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RESEARCH ARTICLE

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GABA-inactivation attenuates colinear facilitation in cat primary visual cortex

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Abstract Neurons in primary visual cortex (V1) respond preferentially to stimuli of a particular orientation falling within a circumscribed region of visual space known as their receptive field (RF). However, the response to an optimally oriented stimulus presented within the RF can be enhanced by the simultaneous presentation of co-oriented, colinearly aligned flank stimuli falling outside the RF which, when presented alone, fail to activate the cell. This type of contextual effect, termed colinear facilitation, presumably forms the physiological substrate for the integration of the line elements of a contour and the perceptual saliency of a contour in a complex environment. Here we show that colinear facilitation in single cells of cat area V1 can be substantially reduced or abolished by focal inactivation of laterally remote cells in the same area which respond strongly to the co-oriented, colinear flank stimulus inducing the facilitatory effect. The results provide evidence that horizontal intrinsic connections between cells with co-oriented and colinearly aligned RFs make a major contribution to colinear facilitation in V1. They imply that the neuronal circuitry underlying contour integration and saliency is already present at the earliest stage of visual cortical information processing.

Keywords Cat V1 · Intrinsic horizontal connections · Focal inactivation · Colinear facilitation · Contour integration

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Introduction

One of the most important tasks the visual system has to perform is to integrate the contour elements of which object boundaries are composed. The integration of an object's component contours into a unified percept makes the object cohere and stand out from its background. Human psychophysical and visual-evoked potential (VEP) studies have demonstrated that the visibility of an oriented stimulus can be markedly enhanced by the simultaneous presentation of flanking oriented stimuli when the target and flanks are positioned close together and are approximately co-oriented and colinearly aligned (Polat and Sagi 1993, 1994; Kapadia et al. 1995; Polat and Norcia 1996; Polat 1999). These effects obey the Gestalt rules of proximity, similarity and continuity that govern the perceptual grouping of contour elements (Wertheimer 1938) and their stimulus dependency is reminiscent of the conditions required for the detection of chains of contour elements embedded in a background field of randomly oriented elements (Field et al. 1993; Polat 1999; Hess and Field 2000). Thus, colinear facilitatory interactions probably mediate the integration of line elements of a contour and serve to enhance the perceptual saliency of contours in a complex environment. Analogous effects to those observed in psychophysical and VEP studies are seen at the level of single cells in cat and monkey V1. A cell's response to a low-contrast, optimally oriented stimulus lying within the RF can be facilitated by the simultaneous presentation of nearby, co-oriented, colinear flank stimuli of high contrast outside the RF (Kapadia et al. 1995; Polat et al. 1998; Mizobe et al. 2001). Similarly, the response to an optimally oriented line presented within the RF can be suppressed when the line is embedded in a background field of randomly oriented line segments, but this suppression is eliminated when some of the segments lying outside the RF are colinearly aligned with the segment within the RF (Kapadia et al. 1995). Thus, the cellular basis for both contour integration and saliency may be found in facilitatory effects from outside the RF in V1. The neuronal

circuitry underlying these effects is not known. They may arise via long-range horizontal excitatory connections within V1 linking cells with co-oriented and colinearly aligned RFs (Ts'o et al. 1986; Gilbert and Wiesel 1989; Bosking et al. 1997; Kisvárday et al. 1997; Schmidt et al. 1997). Alternatively, they may be mediated via feedback projections from higher cortical areas to V1, some of which connect cells with disparate RFs (Nelson et al. 1992; Salin et al. 1993). Here we have explored the contribution of intrinsic horizontal connections to colinear facilitation in cat area V1 by eliciting facilitatory effects in single cells and then using microiontophoresis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) to focally inactivate laterally remote cells in the same area whose RF location and orientation preference corresponded with the location and orientation of the flank stimulus inducing the facilitation. Some of the results have been reported previously in abstract form (Crook et al. 2000).

Materials and methods

Data were obtained from five anaesthetized (70:30% N_2O:O_2+ 0.4–0.6% halothane) and paralysed (0.06 mg/kg/h alcuronium chloride) adult cats which had been prepared acutely using standard procedures (Crook et al. 1991; Crook and Eysel 1992; Kisvárday et al. 2000). The EEG, ECG, pulse rate, end-tidal CO_2 (3.5–4.0%) and body temperature (near 38°C) were monitored continuously as indicators of anaesthetic efficacy. The experiments were performed in accordance with the German law for the protection of experimental animals, which conforms with the corresponding NIH regulations.

The GABA-inactivation paradigm employed in these experiments has been described in detail elsewhere (Crook and Eysel 1992; Crook et al. 1996; Kisvárday et al. 2000; reviewed in Crook et al. 2002). Single units were recorded with micropipettes filled with 3M NaCl (tip diameter 1–2 μm; impedance 1–4 MOhm). An independently driven double-barrel pipette (tip diameter 10–20 μm) was used for local inactivation. One of the barrels contained GABA (0.5 M, pH 3.0) and the other was filled with 3M NaCl to allow recording of multiunit activity. A retaining current (–15 nA) was applied to the GABA-containing barrel. The retaining current and ejecting currents were controlled by an Ionophor-3 iontophoresis unit (Science Products). To achieve cortical stability, a Perspex recording chamber (4×3 mm diameter) whose base was covered with transparent elastic foil was lowered under micromanipulator control into a craniotomy over the central representation of V1 until the foil made contact with the exposed pial surface. Penetrations for recording and inactivation, spaced ~2 mm apart, were made through the transparent foil, approximately normal to the cortical surface, on the crown of the lateral gyrus 3–6 mm posterior to stereotaxic zero. The location of each penetration was marked on an enlarged photograph of the exposed cortical surface, using the branching pattern of blood vessels as landmarks.

Following penetration of the cortical surface with both pipettes, multiunit activity was recorded via the inactivation pipette, its orientation and direction selectivity was determined quantitatively and it was verified that the response to a high-contrast, optimally oriented short moving bar presented within the mapped RF could be reversibly abolished by iontophoresis of GABA. These tests were performed for each monocular input at typically three to four velocities spanning a tenfold range. Thereafter, a single cell was recorded extracellularly, its orientation and direction selectivity determined quantitatively and its RF mapped and classified (Hubel and Wiesel 1962) using both stationary flashed and moving stimuli. Recording and inactivation sites were restricted to the superficial 500 μm of cortex. RFs were mapped as 'minimum response fields' (Barlow et al. 1967): Lateral limits were determined with a long bar of optimal orientation, swept back-and-forth through the RF: these limits were plotted as lines parallel to the bar, at the extreme locations of response as the bar entered and left the RF. RF ends, plotted as lines orthogonal to bar orientation, were defined by systematically varying the trajectory of a short bar through the RF until the response was barely audible. Mapped this way, the RFs at the recording and inactivation sites were always non-overlapping (centre-to-centre spacing: 1.7°–2.9°). This report is concerned only with cases in which the RFs at the two sites were co-oriented (±22°) and approximately colinearly aligned (relative displacement of <0.25° along the axis orthogonal to colinearity). The RFs of recorded cells were located within 7° of the area centralis projection.

To test for contextual influences, we first determined a cell's response to a short, optimally oriented light bar (1.0×0.25°–1.5×0.5°) moving back-and-forth across the RF (test bar) and then compared this response with that evoked by the same bar moving in synchrony with a second light bar of similar dimensions in the surround (flank bar) which was optimally oriented for the inactivation site and presented within its RF. The length of the bars approximated (but did not exceed) the length of each RF. Test and flank bars were moved coherently in both directions along the axis orthogonal to their orientation, with the same phase, amplitude and velocity. Stimulus velocity matched one of the velocities used for quantitative tests at the inactivation site and approximated the preferred velocity of each cell. Velocity was varied by keeping amplitude of motion constant and changing cycle duration. Sweep amplitude always exceeded the width of the wider of the two RFs (usually that at the inactivation site). Stimuli were presented against a background of 1.3 cd/m^2 luminance. The contrast of the flank bar was fixed at 80% $[(L_{max}-L_{min})/(L_{max}+L_{min})]$. The test bar was generally presented at low contrast (8–16%) to yield relatively weak responses, in an attempt to maximize flank-induced facilitation (Kapadia et al. 1995; Polat et al. 1998; Mizobe et al. 2001). However, a higher contrast stimulus (maximum 50%) was used for those cells in which a low-contrast stimulus was inadequate to evoke a consistent response. If presentation of the flank bar caused an obvious enhancement of the response to the test bar, we repeatedly determined the response to the paired presentation of the two stimuli during continuous iontophoresis of GABA (100–200 nA ejecting current) at the inactivation site and following termination of GABA application. In all cases, we verified that the presentation of the flank bar alone did not evoke a significant response in a recorded cell and that cells at the inactivation site did not respond to the test bar. For many of the cells which showed a depressive influence of GABA-inactivation on colinear facilitation, we also determined whether GABA application had an effect on the response to the test bar presented alone. However, in some cases a cell was lost before this test could be performed. Stimuli were always presented monocularly to the dominant eye. Peri-stimulus-time histograms (PSTHs) for a recorded cell were derived from 8–16 stimulus repetitions, with a constant number of stimulus presentations being used in a given cell for all stimulus/experimental conditions.

Stimuli were generated by a 'Leonardo' visual stimulus generator, presented on an Iiyama Vision Master 502 21-inch monitor (refresh rate 85 Hz; resolution 800×600 pixels), and viewed via corrective corneal contact lenses with 3-mm-diameter artificial pupils, at a distance of 57 cm. Data acquisition and analysis were performed using Spike2 software (Cambridge Electronic Design, CED). Stimulus presentation and data acquisition were controlled by a CED-1401-Plus Interface.

Results

The essential result is illustrated in Fig. 1, which documents a dramatic effect of GABA-inactivation on the response facilitation induced by a co-oriented, colinear flank bar in a complex cell. The cell was recorded

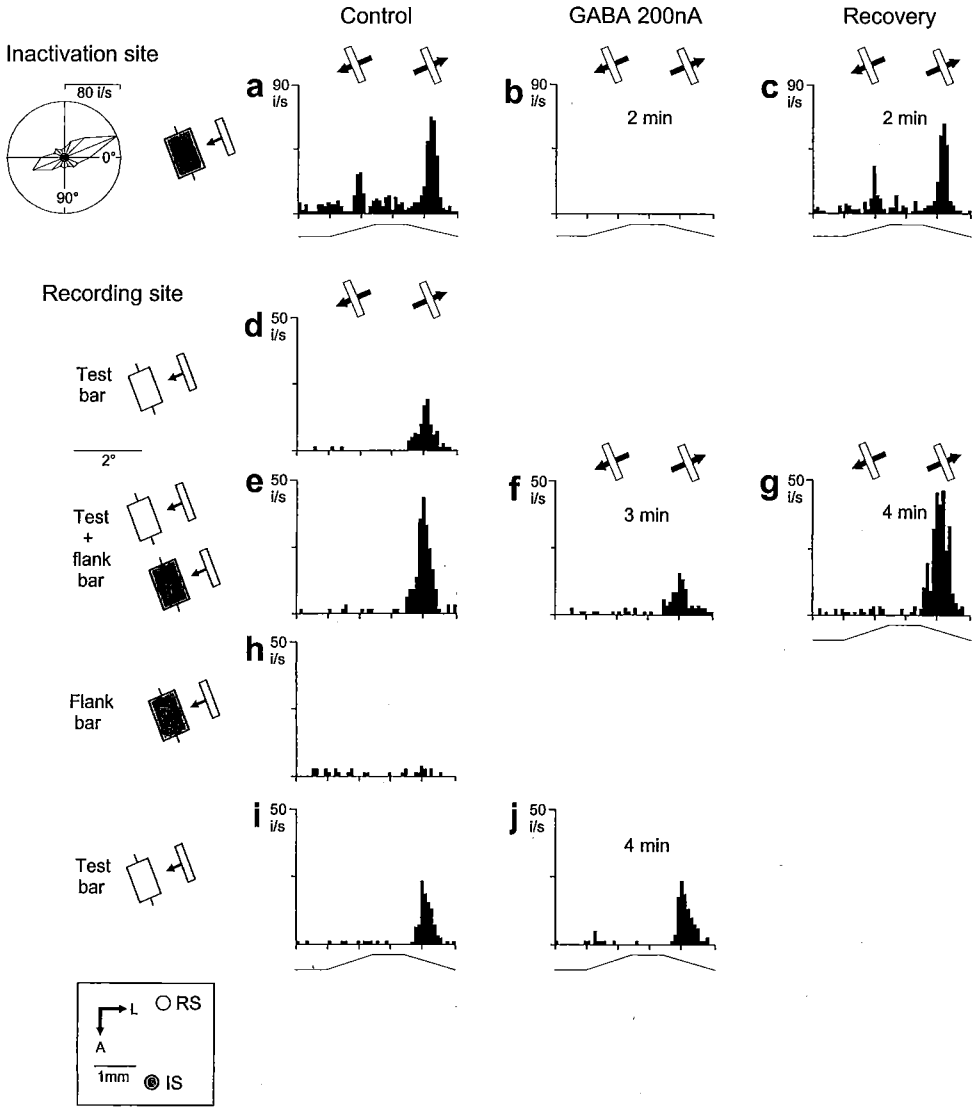

Fig. 1a–j Influence of GABA-inactivation on the response facilitation induced by a co-oriented, colinear flank bar. *Inset (bottom left):* topographical location of the inactivation and recording sites (IS and RS; *shaded and open circles*) and their horizontal separation (*A* anterior, *L* lateral). Polar diagram (*top left*) shows the orientation/direction selectivity of multiunit activity recorded at the inactivation site for a moving light bar. Direction of motion (orthogonal to bar orientation) is plotted as vector angle and response as vector length. **a** Multiunit response to opposite directions of motion of a high-contrast (50%), optimally oriented light bar presented within the RF (*shaded rectangle*; adjoining lines indicate axis orientation). **b** Abolition of visually driven and spontaneous activity during GABA-iontophoresis at the inactivation site. **c** Recovery following termination of GABA application. Bar orientation and direction of motion indicated above, stimulus waveform below each peri-stimulus-time histogram (PSTH). **d–j** PSTHs showing the responses of a complex cell to the stimulus configurations indicated schematically on the left. Conventions as in **a–c**. **d** Response to opposite directions of motion of an optimally oriented light bar (test bar; 30% contrast) presented within the RF (*open rectangle*). **e** Response to the coherent motion of the test bar and a high-contrast (80%) co-oriented, colinear flank bar (test + flank bar), which was optimally oriented for the inactivation site and presented within its RF. Simultaneous presentation of the flank bar caused a marked enhancement of the response to the test bar (colinear facilitation). **f** Abolition of colinear facilitation during GABA-iontophoresis at the inactivation site. **g** Recovery following termination of GABA application. **h** When presented alone, the flank bar evoked no significant response. **i** Re-determination of the response to the test bar alone. **j** Comparable response to the test bar during GABA-iontophoresis at the inactivation site. Time following onset and offset of GABA-iontophoresis indicated within PSTHs *in centre and right columns*. Polar diagram derived from four stimulus presentations per orientation; orientation varied pseudorandomly. PSTHs in **a–c** derived from 8, those in **d–j** from 16 stimulus repetitions. Throughout, iontophoretic ejecting current 200nA, PSTH bin width 100 ms, stimulus velocity 2.7°/s and cycle duration 5 s. All data derived for contralateral eye

~2 mm posterior to the inactivation site (see inset, bottom left). As shown in the schematic to the left of Fig. 1E, this meant that the RFs at the recording and inactivation sites (open and shaded rectangles) were displaced vertically in the visual field, with the RF of the recorded cell located above that at the inactivation site. The two RFs also showed a slight horizontal displacement. They were co-oriented and aligned along an axis in visual space that corresponded with their axis orientation (indicated by short lines adjoining each rectangle).

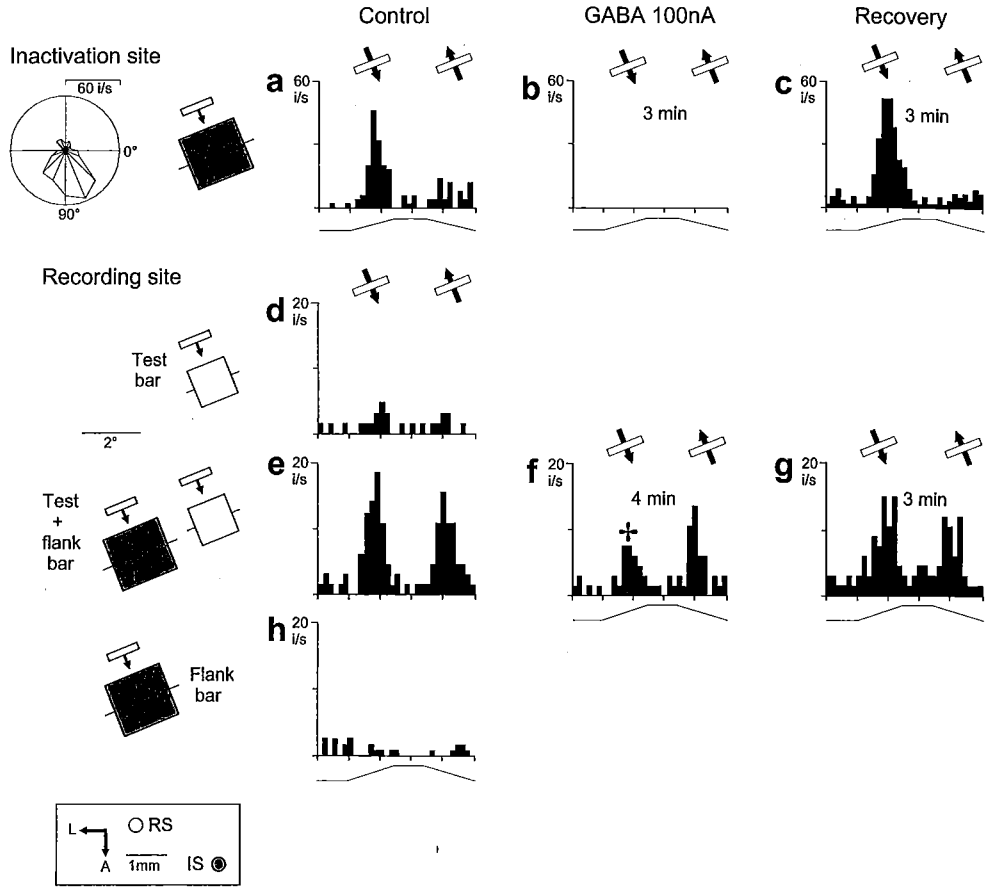

Fig. 2a–h Complex cell showing a predictable, direction-specific decrease in colinear facilitation during GABA-inactivation. Conventions, derivation and layout as in Fig. 1. PSTH bin widths, stimulus velocity and cycle duration: 80 ms, 4°/s and 2.5 s, throughout. Contrast of test and flank bars: 8% and 80%. *Asterisk* in **f** highlights the fact that the GABA-induced attenuation of colinear facilitation was greater for the direction of motion preferred at the inactivation site than for the opposite direction

As can be seen in the polar diagram (top left), multiunit activity recorded at the inactivation site was sharply tuned for orientation and showed a strong direction preference for motion of an optimally oriented bar. GABA-induced reversible abolition of the multiunit response to a short, optimally oriented moving bar of high contrast presented within the RF is documented in Fig. 1A–C, with bar orientation and direction of motion indicated above each PSTH. The recorded cell responded selectively, cells at the inactivation site preferentially to the same direction of motion (cf. polar diagram and Fig. 1A with Fig. 1D). The cell's response to an optimally oriented bar (30% contrast) moving in the preferred direction and presented within the RF (test bar) was markedly enhanced by the simultaneous, in-phase motion of a high-contrast (80%) co-oriented, colinear flank bar (test + flank bar) which was presented within the RF of the inactivation site (cf. Fig. 1D with Fig. 1E). Note that motion of the test + flank combination stimulus in the cell's non-preferred direction did not evoke a significant response. Thus, flank-induced response enhancement occurred without a degradation of direction selectivity. This was a consistent result in cells showing direction selectivity or strong direction-bias, which comprised the vast majority of our sample. Iontophoresis of GABA at the inactivation site (Fig. 1B) abolished response enhancement (Fig. 1F), with the cell now showing a response to the coherent motion of test + flank bars which was of comparable magnitude to that evoked by motion of the test bar alone (cf. Fig. 1F with Fig. 1D). The time course of this effect (3 min) closely paralleled that for the abolition of multiunit activity at the inactivation site (2 min) (cf. Fig. 1F with Fig. 1B). The effect was reversible, response enhancement returning to the control level with a similar time course to that for the recovery of multiunit activity at the inactivation site (cf. Fig. 1G with Fig. 1E and Fig. 1C). When presented alone, the flank bar did not evoke a significant response in the recorded cell (Fig. 1H), indicating that it had a highly non-linear facilitatory effect on the response to the test bar. In cases in which GABA inactivation had a depressive influence on colinear facilitation, an important control was to determine whether it also had an effect on the response to the test bar presented alone. We found that this was not the case, as is documented in Fig. 1I–J. Iontophoresis of GABA with an ejecting current and duration of application which was sufficient to abolish colinear facilitation left the response to the test bar essentially unchanged (cf. Fig. 1J with Fig. 1I and Fig. 1F). This rules out a potential GABA-induced decrease in response to the test bar as a factor contributing to the abolition of colinear facilitation.

In the majority of cases, the recorded cell and cells at the inactivation site were direction-selective or showed strong direction-bias. The results presented in Fig. 1 are representative of those observed in cases where cells at

the recording and inactivation sites showed the same direction preference. However, GABA-inactivation could still cause an attenuation of flank-induced facilitation when cells at the two sites showed strong direction-bias for opposite directions of motion (data not shown), although longer durations of GABA application were generally required to elicit these effects. Exceptionally, when only the inactivation site showed strong direction-bias, a direction-specific decrease in flank-induced facilitation could be observed. An example is shown in Fig. 2. The recorded complex cell and cells at the inactivation site had co-oriented and approximately colinearly aligned RFs (see schematic to the left of Fig. 2E). When presented alone, the test bar (8% contrast) evoked a response which was barely above threshold (Fig. 2D). Simultaneous motion of a high-contrast (80%) co-oriented, colinear flank bar which was presented within the RF of the inactivation site induced a massive facilitation of the response to the test bar (cf. Fig. 2D, E, and H). Iontophoresis of GABA at the inactivation site reversibly reduced (but did not abolish) this effect (Fig. 2F,G). In this case, the recorded cell showed a response of comparable magnitude to opposite directions of motion of the test + flank combination stimulus in the control situation (Fig. 2E), whereas the inactivation site showed strong direction-bias (see polar diagram and Fig. 2A). Note that the effect of GABA-inactivation was predictable in the sense that the attenuation of colinear facilitation was greater for the direction of motion preferred at the inactivation site (see asterisk in Fig. 2F) than for the opposite direction.

A quantitative analysis of the effect of GABA-inactivation on colinear facilitation is presented in Fig. 3. Of 49 recorded cells (40 complex, 9 simple cells), 34 (69%) showed significant flank-induced facilitation. The mean increase in response for these cells was 160% (±13% SEM), with values ranging from 52% to 300%. The flank bar was without significant influence in the other 15 cells. Six of the 34 cells which showed significant flank-induced facilitation were lost before the effect of GABA-inactivation could be tested. As shown in Fig. 3A, 20 (71%) of the remaining 28 cells (3/5 simple cells and 17/23 complex cells) showed a significant attenuation of flank-induced facilitatory effects during GABA-inactivation (filled diamonds below the diagonal line). In 11 of these, facilitation was abolished (filled diamonds closest to the horizontal line). In these cells, there was no statistically significant difference between the response evoked by the test bar presented alone in the control situation and the simultaneous presentation of test + flank bars during GABA iontophoresis. Note that this also held for the three cells in which the response to the combination stimulus during GABA application was slightly lower than that to the test bar alone (filled diamonds below horizontal line).

The population responses for cells which showed a significant effect of GABA-inactivation on flank-induced facilitation are presented in Fig. 3B. The mean firing rate (impulses/s) of each cell was calculated for each stimulus/experimental condition, and these responses were averaged over all cells in the sample. It can be seen that the test bar, when presented alone, elicited a weak response, reflecting the fact that it was typically presented at low contrast. The high-contrast flank bar evoked a negligible response when presented alone, but

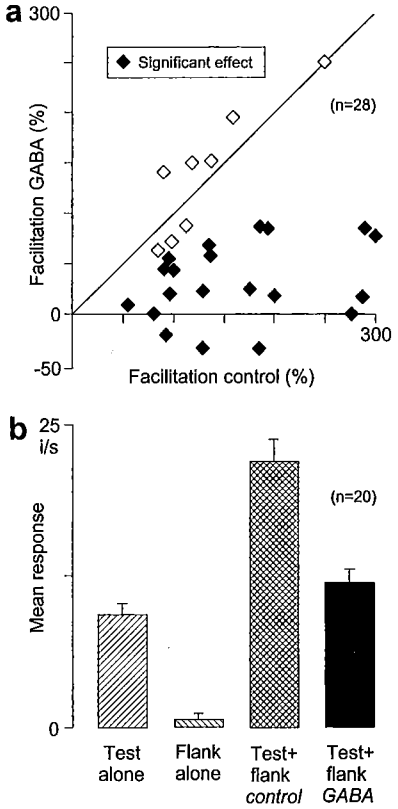

Fig. 3a, b Quantitative analysis of the effect of GABA-inactivation on flank-induced facilitation. Comparisons are for each cell's preferred direction of motion for the test bar presented alone. Responses were quantified as mean firing rate (impulses/s) minus mean spontaneous activity, which was derived from the period preceding each stimulus presentation (see stimulus waveform below PSTHs in Figs. 1, 2). The statistical significance of differences in mean responses across different stimulus/experimental conditions was assessed using the Kolmogorov-Smirnov test ($P<0.05$). **a** For cells in which the response to the simultaneous motion of test+flank bars was significantly greater than that to the test bar presented alone, response facilitation [100×(test + flank response – test response)/test response] prior to GABA inactivation (*abscissa*) is plotted against that during GABA-inactivation (*ordinate*). *Filled diamonds* represent statistically significant GABA-induced effects which always consisted of either an attenuation or abolition of flank-induced facilitation (values *below diagonal line*), and which were always followed by a recovery within the criterion for statistical significance following termination of GABA-iontophoresis. Negative facilitation (*filled diamonds below horizontal line*) denotes a response to the test + flank combination stimulus during GABA application which was smaller than that evoked by the test bar presented alone in the control situation. In no case was this difference in response magnitude statistically significant. **b** Population responses of cells which showed a significant effect of GABA-inactivation on flank-induced facilitation. Histograms plot the averaged responses of all cells for each stimulus/experimental condition indicated below. *Vertical lines*: standard errors of these mean responses (drawn in one direction only)

it induced a 136% increase in the mean response to the test bar when the two bars were presented simultaneously in the control situation. This emphasizes that the flank bar had a highly non-linear, facilitatory influence on the response to the test bar. Flank-induced facilitation was reduced to just 30% during GABA-inactivation. Importantly, in none of the nine cells for which direct comparisons were made did GABA-iontophoresis at the inactivation site significantly influence the response to the test bar presented in isolation (mean responses ± SEM before and during GABA-inactivation: 10.0±2.0 and 9.7±1.3 i/s). Thus, a potential GABA-induced decrease in response to the test bar can be essentially excluded as a factor contributing to the attenuation of colinear facilitation.

Discussion

In the present study, we have demonstrated that colinear facilitatory effects elicited in single cells of cat area V1 can be severely attenuated or abolished by focal inactivation of laterally remote cells in the same area whose RF location and orientation preference correspond with the location and orientation of the flank stimulus inducing the facilitation. Iontophoresis of GABA with ejecting currents and durations of application which were sufficient to abolish multiunit activity at the inactivation site inactivates a region of cortex ~300–400 μm in diameter (Crook et al. 1998; Hupé et al. 1999). In cat V1, cells with similar orientation preferences are organized in iso-orientation domains which extend a few hundred microns parallel to the cortical surface and throughout the depth of the cortex (Hubel and Wiesel 1962; Bonhoeffer et al. 1995). Superficial-layer cells located within the same iso-orientation domain show largely superimposed RFs (Das and Gilbert 1997). Our applications of GABA thus inactivated large populations of cells whose orientation preference and RF location corresponded approximately with that recorded at the inactivation site and which therefore responded strongly to the flank bar inducing facilitatory effects in a recorded cell. GABA-inactivation attenuated or abolished facilitation induced by the flank bar without significantly influencing the response to the test bar. These GABA-induced depressive effects are thus commensurate with a loss of excitatory interactions between cells with approximately co-oriented, colinearly aligned RFs which normally contributed to flank-induced facilitation in a recorded cell.

The anatomical substrate for the GABA-induced effects lies in the plexus of long-range horizontal connections within the superficial layers of V1. The horizontal distance between the recording and inactivation sites (~2 mm) is well within the lateral extent (3–4 mm) of superficial-layer excitatory projections (Gilbert and Wiesel 1983; Martin and Whitteridge 1984; Kisvárday et al. 1997). Long-range (≥2 mm) lateral projections in the superficial layers derive exclusively from pyramidal (excitatory) cells and they make contact predominantly with other pyramidal cells (Gilbert and Wiesel 1983; Martin and Whitteridge 1984; Kisvárday et al. 1986, 1997). These projections preferentially link cells with similar orientation preferences and with RFs which are topographically aligned along an axis of colinearity (Ts'o et al. 1986; Gilbert and Wiesel 1989; Bosking et al. 1997; Kisvárday et al. 1997; Schmidt et al. 1997). Responses elicited within the RF result from intracortical amplification of the thalamocortical input via dense local recurrent excitatory connections (Hubel and Wiesel 1962; Douglas et al. 1995). Compared with local intracortical connections, long-range lateral projections are relatively weak, explaining why they are unable to elicit suprathreshold responses in their target cells (Gilbert and Wiesel 1983; Martin and Whitteridge 1984; Kisvárday et al. 1986, 1997; Hirsch and Gilbert 1991). However, in vitro intracellular studies (Hirsch and Gilbert 1991; Yoshimura et al. 2000) have demonstrated that electrical stimulation of superficial-layer lateral projections in cat V1 elicits non-linear facilitatory effects in target pyramidal cells when the cell's membrane is depolarized, as would be the case when a visual stimulus is presented inside the RF. Moreover, in vivo optical imaging (Das and Gilbert 1995) and intracellular (Bringuier et al. 1999) studies in cat V1 have provided strong evidence that horizontal intrinsic connections mediate iso-orientation integration of subthreshold excitation originating outside a cell's RF. Thus, the most direct interpretation of the GABA-induced depressive effects is that they were due to the inactivation of monosynaptic excitatory inputs from cells in the vicinity of the inactivation site to the recorded cells. However, they may have additionally reflected a reduction in the strength of colinear facilitation in populations of cells in the vicinity of the recording site which had recurrent excitatory connections with each other (Douglas et al. 1995) and a recorded cell. Both of these explanations involve a loss of laterally directed excitatory interactions between cells in the superficial layers of V1 with co-oriented and colinearly aligned RFs. The present results bear some resemblance to those of Bolz and Gilbert (1989), who reported that in layer VI cells of cat V1, the extent of conventional length summation for a *single* bar stimulus along the RF axis orientation could be reduced by GABA-inactivation of cells in layer V with co-oriented and colinearly aligned RFs.

As in previous studies of colinear interactions in cat V1 (Polat et al. 1998; Mizobe et al. 2001), we mapped RFs as classical minimum response fields, that is by delimiting the area of visual space that elicits spike discharges. Mapped this way, the RFs at the recording and inactivation sites were always non-overlapping. The influences we have revealed must therefore have derived from outside a cell's classical RF and involved interactions between cells whose RFs were non-overlapping. We are, of course, aware that the minimum response field method may underestimate RF length as assessed by length summatory behaviour (Kato et al. 1978; Hammond and Ahmed 1985; Crook 1990) and that, in some cases, the flank bar may have been placed within a

cell's length summation zone. The extent to which colinear facilitatory effects can be explained on the basis of conventional length summatory behaviour remains to be determined. In monkey V1, it was initially reported that facilitatory effects can be induced by the presentation of colinear flank stimuli in regions beyond the length summation zone (Kapadia et al. 1995). Subsequently, however, it was demonstrated that length summation is contrast dependent, being far more extensive for low-contrast than for high-contrast stimuli (Kapadia et al. 1999; Sceniak et al. 1999). This introduces the possibility that colinear facilitatory effects, which occur most frequently when stimuli of low contrast are presented at the receptive field centre (Kapadia et al. 1995; Polat et al. 1998; Mizobe et al. 2001), reflect placement of flank stimuli within a cell's length summating zone for low-contrast stimuli. We are currently investigating this issue.

Previous psychophysical (Polat and Sagi 1993, 1994; Kapadia et al. 1995), VEP (Polat and Norcia 1996) and single-unit (Kapadia et al. 1995; Polat et al. 1998) studies have demonstrated colinear facilitatory interactions using stationary test and flank stimuli (bars or gratings). Here we have shown that colinear facilitation can be elicited with coherently moving stimuli, conforming to the Gestalt perceptual grouping criterion of common fate (Wertheimer 1938). These effects would seem to require excitatory connections between cells with similar orientation *and* direction preferences. Iso-orientation domains are typically subdivided into regions preferring opposite directions of motion (Weliky et al. 1995; Shmuel and Grinvald 1996), and there is evidence that lateral excitatory connections between visuotopically non-corresponding locations in V1 preferentially link regions of similar direction preference (Roerig and Kao 1999). Direct inactivation of such projections could account for the attenuation of colinear facilitation in cases where the recording and inactivation sites showed the same direction preference (Figs. 1, 2). The effects that occurred when the two sites showed opposite direction preferences, which generally required longer durations of GABA application, presumably reflected diffusion of the drug to an adjacent iso-direction domain whose direction preference was opposite that recorded at the inactivation site.

It has been variously argued that facilitatory contextual effects in V1 are mediated via intrinsic lateral interactions or via feedback pathways from higher-order cortical areas. Until now, there has been no direct evidence to support either of these hypotheses. The high occurrence and large magnitude of the GABA-induced effects reported here provide evidence that intrinsic horizontal connections make a major contribution to colinear facilitatory effects in V1. Facilitation via long-range horizontal connections in V1 could enhance the neural activity related to stimulus elements that form smooth contours, resulting in increased saliency of these elements relative to other features in the surround. Indirect evidence for the involvement of intrinsic connections in colinear facilitatory effects derives from a recent intracellular study in cat V1 which demonstrated the presence of a large visually evoked subthreshold depolarizing field extending over a much larger area than that within which action potentials could be elicited (Bringuier et al. 1999). This depolarizing field presumably underlies facilitatory surround effects in V1, including colinear facilitation. It was concluded from the latency of the depolarizing responses that they result from the integration of activity relayed by slowly conducting horizontal axons within V1 rather than via feedback projections from extrastriate areas (Bringuier et al. 1999). The present results are also consistent with those of a study in V1 of the alert monkey (Ito and Gilbert 1999), which suggest that feedback projections from extrastriate areas regulate the efficacy of intrinsic horizontal connections mediating colinear facilitation depending on attentional state. Our results to date do not, however, exclude a direct contribution of feedback projections to colinear facilitation in V1. Feedback projections may have made a major contribution to the facilitatory effects observed in the minority of recorded cells which were not significantly influenced by GABA-inactivation (open diamonds in Fig. 3A). Additionally, in the present study the recording and inactivation sites were intentionally confined to the superficial layers where the orientation and topographic specificity of intrinsic horizontal connections is most pronounced. Colinear facilitation has, however, been demonstrated in deep-layer cells of cat V1 (Mizobe et al. 2001), and feedback projections may play a role in these effects. Clearly, additional experiments comparing the effect of inactivating topographically corresponding sites in V1 and multiple extrastriate areas are required to assess the relative contribution of intrinsic connections and feedback pathways to colinear facilitation in V1. The important implication of the present results, however, is that the neuronal circuitry underlying perceptual phenomena normally associated with 'higher-level' vision, such as contour integration and saliency, is already present at the earliest stage of visual cortical information processing.

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