers suppress the response to the central stimulus that is measured with optical imaging of intrinsic signals (Kinoshita et al., 2009). Thus, not all neurons in the primary visual cortex respond as would be predicted by human psychophysics (Mizobe et al., 2001). Furthermore, most psychophysical studies were performed in central vision, while most neurophysiological studies recorded neuronal activity at parafoveal or more eccentric representations. As was mentioned above, the psychophysical studies that compared the effect of flankers at the fovea to that in peripheral vision found that flankers improve contrast detection at the fovea, but tend to reduce the sensitivity in the periphery of the visual field (Zenger-Landolt and Koch, 2001; Shani and Sagi, 2005). Most, if not all, neurophysiological results come from more eccentric representations where the perceptual effects of flankers are largely suppressive, and it is therefore remarkable that these neurophysiological studies mostly revealed facilitatory flanker effects. Why do flankers in the periphery reduce contrast sensitivity at the behavioral level but facilitate neuronal responses to low contrast image elements?

One possibility is that this apparent discrepancy is related to differences in behavioral state. Some of the neurophysiological studies were carried out under anaesthesia (Polat et al., 1998), and this has been shown to influence contextual effects in other tasks (Lamme et al., 1998), likely because GABAergic and cholinergic mechanisms, which are involved in attention and perception (Herrero et al., 2008), are affected by the anesthetics (Anthony et al., 1989; Zimmerman et al., 1994; Tassonyi et al., 2002). In the other studies, animals were awake, but not engaged in a contrast detection task (Kapadia et al., 1995), while the strength of contextual effects on contrast sensitivity and on neuronal responses in area V1 depends on the subject’s attention (Freeman et al., 2001; Crist et al., 2001; Roberts et al., 2007).
The present study has two major goals: we will try to clarify these unresolved issues regarding the neuronal correlates of flanker effects and will also record neuronal activity in animals engaged in a contrast detection task so that we can relate the neuronal responses to the behavioural effects of flankers. Towards the first goal, we recorded single V1 neurons in awake monkeys and systematically varied the contrast of the central Gabor so that we could directly measure the effect of flankers on contrast response functions. In a second set of experiments we tested monkeys in a contrast detection task in the presence or absence of collinear flankers while we monitored the neuronal activity in area V1. We find that the collinear flankers increase ongoing activity and suppress neuronal responses evoked by the central target and that these effects are associated with a decrease in contrast sensitivity at the behavioural level.

**Results**

**Effects of collinear flankers on single-unit activity of V1 neurons**

In the first set of experiments, we recorded the responses of 67 single neurons in area V1 of two monkeys (19 cells in monkey H and 48 cells in monkey D). For each cell, we first mapped the receptive field (see Experimental Procedures) and determined the preferred orientation and spatial frequency. We subsequently recorded the activity of the neuron while the monkey performed a fixation task (Figure 1A). A trial started when the monkeys fixated a central fixation point. After 500 ms four stimuli were sequentially presented on the screen. Each stimulus consisted of a central Gabor patch that was placed at the neuron’s CRF and matched a neuron’s preferred orientation and spatial frequency. The central Gabor target was either presented alone or together with two collinear Gabor flankers at one of three distances (1, 2 and 3\(\lambda\), where \(\lambda\) is the wavelength of the Gabor) from the central target. We chose these distances (as measured from the centre of the flanker to the centre of the target) because previous studies in human observers demonstrated that collinear flankers at a distance of 1\(\lambda\) decrease contrast sensitivity while flankers at 2 and 3\(\lambda\) increase sensitivity if the target stimulus is of low contrast. We intentionally did not adapt the target-flanker separation to the size of the neurons’ receptive fields, and therefore were able to measure the neuronal responses under conditions that resemble the conditions used in human psychophysics. We expected that the flankers stimulate the neurons’ receptive fields at the smaller target-flanker distances and we investigated the consequences for the response evoked by the target stimulus. We systematically varied the contrast of the central target (Gabor at 8 contrast
levels, 0, 4, 8, 12, 16, 24, 32 and 64% Michelson contrast), while flanker contrast was fixed at 48%. Each stimulus was presented for 700 ms followed by a 300 ms blank period. The order of the four stimuli (center-only, center + flankers at 1, 2 and 3\(\lambda\)) and the contrast of the central Gabor were randomized across the trials. After the presentation of the last stimulus and the subsequent blank, the trial ended and the monkey was rewarded if steady fixation had been maintained throughout the trial. For the initial recordings we used two target-flankers spatial distances (1 and 3\(\lambda\)) instead of three (31 cells out of 67 cells, all recorded in monkey D), but in our later recordings we used three flanker distances to increase the flanker-distance sampling density.

Figure 1B illustrates the peri-stimulus time histograms (PSTHs) of an example neuron to the central target stimulus at 8 contrast levels when it was presented alone or together with two collinear flankers. In the absence of the flankers and without central stimulation of the receptive field, the neuron’s firing rate remained at the level of the spontaneous activity.
Figure 1. Effects of collinear flankers on the activity of V1 neurons

A) The fixation task. The monkeys fixated a central fixation point (red) and passively viewed a sequence of 4 stimuli. Each stimulus consisted of a central target Gabor that was presented either in isolation or together with two collinear flankers. Stimulus presentation was 700ms with an interstimulus interval of 300ms. RF denotes receptive field location.

B) Activity of a V1 neuron evoked by the presentation of the centre stimulus alone or in the presence of collinear flankers located at one of three distances (1, 2 and 3). The grey shaded area represents the net response evoked by a target with a contrast of 8%.

C) Contrast response function of the neuron shown in B. Note that the cell shows suppression at all contrast levels and every flanker distance, as was typical in our sample. Curves show fits of a hyperbolic ratio function.

D) Contrast response function of a different, more exceptional neuron that showed some degree of facilitation for high contrast stimuli in the presence of far (2 and 3) flankers.
Without the flankers the appearance of a central Gabor elicited a response that increased with the contrast of the stimulus. When two collinear flankers were presented without the central target, the neuron’s responses also increased above the spontaneous activity level. This response was significant for close flankers (1 and 2λ) \((P<0.001\) and \(P<0.02\) respectively, signed rank test). The responses to the far flankers (3λ) were not significantly different from the neuron’s spontaneous activity \((P>0.3,\) signed rank test). In order to determine the amount of activity evoked by the central target in the presence of flankers \((\text{Resp}_{\text{target}})\), we subtracted the responses to the flankers only condition \((\text{Resp}_{\text{flankers}})\) from responses to the central target accompanied by the flankers \((\text{Resp}_{\text{target+flankers}})\). The shaded grey area below the X-axis of Figure 1B depicts \(\text{Resp}_{\text{target}}\) for the stimulus of 8% contrast. Compared to the center-only condition, the presence of the nearest flankers (at 1 and 2λ) reduced responses to the central target. We constructed neurons’ contrast response functions by computing the average \(\text{Resp}_{\text{target}}\) in a time window from 200 to 700 ms after the stimulus onset for every contrast level (Figure 1C). Flanker induced facilitation/inhibition was quantified by fitting a hyperbolic ratio function to the neurons’ contrast response function (see Methods). We anticipated two possible neuronal correlates of the flanker effect. Firstly, the flankers might increase or decrease the maximal response maximum \((\text{R}_{\text{target max}})\). Secondly, the flankers could increase or decrease the contrast \((c50)\) at which the half maximal response is obtained. An increase in \(\text{R}_{\text{target max}}\) or a decrease in \(c50\) would indicate flanker facilitation and the reverse would indicate flanker suppression. In the example cell in figure 1C the flankers caused a reduction in the \(\text{R}_{\text{target max}}\) at all target contrast levels. Fitted \(\text{R}_{\text{target max}}\) was reduced from 24 spikes/s in center-only condition to 6.6, 18 and 23 spikes/s in the presence flankers at 1, 2 and 3λ, respectively. To determine whether \(\text{R}_{\text{target max}}\) or \(c50\) differed significantly when the flankers were introduced, we fitted each function independently with the hyperbolic ratio function and determined the chi-square error of the individual fits, and also when fits were forced to obtain the same \(\text{R}_{\text{target max}}\) \((c50)\). The difference of the chi-square errors for the two approaches can be used to test whether the parameter of interest significantly changes when flanker are presented (Watson, 1979). \(\text{R}_{\text{target max}}\) was significantly reduced for all three flanker distances \((P<0.001)\). Moreover, the flankers significantly increased \(c50\) for all three flanker distances \((P<0.001)\). The value of \(c50\) increased from a value of 10.2% in the absence of flankers to 17.8, 22.2, and 16.2% in the presence of flankers at 1, 2 and 3λ, respectively.

Figure 1D shows the contrast response function of another neuron. The cell was strongly suppressed by flankers at a distance of 1λ, but we observed a facilitatory effect of the far flankers (2 and 3λ) as reflected by an increase in \(\text{R}_{\text{target max}}\) from 30 spikes/s in the absence of
flankers to 34 and 36 spikes/s for flankers at 2 and 3λ, respectively (P<0.01, chi-square fitting, see above). Flankers had variable effects on c50 for this neuron, it changed from 10.4% in the absence of flankers to 16.4% for a flanker distance of 2λ (P<0.001) and to 11.3% for a flanker distance of 3λ (P=0.082). However, the facilitation at larger flanker distances that was observed in this neuron was exceptional in our sample. In most of the cells, flankers reduced responses to centre targets at all distances tested (see below). Thus, the contrast response functions of the cell shown in Figure 1C are more representative of the effects observed at the population level.

As shown for our example neurons, nearby collinear flankers often induced responses in the absence of the central target. The degree of flanker induced activation depended on flanker distance as well as the neuron’s tuning to spatial frequency. This is due to the fact that flanker distance was measured in wavelength λ, the inverse of spatial frequency. For example, one wavelength corresponds to an absolute distance from the centre of the target to the centre of the flanker of 0.25° for a spatial frequency of 4cyc/°, and the flankers therefore infringe on the neurons’ CRF, while one wavelength corresponds to a distance of 1° for a spatial frequency of 1cyc/°, which may not infringe on the CRF if it is small (e.g. 0.5° diameter). We characterized this flankers-induced activity (RespFlankers) across the population of V1 neurons (Figure 2A). We computed the magnitude and the latency of responses to the collinear flankers when they were presented without the central target and compared these responses to the activity evoked by the target. A target placed inside the neurons’ CRF (contrast 32%) caused a strong response with a latency of 80 ms (95% confidence interval: 76-84 ms). Flankers of 1 and 2λ presented in isolation (no centre stimulus) elicited responses that were significantly higher than the baseline activity (both Ps < 10^{-5}, Mann-Whitney U-test) but weaker (both Ps<10^{-10}, U-test) than responses to the central target. The onset latencies of these responses (mean: 84 ms, 95%-confidence interval: 75-90 ms for 1λ and mean: 90 ms, confidence interval: 80-100 ms for 2λ) were not significantly different from the latency of the response evoked by the central target (two-sided bootstrap test, P > 0.05). At a distance of 3λ, flanker responses dropped to a level that was barely different from the baseline (P=0.048, U-test). The onset of responses to the far flankers was significantly later (mean: 109 ms, confidence interval: 90-258 ms) than the onset of the responses to the central target (two-sided bootstrap test, P < 10^{-10}). These results are consistent with the suggestion that the farthest flankers (3λ) were generally located outside neurons’ CRF since they elicited very weak responses that occurred later in time than responses the central target (Bringuier et al., 1999; Li et al., 2000). We note, however, that the extent of the CRF depends on the method
used for mapping (Angelucci and Bullier, 2003) and that we cannot exclude that the far flankers fell into the CRF of some of the cells had we measured them with other methods. Figure 2B summarizes the average activity evoked by the central targets and flankers at different separations from the CRF.

**Figure 2.** Population analysis of the effects of collinear flankers in area V1

A) Comparison of the V1 population responses elicited by the central target to the response evoked by the flanker-only conditions. The dashed lines show the fit to the data used to compute the response latency that was defined as the time that the fitted function reached 33% of its maximum. Coloured arrows on the x-axis denote onset-latencies. B) Average responses to different stimuli in a window from 50-350 ms after the stimulus onset. Arrow: ongoing activity in the same time window in the absence of gabor stimuli.

C) Population responses evoked by the central stimulus alone or in the presence of the collinear flankers located at three different spatial distances (1, 2 and 3λ). The shaded grey area denotes the responses (Resp\text{target}) evoked by a target contrast of 24%, after subtraction of the response evoked by the flankers.

Figure 2C shows the population responses to the central Gabor stimuli of different contrasts presented either alone or together with the collinear flankers. As expected, the
neuronal responses to the central target increased with stimulus contrast. We illustrated the target-evoked responses, $\text{Resp}_{\text{Target}}$, for one of the intermediate stimulus contrast levels (24%) in Figure 2C (grey area below X-axis). The flankers reduced the target response, and this reduction was most profound at the smaller flanker distances. We measured the $\text{Resp}_{\text{Target}}$ as the difference between the response of the target plus flankers and the flankers only conditions (averaged in a time window from 200-700ms after the stimulus onset) to construct the population contrast response functions (Figure 3A) and to these we fitted a hyperbolic ratio function. The main effect of the collinear flankers was a suppression of the response induced by the centre target, which was observed at all contrast levels and at every target-flanker separation. The suppressive effect of the flankers was strongest at the smaller flanker distances. Figure 3B shows the effect of the flankers on the $R_{\text{target max}}$ and $c_{50}$ across all the recorded neurons, by comparing the responses in the absence (abscissa) or presence (ordinate) of collinear flankers. At 1 and 2$\lambda$, flankers induced a strong suppression indicated by a significant decrease in $R_{\text{target max}}$ and a significant increase in $c_{50}$ compared to the no-flanker condition (paired t-test, all $P$s< 0.05). Far flankers (3$\lambda$) did not have a significant effect on $R_{\text{target max}}$ ($P>0.05$) but caused a highly significant increase in $c_{50}$ ($P<5.10^{-6}$).

Our results so far demonstrate that flankers reduce the neuronal responses evoked by a central target. However, since neuronal responses vary across trials, we also quantified the flankers’ effects with a measure that takes this variability into account. To this end, we measured the detection sensitivity (neuronal $d$-prime) of every neuron, which is computed as the difference between the responses to the central stimulus and ongoing activity in the absence of the central stimulus (i.e. $\text{Resp}_{\text{Target}}$), normalized to the standard deviation of the response across individual trials (see Experimental Procedures). Figure 4 compares the $d$-primes of single neurons in the flanker condition to those in the no-flanker condition. At all the target-flanker distances the majority of data points lie below the line of unity, in accordance with the predominantly suppressive effect of the flankers. We computed the best fitting regression line to the distribution of $d$-primes (dashed lines in figure 4) and used the slope to measure the flanker effect on $d$-prime. At all target-flanker distances the slope of the best fitting line was smaller than one. At a distance of 1$\lambda$ the slope was 0.29 and the $d$-prime distributions for the centre only vs. centre plus flanker were significantly different ($P<0.001$, paired t-test), the slope increased to 0.62 at 2$\lambda$ ($P<0.001$), and it increased to 0.82 at 3$\lambda$ ($P>0.5$).
Figure 3. Quantification of flanker modulation at the population level

A) Population contrast response functions in area V1. The error-bars denote the s.e.m. across the neurons. The curves are hyperbolic ratio functions.

B) Distribution of $R_{\text{max}}$ and $c_{50}$ values derived from the fits to the responses of all V1 single cells. Each point represents $R_{\text{max}}$ (upper panels) or $c_{50}$ (lower panels) of a single cell in the presence (ordinate) or absence (abscissa) of flankers. $P$-values are derived from a paired $t$-test.
The effect of collinear flankers on contrast detection

We were surprised by the consistency of the suppressive effects of collinear flankers across our population of V1 neurons, as this result appears to be in conflict with previous studies that reported predominantly facilitatory effects (Kapadia et al., 1995) or a mixture of facilitatory and suppressive effects (Chen et al., 2001). We considered a number of possibilities for the discrepancy. Firstly, it is possible that we did not observe facilitatory effects of flankers since monkeys were not actively performing a detection task. In the second experiment we therefore examined the effects of flankers on the performance of monkeys in a contrast detection task, while we simultaneously monitored the activity of V1 neurons using a multi-electrode recording technique. This approach will also permit us to link the behavioural effects of the flankers to the effects on neuronal activity. Secondly, it is possible that we positioned the flankers too close to the neurons’ receptive fields to observe facilitatory effects, although the flankers at $3\lambda$ did not evoke a response in most cells of the first experiment and $3\lambda$ was previously shown to be the flanker distance with strongest facilitatory effects (Polat and Sagi, 1993; Williams and Hess, 1998). In the second experiment we tested larger distances between the target and the flankers. Thirdly, we focused on stimuli with lower contrasts and presented the central target later than the flankers to optimise the conditions for facilitation (Polat and Sagi, 2006).

Figure 4. The effects of collinear flankers on neuronal sensitivity: Comparison of the neuronal d-primes in the presence and in the absence of the collinear flankers. Note that most points lie below the line of unity at all flanker distances and that the slope of the best-fitting regression line is lower than 1, indicating that the flankers reduce the neuronal d-primes.
We used a contrast detection task in which the monkeys had to report the presence of a small Gabor target (see Figure 5 and Experimental Procedures). A trial started as soon as the monkey fixated a fixation point. After 500 ms of fixation, a Gabor patch with a horizontal orientation was presented in the center of the neurons’ receptive fields. We used targets with 6 levels of contrast, 0%, 3%, 4%, 6%, 9%, 14% and 20% Michelson contrast, and added a 7th contrast of 60% in monkey A. We will refer to trials with zero contrast of the target as target-absent trials (50% of trials) and refer to the other trials as target-present trials (in the other 50% of trials the various non-zero contrasts occurred with equal likelihood). The targets were in view for 200 ms, after which they disappeared but the monkeys had to maintain fixation for an additional 400 ms. Then the fixation point disappeared cueing the monkeys to report their choice by either making an eye movement towards a marker (black circle) at the RF if they had seen the target or by maintaining fixation if not. Six (or seven) different contrast levels (3-20%) of the central stimulus were tested. Trials with and without the flankers were presented in separate blocks.

Figure 5. The contrast detection task

The monkeys performed a contrast detection task in the presence or absence of two collinear flankers. We presented collinear flankers or no flankers during the initial fixation interval. After 500 ms of fixation, a Gabor target appeared at the receptive location (RF) for 200 ms or no Gabor stimulus was presented. After an additional 400 ms the monkey reported his choice by either making an eye movement towards a marker (black circle) at the RF if he had seen the target or by maintaining fixation if not. Six (or seven) different contrast levels (3-20%) of the central stimulus were tested. Trials with and without the flankers were presented in separate blocks.

We used a contrast detection task in which the monkeys had to report the presence of a small Gabor target (see Figure 5 and Experimental Procedures). A trial started as soon as the monkey fixated a fixation point. After 500 ms a Gabor patch with a horizontal orientation was presented in the center of the neurons’ receptive fields. We used targets with 6 levels of contrast, 0%, 3%, 4%, 6%, 9%, 14% and 20% Michelson contrast, and added a 7th contrast of 60% in monkey A. We will refer to trials with zero contrast of the target as target-absent trials (50% of trials) and refer to the other trials as target-present trials (in the other 50% of trials the various non-zero contrasts occurred with equal likelihood). The targets were in view for 200 ms, after which they disappeared but the monkeys had to maintain fixation for an additional 400 ms. Then the fixation point disappeared cueing the monkeys to report their choice by either making an eye movement towards a marker (black circle) if they had seen the Gabor target or by maintaining fixation if they had not. Trials with and without flankers were identical in all respects except for the presence of the two collinear flankers (contrast 13%, horizontal orientation) that were located at one of three distances from the target (2.8, 3.5 and 4.2λ). We presented trials with and without flankers in different blocks, since the monkeys might employ a similar response criterion when stimuli are mixed (Gorea et al., 2005) and this would undermine their accuracy. The data was collected across several recording sessions...
where each of the three flanker distances (2.8, 3.5 and 4.2λ) was repeatedly tested with an average of five recording sessions. In order to evaluate the behavioural effects of flankers, we first pooled across all trials that had been recorded at each flanker distance and then derived estimates of the monkeys’ performance by a bootstrapping method (see the Experimental Procedures). We first investigated how flankers influenced the probability of false alarms (Figure 6A), i.e. reports of seeing the target in the absence of the target. Flankers increased the probability of false alarms for every target-flanker separation. At a flanker distance of 2.8λ monkey A had a false alarm rate of 14% without flankers that increased to 18% in the presence of flankers; the false alarm rate increased from 19% to 22% at a flanker distance of 3.5λ and from 18% to 21% at a distance of 4.2λ (P<10^-10 at every target-flanker distance, two-sided bootstrap test). A similar result was obtained in monkey G where the flankers increased the false alarm rate from 16% to 26% at a flanker distance of 2.8λ, from 9% to 16% at 3.5λ and from 18% to 23% at a distance of 4.2λ (P<0.01 at all target-flanker distances, two-sided bootstrap test). We suspect that the relatively high rate of false alarms was related to the inclusion of trials with low contrast targets (<10%) that were difficult to perceive given the short presentation times. These target-present trials presumably induced a low response criterion at the cost of an increased false alarm rate.
Figure 6. Behavioural Performance

A) Probability of false-alarms with (red bars) and without flankers (green bars) in the two monkeys. Note that the various flanker distances were tested on different days and that the green bars denote false alarm rates in the corresponding sessions for the no-flanker trials. The error bars indicate the 95% confidence intervals of the bootstrapped data. Asterixes denote significant difference (P < 0.05) in FA rate between trials with and without flankers measured by a two-sided bootstrap test.

B) Detection sensitivity (d-prime) at different contrast levels with and without the flankers. The vertical dashed lines represent the behavioral contrast thresholds. Error bars represent the 95% confidence intervals. A plus (+) sign indicates that the contrast thresholds with and without the flankers were significantly different. Asterixes (*) denote that the d-primes with and without the flankers were significantly different (two-sided bootstrap test, P<0.05 with Bonferroni correction for multiple comparisons).
To measure the monkeys’ accuracy while accounting for variations in response criterion, we computed $d$-prime values (see Experimental Procedures). Figure 6B illustrates the $d$-prime of the monkeys for different contrast levels with and without the collinear flankers. As expected, $d$-prime generally increased when the stimulus contrast was higher. Monkey A had a lower performance than monkey G and even missed some of the high contrast stimuli, which is why we added one extra high contrast level (60%) to the stimulus set of this animal, although we limited our analysis to the contrast levels used in both monkeys.

The main effect of collinear flankers was a reduction in $d$-prime, especially at higher stimulus contrast and at the smaller flanker distances (the significance of difference between $d$-primes with and without flankers was tested by a two-sided bootstrap test, the asterisks in figure 6B indicate a significant difference; $P < 0.05$, where $P$ is adjusted for multiple comparisons across 6 contrast levels). The only exception was for the far flankers in monkey G (4.2$\lambda$), where the $d$-prime at intermediate contrasts (6, 8, and 16%) was higher in the presence of flankers. Contrast thresholds (dashed vertical lines in figure 6B) were computed by fitting a Weibull function to the performance data and by measuring the contrast value for which the function reached a $d$-prime value of 1.5 (i.e. a correct rate of $\sim$70% if the false-alarm is about 15%). Collinear flankers significantly increased contrast thresholds at a flanker distance of 2.8$\lambda$ in both monkeys and at 3.5 and 4.2$\lambda$ in monkey A (two sided bootstrap test, all $Ps < 0.05$). These results, taken together, indicate that collinear flankers increased the false alarm rate of the monkeys and decreased their contrast sensitivity.

The effects of collinear flankers on activity of V1 neurons

Similar to our first experiment, we initially determined whether collinear flankers placed at the selected range of distances (2.8-4.2$\lambda$) induced a noticeable response in V1 neurons while the monkeys were engaged in a passive fixation task (see Experimental Procedures for details). We compared the magnitude and the latency of the responses evoked by a central Gabor (13% contrast) to the responses evoked by collinear or orthogonal flankers presented alone with the same contrast of 13% and placed at a distance of 2.8, 3.2 and 4.2$\lambda$ from the center of the receptive field (figure 7A and 7B). Note that in these experiments the stimulus (either the central target or the flankers) appeared at time 0 and disappeared at 200 ms. We normalized the responses to the maximum activity evoked by the central Gabor (13% contrast) in a time window from 50 to 150 ms after the stimulus onset (see figure 7A). To compare neuronal activity across flanker conditions, we averaged responses in a time window
between 50-350ms, while normalizing activity to the peak response (Figure 7A) and this explains why the average response in the absence of flankers was smaller than 1 (Figure 7B). Collinear flankers placed at 2.8 and 3.5λ from the CRF center elicited neuronal responses that were significantly higher ($P < 0.01$, Mann-Whitney $U$-test) than the baseline, but weaker ($P < 0.01$, Mann-Whitney $U$-test) and delayed ($P < 0.05$, two-sided bootstrap test) compared to the responses to the central Gabor (onset latencies: central target, 58ms; flankers at 2.8λ, 70ms; flankers at 3.5λ, 71ms). Responses evoked by the collinear flankers decreased at larger distances from the CRF, and the flankers did not evoke a significant response if they were at a distance of 4.2λ ($P > 0.05$, Mann-Whitney $U$-test). Neuronal responses to the orthogonal flankers at 2.8 and 3.5λ were significantly weaker ($P < 0.01$, $U$-test) than responses to the collinear flankers at the same location but had a similar latency (flankers at 2.8λ = 66 ms and flankers at 3.5λ = 78ms; $P > 0.05$, two-sided bootstrap test). At a distance of 4.2λ responses to the orthogonal flankers became indistinguishable from the baseline ($P > 0.05$).

The activity induced by the collinear flankers depended on the preferred orientation of the recording sites. The orientation tuning of the recording sites was determined by measuring the responses to a moving bar stimulus (see Experimental Procedures). We measured the orientation selectivity of the recording sites by computing an orientation selectivity ratio (OSR), defined as the ratio between the response to the best and the worst orientations. OSRs ranged from 1.2 to 4.3 with a median of 1.7 ($N = 38$). For every recording site we determined how well the horizontal orientation used in our experiments matched the preferred orientation of that recording site. In our sample of 38 recording sites, 15 sites the flankers had an orientation preference within 0-30° from horizontal, 15 sites had a preferred orientation that differed by 30-60° and 8 sites had an orientation preference differing by 60-90° from the horizontal orientation. Figure 7C shows the responses evoked by the collinear flankers at these three groups of recording sites in absence of a target stimulus, normalized to the responses evoked by the horizontal stimulus centered on the RF. At all target-flanker distances the responses to the collinear flankers were highest when they matched the recording site’s preferred orientation, they were at an intermediate level when the orientation of the flankers differed by 30-60 degrees from the preferred orientation and weakest when the flankers were approximately orthogonal to the preferred orientation.

We performed an ANOVA with factors distance (3 distances) and the orientation of the flankers relative to the preferred orientation of the recordings sites (3 degrees of offset from the preferred orientation: 0-30°, 30-60° and 60-90° offset) to investigate the effect on the magnitude of the responses to the collinear flankers. The effect of distance ($F_{2,87} = 3.6$, $P =$
0.03), and the relative flanker orientation ($F_{2,87} = 6.2, P = 0.003$) were significant while their interaction did not reach statistical significance ($F_{4,87} = 0.02, P = 0.9$). Taken together, these results show that the flankers induced stronger activity if they were close to the receptive field and if they matched the neurons’ preferred orientation.

We next examined the neuronal responses while monkeys performed the detection task. Figure 7D illustrates the population responses (average across 38 recording sites) evoked by the central target alone as well as well as by the target plus collinear flankers at the three flanker separations. These data were normalized to the 20% contrast stimulus in the no-flanker condition that was repeated on every day of recording, but as different target-flanker distances were tested in different recording sessions the data of the various flanker distances are shown in separate panels. The flankers that were presented 500ms before the target (i.e. at $t=-500$ms in Figure 7D) caused an increase in activity even in the absence of the target (note the grey areas in Figure 7D) that was strongest if they were close to the CRF. As expected, the strength of the response evoked by the central Gabor targets increased with stimulus contrast. To compute the responses that were evoked by the central target ($Resp_{Target}$), we subtracted the responses in target-absent trials from the responses in target-present trials. As an example, we illustrated the responses evoked by a target with a contrast of 9% as the yellow area in figure 7D. It can be seen that the flankers decreased the responses to the central target, because it was evoked on top of a higher baseline activity.
Figure 7. Effects of flankers on the population responses in area V1

A) The neuronal responses evoked by a central target (green) are compared to the responses elicited by the collinear (red) and orthogonal flankers (blue) placed at three different distances (2.8, 3.5 and 4.2λ). The arrows on the x-axis denote the response latencies.

B) Average neuronal activity in a time window from 50-350 ms after stimulus onset (same data as in A).

C) Activity induced by collinear flankers as a function of the difference between the presented and preferred orientation (0-30°, 30-60°, and 60-90°).

D) Responses evoked by the central target at different contrast levels. The yellow response shown below represents the difference between the activity evoked by a central Gabor with 9% contrast and the activity evoked by the flankers on target-absent trials. Note that the flankers reduce the response.
Figure 8. Quantification of flanker modulation in a population of V1 recording sites

A) Contrast response functions averaged across all recording sites in the presence and absence of the flankers. The error bars represent the s.e.m. Asterixes (*) denote a significant difference in the response evoked by the target in the presence and absence of flankers (Mann-Whitney U-test with Bonferroni correction for multiple comparisons).

B) Distribution of $R_{target\_max}$ and $c50$ values derived from hyperbolic fit to the responses of all V1 recording sites in the presence (ordinate) or absence (abscissa) of flankers. $P$-values are computed with a paired $t$-test.
Figure 8A shows the effect of the flankers on responses evoked by the target in the time window from 100-600 ms after target appearance for the various contrast levels. In both monkeys, the flankers at every distance decreased the magnitude of the response evoked by the target. The suppressive effect of flankers was largest at small distances and for targets with higher contrasts. Similar to our single cell experiments, we quantified the flanker effect by fitting a hyperbolic ratio function to the contrast response curve of every recording site (figure 8B). The flankers significantly decreased the $R_{target\_max}$ values at all distances (paired t-test, all $P_s < 0.001$) compared to the no-flanker condition. Closer flankers (2.8$\lambda$) also significantly increased the $c_{50}$ values (paired t-test, $P < 0.01$) but at larger flanker distances the increase in $c_{50}$ was not statistically significant. Therefore, the major effect of flankers in the contrast detection task was a decrease in the $R_{target\_max}$.

To examine the distribution of the modulatory effect of flankers across the population of V1 neurons, we computed neuronal $d$-prime for every individual recording site. In figure 9 neuronal $d$-primes in the presence of the flankers (ordinate) are plotted against neuronal $d$-primes in centre-only condition (abscissa). Each point represents the neuronal $d$-prime of an individual recording site at a certain contrast level. For all target-flanker distances, the data points lie below the line of unity indicating that collinear flankers decreased the neuronal sensitivity. The slope of the best fitting regression line is an indicator of the direction and magnitude of the flanker effect and it was invariably smaller than 1 (neuronal $d$-primes without flankers > neuronal $d$-primes with flankers, all $P_s < 0.01$, paired t-test). The data were well fitted by a line, which suggests that the flankers decreased neuronal sensitivity by a constant fraction. Moreover, the decrease in $d$-prime was most pronounced at smaller target-flanker separations.

**Comparison of flanker effects on neuronal $d$-prime between experiments**

In our behavioral experiment we used larger flanker separations than in the first experiment and recorded multi-unit activity rather than single units. Are the effects of flankers comparable across the two experiments? To address this question, we computed the slope of the regression lines for the effect of the flankers on neuronal $d$-prime (Figures 4 and 9A). In all instances collinear flankers resulted in a suppression of the neuronal $d$-primes. In the first experiment the $d$-prime was reduced to 29%, 62% and 82% of the value in the no-flanker condition for flankers at a distance of 1, 2, and 3$\lambda$, respectively, while the $d$-prime of the second experiment was reduced to 81%, 90% and 91% for distances of 2.8, 3.5 and 4.2$\lambda$. 
respectively. It can be seen that the flankers at similar distances evoked a comparable degree of suppression of neuronal sensitivity in the two experiments.

**Figure 9. The effects of collinear flankers on neuronal sensitivity (d-prime) in the detection task**

A) The distribution of the neuronal $d$-primes in the presence (ordinate) and absence (abscissa) of the collinear flankers. The slope of the best-fitting regression line is lower than 1 at all flanker separations, indicating that the flankers reduce the neuronal sensitivity to the central target.

B) Comparison of the reduction in neuronal $d$-prime across the two experiments (estimated as the slope of the regression line, see A). Error bars are the s.e.m. of the regression slopes.
Discussion

Here we investigated the contextual effects of flankers on the accuracy of monkeys in a contrast detection task as well as on the activity of neurons in area V1. Our results show that collinear flankers at distances which have commonly been associated with an increase in contrast sensitivity induce a decrease in the contrast sensitivity of neurons in area V1. When a central target was presented together with collinear flankers, the response to the target plus flankers was equal to or less than the response evoked by the target alone. Flankers at distances up to $3.5 \lambda$ increased the ongoing activity of V1 neurons and the response evoked from the enhanced baseline activity was smaller than the response evoked by the same target in the absence of flankers. This decreased responsiveness caused a decrease in the neuronal $d'$ and was also accompanied by a decrease in contrast sensitivity at the behavioural level.

At first sight, these results are at odds with psychophysical experiments showing that flankers improve contrast detection and also with earlier neurophysiological studies reporting mainly facilitatory flanker effects (Kapadia et al., 1995; Polat et al., 1998; Mizobe et al., 2001; Chen et al., 2001; Kasamatsu et al., 2001; Crook et al., 2002). Why did we find suppression where previous studies tended to observe facilitation? In our discussion we will first address the effects of the flankers on the ongoing activity and then discuss the possible reasons for the suppressive effects of the flankers that were observed in the present study.

Effects of flankers on the false alarm rate and on ongoing neuronal activity

We observed that the collinear flankers increased the probability of false-alarms (Fig. 6A). This result is reminiscent of a recent study in human observers showing that flankers increase the false alarm rate, an effect that is particularly strong if trials with and without flankers are interleaved (Polat and Sagi, 2007). Polat and Sagi (2007) speculated that the false alarms are caused by a perceptual process that fills in the space between the flankers. They suggested that this process might induce activity in neurons with receptive fields on the background region between the flankers. In their study, the increase in the false alarm rate was smaller if the flanker and no-flanker conditions were presented in different blocks so that subjects could adjust their response criterion for the central target, and could be more conservative in reporting targets in the presence of the flankers. However, even if the flanker and no-flanker trials were presented in different blocks, flankers induced extra false alarms, just as we observed in the present study.
In accordance with the suggestion of Polat & Sagi (2007), we found that the flankers presented at distances smaller than ~3.5λ indeed increased the activity of neurons with a receptive field between the flankers. Collinear Gabor elements induced a stronger increase of the ongoing activity than orthogonal flankers, which is in accordance with the finding that non-collinear flankers also cause a smaller increase in the false alarm rate in human observers (Polat and Sagi, 2007). The proposed filling-in process is also in line with a ‘visual phantom’ phenomenon that occurs if two collinear gratings are separated by a gap (Meng et al., 2005). Under some conditions, subjects perceive that the gap is filled with a grating and this illusory percept is accompanied by neuronal activity at the retinotopic location of the gap in early visual areas. Interestingly, the illusory grating percept only occurs for collinear gratings and not for gratings with an orientation that is parallel to the gap, in line with the orientation dependence of the flankers on ongoing activity observed in the present study.

Here we chose flanker distances that are known to induce facilitation in human foveal vision and we were not surprised to find an effect of ongoing activity for the shorter distances (1 and 2λ) where it is likely that the flankers encroached on the CRF, at least in some of our experiments. Flankers at 3λ and larger distances caused only a weak effect on the ongoing activity, and this effect was strongest if the flankers had a collinear orientation. These flankers avoided the most sensitive regions of the CRF. We do not wish to claim, however, that the flankers at a distance of 3λ were always entirely outside the CRF for at least two reasons. Firstly, the boundary of the CRF can be defined in a number of ways (Angelucci and Bullier, 2003) and the receptive field size has been shown to be larger for stimuli with a lower contrast (Kapadia et al., 1999; Sceniak et al., 1999). We did not perform the CRF measurement at all possible contrasts and even the more distant flankers may have fallen into an excitatory region contiguous with the CRF. Secondly, if the receptive field size is determined with pairs of flanker bars symmetrically arranged across the CRF centre, the observed CRF is larger than when it is mapped with more standard techniques (Angelucci and Bullier, 2003). Nevertheless, we found that the activity evoked by flankers farther from the CRF centre was weaker and had a later onset compared to the responses elicited by the central target, in line with previous findings (Li et al., 2000). This delay has been proposed to reflect the horizontal propagation of the signals from the adjacent neurons (Bringuier et al., 1999), although some authors have suggested that the dynamics of surround effects better match with the feedback signals from extra-striate cortex (Angelucci and Bullier, 2003). However, it is also possible that the weaker response and increased delay is caused by less efficient feedforward input
when the flankers are placed outside the central CRF causing an extra delay before the neurons reach firing threshold.

**Effects of flankers on neuronal contrast sensitivity**

In the presence of the flankers, the centre target had to evoke a response on top of the enhanced baseline firing rate while we found that the response to maximal contrast remained the same or was even reduced. The flankers therefore decreased the dynamic range ($R_{Max}$) of the V1 responses to the targets and this decrease in evoked activity was larger for targets with higher contrasts (Figure 3, 8A). Accordingly, the neuronal $d$-primes were reduced by a fraction that was relatively constant for a specific flanker separation (Figure 4, 9A). The magnitude of this fractional decrease in $d$-prime was largest for flankers closest to the central Gabor element (Figure 9B) that caused highest activity if presented alone. In addition, the flankers increased $c_{50}$, in particular at the smaller distances ($<3.5 \lambda$), and this effect corresponds to a rightward shift of the contrast response function.

The finding of a general decrease in neuronal contrast sensitivity appears to be in conflict with earlier studies that uncovered facilitatory flanker effects (Kapadia et al., 1995), especially at the lower contrasts (Polat et al., 1998), or a mixture of facilitatory and inhibitory effects (Kapadia et al., 2000; Mizobe et al., 2001; Kasamatsu et al., 2001), although we also did find facilitatory interactions in a small number of cells (e.g. the example cell in Figure 1D). Why did we find such a predominance of suppressive effects while previous studies found much more evidence for facilitation? One possible explanation is that we fixed the distances between the flanker and central targets at multiples of the Gabor wavelength ($\lambda$), in accordance with the procedures used in most psychophysical studies, while previous neurophysiological studies usually ensured that the flankers did not induce any response above the baseline. We note, however, that we also observed a small but highly consistent suppression at the largest flanker distance of 4.2$\lambda$ that did not induce a significant increase in the baseline firing rate in the passive fixation task. Another possibility is that we used Gabor stimuli while the studies that observed a predominance of facilitatory effects used short light bars (Kapadia et al., 1995; Kapadia et al., 1999; Kapadia et al., 2000). However we do not consider this possibility likely, because mixtures of facilitatory and suppressive effects were also observed in studies in anesthetized cats that used Gabor stimuli (Mizobe et al., 2001; Chen et al., 2001; Kasamatsu et al., 2001; Crook et al., 2002). Furthermore, our results fit well with a recent study that used short bars and measured the effects of flankers in awake monkeys with optical imaging of intrinsic signals (Kinoshita et al., 2009). This study
demonstrated that flankers caused a robust reduction in the intrinsic signals evoked by targets of all contrasts. We therefore feel confident about the predominance of suppressive effects of the flankers on contrast sensitivity of spiking activity in area V1 that was observed by us in two experiments with different recording techniques. Furthermore, the present study is the first to measure the effect of flankers on behavioral contrast sensitivity in monkeys during the recordings. We also observed a suppression of contrast sensitivity, which is in accordance with the effects on the neuronal activity in area V1.

**Flanker effects on contrast sensitivity in behavior**

In human psychophysics the predominant effect of flankers is an increase of the sensitivity for targets with a low contrast. This facilitatory effect of flankers was first observed by Polat & Sagi (2003) and has since been replicated in a large number of studies (Polat and Sagi, 1994; Morgan and Dresp, 1995; Wehrhahn and Dresp, 1998; Chen and Tyler, 2002). Here we observed that the flankers rather reduced the contrast sensitivity in the monkeys, with largest decreases in sensitivity for the smaller flanker distances. Our findings therefore appear, at first sight, to be also in conflict with previous psychophysical findings. We note, however, that the reduction in contrast sensitivity observed here is in line with most of the human psychophysical work that compared flanker effects in the fovea to their effects at more peripheral locations in the visual field. While flankers increase the contrast sensitivity in human observers at the fovea, they have less of an effect or decrease contrast sensitivity at parafoveal and more eccentric locations (Williams and Hess, 1998; Zenger-Landolt and Koch, 2001; Shani and Sagi, 2005).

Two hypotheses have been proposed to account for the effect of eccentricity on flanker facilitation. The first hypothesis holds that subjects allocate less attention to the flankers at eccentric locations compared to when they are presented close to the fovea. This view received support from findings that the facilitatory effects can be restored by directing attention to the flankers in the periphery (Giorgi et al., 2004; Shani and Sagi, 2005), which is in accordance with a study showing that flanker effects at the fovea also depend on attention (Freeman et al., 2001), and also with findings of Roberts et al. (2007) who showed that attention increases spatial integration, especially at peripheral locations.

The second hypothesis is that the interactions between adjacent V1 neurons in the fovea and the periphery are fundamentally different, with stronger facilitation at the fovea and stronger inhibitory interactions in the periphery (Xing and Heeger, 2000; Zenger-Landolt and Koch, 2001; Petrov et al., 2005). This view is supported by ‘pathfinder’ studies where
subjects have to detect a string of collinearly aligned Gabor elements in a background of elements with random orientations. The efficiency of this path-finding process is much lower in the periphery of the visual field than at the fovea (Hess and Dakin, 1997; Hess and Field, 1999), in accordance with the suggestion that lateral interactions in the periphery and at the fovea have different properties.

In conclusion, our results indicate that the predominant effect of collinear flankers at parafoveal locations is suppressive. Flankers cause an increase of the ongoing activity that scales down the activity evoked by a central target, and these suppressive effects are accompanied by a reduction of contrast sensitivity at the behavioral level. Future neurophysiological studies could compare the flanker effects at foveal locations to those in the periphery to further bridge the gap between the neurophysiology and the psychophysics of interactions between the representations of adjacent image elements in early vision.

**Experimental Procedures**

The experiments were carried out in two labs. The first, single unit experiment was carried out in Newcastle and the experiment where behavioural contrasts sensitivity was measured in combination with multi-unit recording in Amsterdam. We will first describe the methods for single unit recordings and then describe the methods for the multi-unit recordings in combination with the measurement of contrast detection performance.

**Single-cell recordings during visual fixation (Newcastle data)**

All experiments were carried out in accordance with the European Communities Council Directive 1986 (86/609/EEC), the US National Institutes of Health Guidelines for the Care and Use of Animals for Experimental Procedures. The Newcastle experiments were carried out in accordance with the UK Animals Scientific Procedures Act.

We recorded neurons in two male adult rhesus monkeys (Macaca mulatta). After initial training, monkeys were implanted with a head holder, eye coil, and recording chambers above V1 under general anaesthesia and sterile conditions. All details of surgical procedures, postoperative care and the cleaning of the implant and recording chambers have been published elsewhere (Thiele et al., 2006).

Electrophysiological recordings and behavioural procedures

Once monkeys could perform the task reliably, a craniotomy was made above V1. Single-cell discharges were recorded extracellularly using tungsten-in-glass microelectrodes (0.5–
Stimulus presentation and behavioral control was managed by Remote Cortex 5.95 (Laboratory of Neuropsychology, National Institute for Mental Health, Bethesda, MD). Neuronal data was collected by Cheetah data acquisition (Neuralynx, 30-kHz sampling rate) interlinked with Remote Cortex 5.95.

Monkeys were trained to keep fixation (eye window 1.2° in diameter) while a small oriented Gabor was presented in the periphery of their visual field, with or without two collinear flankers (Figure 1). The fixation point (FP, 0.1° diameter) was presented centrally against a grey background (21 cd/m²) on a 20” analogue cathode ray tube monitor (100 Hz, 1,600 x 1,200 pixels, 57 cm from the animal). Eye position was recorded with an infrared based camera system (Thomas Recording GmBH) and sampled at a rate of 250Hz.

A trial started as soon as the monkey’s eye position was within a fixation window centered on the fixation point. After 500 ms of the fixation onset, a set of 4 oriented Gabor stimuli was presented for 700 ms each with 300 ms gaps between presentations. At the end of the four presentations, the fixation point disappeared and monkeys were rewarded if their eye position had been within the fixation window for the trial duration. If the monkey broke fixation before the FP disappeared the condition was repeated later in the block. Twenty trials per stimulus and contrast condition were recorded in most recordings. Cells were excluded if fewer than 10 trials per stimulus and contrast were available.

**Stimuli**

Stimuli consisted of either central Gabor elements presented in isolation or flanked by two iso-oriented flankers. The orientation and spatial frequency of the Gabors matched the neuron’s preference (see below). Each Gabor moved within a Gaussian aperture at 4Hz temporal frequency. The motion was perpendicular to the Gabor’s orientation, and reversed direction at a frequency of 4Hz. Within the sequence of 4 presentations per trial the order of stimulus presentation within a trial and between trials was randomised.

Central and flanker Gabors were identical in all respects except for their contrasts. The contrast of the central Gabor was varied between 0, 4, 8, 12, 16, 24, 32, and 64% (Michelson contrast), while the contrast of the flanking Gabors was fixed at 48%. The distance between the central and flanking Gabors could be 1, 2 or 3-4 times the Gabor wavelength ($\lambda$). The exact wavelength varied slightly with the spatial frequency preference. High spatial frequencies of e.g. 6 cyc/° would result in centre-flanker distances of 0.166° at a distance of 1$\lambda$, whereby the receptive field centre would be filled by flankers. To account for this we used distances of 1.5, 2.8, and 4$\lambda$ for spatial frequencies of $\geq$6 cyc/°, and distances of 1, 2, and
3\lambda for spatial frequencies of <6 cyc/°. The large majority of our neurons preferred spatial frequencies of <=4 cyc/° and only 8 neurons were measured with >=6 cyc/°. We included them in our overall sample and treated them as if the wavelengths had been 1, 2, and 3\lambda, respectively. We also scaled the size of our stimuli, whereby the half width at half height of the Gaussian envelope was 0.3 times the spatial frequency for spatial frequencies of <2 cyc/°, it was 0.4 times the spatial frequency for spatial frequencies between 2 cyc/° and 4 cyc/°, 0.5 times the spatial frequency for spatial frequencies between 4 cyc/°, and 6 cyc/°, and 0.6 times the spatial frequency for spatial frequencies of >=6 cyc/°.

Receptive field characterization
Receptive fields were mapped by presenting a 0.1° black (100% contrast) square at pseudo-random locations on a 10x10 grid (a 1x1° area; 5 repetitions at each location; 100-ms presentation time with 100-ms gaps), while monkeys fixated centrally on the CRT monitor. The mean response at each stimulus location (calculated from 30 to 100 ms after stimulus onset) was determined, and a two-dimensional Gaussian was fitted to the response distribution. The RF centre was taken as the location of the peak of the fitted Gaussian (Roberts et al., 2007). The mean receptive field eccentricity was 3.25° in monkey D and 5.6° in monkey H.

Optimal spatial frequency (in conjunction was orientation and phase) was determined by a reversed correlation technique (DeAngelis et al., 1994). Monkeys fixated centrally on the CRT monitor while 336 circular patches of static sinusoidal gratings (1° diameter) were presented for 60 ms in a pseudo-randomized order. The patches were centered over the minimum response field and varied in orientation (12 orientations, 0-165), spatial frequency (1, 3, 5, 7, 8, 9, 10 cycles per degree) and phase (0, 0.5\pi, 1\pi, 1.5\pi). Responses were averaged in a time window from 30-90ms (60-120ms for neurons with a late onset response) with 5-10 repetitions per stimulus. The stimulus that yielded the peak response was taken to represent the preferred orientation, spatial frequency, and phase of the neuron under study. The obtained parameters were used to determine the spatial frequency and orientation of the central and the flanking Gabors which had identical properties.

Analysis of the physiological data
In a total of 72 cells from two monkeys we tested whether the contrast of the central Gabor or the presence of flankers significantly affected neuronal activity, and whether there was a significant interaction between these factors. We used the response period from 200 to 700ms after stimulus onset for our analysis. Neurons were analysed further if contrast and flanker
presence significantly affected firing rates, or if a significant interaction between contrast and flanker occurred (ANOVA, p<0.05). A total of 67 out of 72 cells (48 from monkey D and 19 from monkey H) passed the basic statistical test (two-factor ANOVA: factor 1, contrast and factor 2, flanker presence).

We quantified the target-evoked response for each contrast level $c$ at three target-flanker separation distances. This was done by subtracting neuronal responses elicited in a period 200-700 ms after stimulus onset by the presence of flankers alone ($R_{\text{Flankers}}$) from the responses when the flankers were presented in conjunction with the target, $R_{\text{Target}}(c) = R_{\text{Target+Flankers}}(c) - R_{\text{Flankers}}$ (after subtraction of the ongoing response, $R_{\text{Baseline}}$, in the absence of any stimulus).

To determine the effect of the flankers on contrast tuning, contrast response functions were obtained for each neuron. Each contrast response function was based on the $R_{\text{Target}}(c)$ to 10-30 repetitions of each contrast and a total of 80-160 stimulus repetitions for each flanker condition. Contrast response functions were fitted for each flanker condition with a hyperbolic ratio function of the following form:

$$R_{\text{target}}(c) = R_{\text{target\_max}} \times \left( \frac{c^n}{[c^n + c_{50}^n]} \right) + M$$

where $R_{\text{target\_max}}$ is the saturated response, $c_{50}$ is the contrast at which the half maximal response is reached, $n$ determines the slope of the contrast response function, and $M$ corresponds to the spontaneous activity. This model provides a good approximation of contrast response functions in monkey visual cortex (Albrecht and Hamilton, 1982; Thiele et al., 2004; Williford and Maunsell, 2006) and we used multidimensional unconstrained nonlinear minimization (Nelder-Mead) to minimize the summed squared difference between data and model (Matlab 7.1, Mathworks).

**Multi-unit recording in the contrast-detection task (Amsterdam)**

All procedures were carried out in accordance with the European Communities Council Directive 1986 (86/609/EEC), complied with the US National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the institutional animal care and use committee of the Royal Netherlands Academy of Arts and Sciences.

**Behavioural task**

The monkeys were trained to detect a small oriented Gabor target presented with or without two collinear flankers (contrast detection task depicted in Figure 5). The monkeys
were trained on this task for 1-2 months until their performance stabilized. The stimuli were presented on a CRT color monitor (21", with a resolution of 1024 × 768, and frame rate of 75 Hz) calibrated (gamma correction) by using a 8-bit RGB lookup table. The stimuli were viewed binocularly from a distance of 75cm from the monitor. The eye position was monitored with the double induction technique (Bour et al., 1984) and sampled at a rate of 900Hz. At the beginning of each trial, a black fixation point (0.2° in monkey G and 0.4° in monkey A) appeared in the central position against a homogenous grey background (luminance 16.3 cd/cm²). In trials with the flankers, two horizontal Gabor elements appeared on the screen at the same time as the fixation point. A trial started as soon as the monkey’s eye position was within a 1.1° × 1.1° window centered on the fixation point. An initial fixation interval of 500ms was followed by a stimulus presentation epoch. During this epoch in half of the trials (target-present trials) a Gabor stimulus of varying contrasts was displayed while in the other half of the trials the target was absent. The stimulus presentation epoch lasted for 200ms and was followed by a second fixation interval of 400ms. At the end of this interval the fixation point disappeared and the monkeys were required to respond by either making an eye movement to a black circle (size: 0.6°) displayed at the location where the Gabor target had been presented if they had seen the target or by maintaining gaze within the fixation window for 500 ms if they had not seen the target. Correct responses were rewarded by a drop of apple juice.

Target-present trials with the various contrasts (in total 50% of trials) and target-absent trials (the other 50%) were randomly interleaved. Trials with and without collinear flankers were presented in separate blocks with blocks alternating between the two conditions. Trials with the three different distances between target and flankers were run on different days with 10-25 blocks of trials per day, each consisting of 60-100 trials. Across all the recording sessions, we collected at least 70 trials for each stimulus condition. To derive the population data (Figure 7B-D) we pooled across different recording sessions where the same stimulus configuration had been used.

The data shown in figure 7A was recorded in separate sessions where the monkeys performed a passive fixation task. After 500 ms of steady fixation, Gabor targets were presented on the screen. Note that unlike in the main experiment, the central target or the flankers appeared at the same time, after 500 ms of fixation. The stimuli disappeared after 200 ms while the monkeys had to maintain fixation for another 400 ms.
Stimuli

Gabor stimuli were Gaussian-windowed, sinusoidal luminance gratings (carrier wavelength $\lambda = 0.44^\circ$; Gaussian $\sigma = 0.2^\circ$ in monkey G and $0.3^\circ$ in monkey A and carrier spatial frequency 2.3 cyc/deg) added to a uniform background. We used six centre contrasts, 0%, 3%, 4%, 6%, 9%, 14% and 20% (Michelson contrast) in monkey G and added a seventh contrast of 60% in monkey A and all contrast levels were presented with equal probability. The flanker contrast was fixed at a value of 13%. The stimuli were placed at the centre of the receptive fields, at an eccentricity of 1.5 degree in monkey G and at 2.5 degree in monkey A. The horizontal axis of the Gaussian envelop was scaled by a factor of 1.5 relative to the vertical axis because this increases the facilitatory effects of flankers on contrast perception in human perception. The orientation of the Gabors was 0 (vertical) or 90 (horizontal) degrees. Target and flanker Gabors were identical in all respects except for their contrasts. The center-to-center distance between target and flankers was 2.8, 3.5 or 4.2$\lambda$ (corresponding to 1.25, 1.55 and 1.85 degrees of visual angle, respectively).

Recording of multi-unit activity in area V1

We used two macaque monkeys for our electrophysiological and behavioural recordings. In a first operation a head holder was implanted and a gold ring was inserted under the conjunctiva of one eye for the measurement of eye position. In a second operation, arrays of 4x5 or 5x5 electrodes (Cyberkinetics Neurotechnology Systems Inc.) were chronically implanted in area V1. The operations were performed under aseptic conditions and general anaesthesia. Details of the surgical procedures and the postoperative care have been described elsewhere (Roelfsema et al., 1998). Extracellular activity was recorded with TDT (Tucker-Davis-Technologies) multi-channel recording equipment. For the detection of multiunit activity (MUA), the signal was amplified, band-pass filtered (300-9000Hz) full-wave rectified, low-pass filtered (< 200Hz) and sampled at a rate of 760Hz. The MUA provides an instantaneous measure of the number and the size of action potentials of neurons in the vicinity of the electrode tip (Super and Roelfsema, 2005).

When the monkeys had fully recovered from the operation, we first mapped the dimensions of the receptive fields by measuring the onset and offset times of the visual response to a slowly moving light bar, for each of the eight movement directions (Super and Roelfsema, 2005). In both monkeys the receptive fields were located at the lower left visual field. The median area of the receptive fields was 0.76 deg$^2$ (range 0.12 deg$^2$ to 2.03 deg$^2$). The median receptive field eccentricity was 2.5° in monkey A (range from 2.1° to 2.83°) and
1° in monkey G (range from 0.87° to 1.87°). We determined the orientation tuning of the recording sites by using the responses to the moving bar stimulus. The responses were averaged in a time window extending from the onset to the offset of the bar. The responses to the bars that had the same orientation but moved in different directions were averaged together. The preferred orientation of a recording site was determined as the orientation that induced the maximum response.

**Analysis of the behavioral data**

As a measure of detection sensitivity, we computed $d'$-prime as $d' = Z(P_{hit}) - Z(P_{FA})$, where $Z(P_{hit})$ denotes the $Z$-transform of the probability of hit and $Z(P_{FA})$ denotes the $Z$-transform of the probability of false-alarm. Psychometric curves (Figure 6B) were generated by computing the $d'$-prime values for the various contrast levels and were fitted by a Weibull function $F(c)$:

$$F(c) = \delta \left[1 - e^{-\left(c/\alpha\right)^\beta}\right]$$

Here $c$ is the contrast level, $F(c)$ is the estimated $d'$-prime for each contrast level and the three free parameters $\delta$, $\alpha$ and $\beta$ correspond to the function’s asymptote, offset and slope, respectively. The contrast thresholds were defined as the contrast level $c$, where $F(c)$ reaches a $d'$-prime value of 1.5 (i.e. a correct rate of ~70% if the false-alarm is about 15%). We used a multidimensional unconstrained nonlinear minimization (Nelder-Mead) algorithm for minimizing the summed squared difference between data and model (Matlab 7.1, Mathworks).

To assess whether flankers significantly affected the behavioral performance, for each measure of behaviour (probability of false-alarm, $d'$-prime, contrast threshold) we generated a bootstrap distribution by resampling the data with replacement ($N=1000$). This procedure was performed for the trials with and without flankers and a distribution of differences between them was computed to determine statistical significance (two-sided test). An effect was considered significant if P-value was < 0.05.

**Analysis of the neuronal activity**

Peri-stimulus time histograms (PSTHs) were calculated in a time window from 500ms before stimulus onset to 600ms thereafter, and were normalized to the peak response ($Pe$) after subtraction of the spontaneous activity ($Sp$). The spontaneous response ($Sp$) was computed as the average response in the 500ms fixation interval prior to the stimulus onset. The peak response ($Pe$) was determined as the maximum evoked response by the central
target (centre only condition) with a contrast of 20% in a time window from 50 to 150ms after the stimulus onset. The average PSTHs and the single trial responses were normalized by first subtracting $S_p$ and dividing the result by the peak response ($P_e - S_p$). We included a recording site in our analysis if the ratio of the maximum stimulus-evoked response ($P_e - S_p$) to the standard-deviation of the spontaneous activity, $\sigma_{S_p}$, reached a criterion value ($> 1$).

Contrast-response functions (Figure 8) were generated by subtracting the responses on target-absent trials from the responses on target-present trials (averaged in a window from 50-350 ms after the stimulus onset). The $R_{\text{target}\_\text{max}}$ and $c50$ values were estimated by fitting a hyperbolic ratio function to the contrast-response function of each recording site, similar to the procedure used for the single cell data.

We computed the neural $d$-prime as:

$$d' = \frac{(\mu_2 - \mu_1)}{\sigma}, \text{ with } \sigma = \sqrt{\frac{(n_1 - 1)\sigma_1^2 + (n_2 - 1)\sigma_2^2}{n_1 + n_2 - 2}}$$

$\mu_2$ and $\mu_1$ represent the mean activity and $\sigma_2$ and $\sigma_1$ denote the standard deviation of the activity in target-present and target-absent trials, respectively.

**Measurement of the latency of the neuronal responses**

We estimated the latency of responses by fitting a function $f(t)$ to the single unit (figure 2A) or multiunit (figure 7A) responses (Roelfsema et al., 2003; Roelfsema et al., 2007). The shape of $f(t)$ was derived from the following two assumptions: (i) the onset of response (or the response modulation) has a gaussian distribution across trials and across neurons, and (ii) a fraction of the response (modulation) dissipates exponentially. These assumptions yield the following two differential equations: $\frac{\partial m_1(t)}{\partial t} = -\alpha m_1(t) + g(t, \mu, \sigma)$ for the dissipating modulation, and $\frac{\partial m_2(t)}{\partial t} = g(t, \mu, \sigma)$ for the non-dissipating modulation. Here, $m_1(t) + m_2(t) = f(t)$ is the total response (modulation), $g(t, \mu, \sigma)$ is a gaussian density with mean $\mu$ and standard deviation $\sigma$, and $\alpha^{-1}$ is the time constant of dissipation. The solution to these equations is the sum of an ex-gaussian and a cumulative gaussian, which was fitted to the response difference:
\[ f(t) = d \cdot \exp(\mu \alpha + 0.5 \sigma^2 \alpha^2 - \alpha t) \cdot G(t, \mu + \sigma^2 \alpha, \sigma) + c \cdot G(t, \mu, \sigma) \]  

Thus, \( f(t) \) is determined by 5 parameters, \( \mu, \sigma, \alpha, c, \) and \( d; \) \( G(t, \mu, \sigma) \) is a cumulative gaussian, and \( c \) and \( d \) are the contributions of non-dissipating and dissipating modulation, respectively. The latency of the visual response (\( \text{lat}_{\text{onset}} \)) was (arbitrarily) defined as the point in time that the fitted function reached 33\% of its maximum (\( \text{lat}_{33} \)). To compute a 95\% confidence interval for the latency of the visual response and of the attentional modulation, we used a bootstrapping procedure. If there are \( N \) recording sites, we randomly selected \( N \) cases with replacement, and determined the latency in the simulated sample using the curve-fitting method described above. We repeated this procedure 10,000 times to estimate the 95\% confidence interval.

Reference List


