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LATERAL INTERACTIONS AT DIRECTION-SELECTIVE STRIATE NEURONES IN THE CAT DEMONSTRATED BY LOCAL CORTICAL INACTIVATION

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SUMMARY

1. Single neurones were recorded with glass-coated tungsten electrodes from area 17 of the cat's visual cortex. The cats were anaesthetized and artificially respirated with a mixture of halothane, nitrous oxide and oxygen.

2. For local cortical inactivation a multibarrel pipette was placed 0·5–2·5 mm posterior (or anterior) to the recording site, at a depth of 400–600 μm. Four separate barrels of the pipette were filled with γ-aminobutyric acid (GABA); the fifth was filled with Pontamine Sky Blue for labelling of the centre of the inactivation site.

3. Direction-selective cells, of differing optimal orientations and preferred directions of motion, were classified as simple or complex and tested with computer-controlled stimuli presented on an oscilloscope.

4. During continuous recording GABA was microionophoretically applied for different durations and with different ejection currents. The effectiveness of GABA microionophoresis was evident from the direct GABAergic effects (strong overall inhibition of the recorded cells) observed with high ejection currents and prolonged application.

5. Two discrete effects could be observed during local inactivation distant from the cortical cell under study: an increase of the response in either the non-preferred or the preferred direction; or a decrease of the response in the preferred direction. All GABA-induced changes were reversible.

6. The depressant action of GABA was independent of the relative topography between recording and inactivation site and affected mainly the response to the preferred direction of stimulus motion.

7. Disinhibition was only observed when the stimulus-evoked response moved on the cortical map in a direction from the GABA pipette towards the recording electrode. It is concluded that GABA reversibly silences inhibitory interneurones that are situated in the vicinity of the micropipette tip and are involved in generation of direction selectivity.

8. No fundamental differences between cells from different cortical layers were observed. The disinhibitory effects of GABA inactivation were more pronounced and

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more frequently seen in simple cells (61%) than in complex cells (38%), while the opposite was true for reduced excitation during lateral GABA inactivation (observed in 62% of the complex *vs.* 39% of the simple cells). Accordingly, lateral inhibition statistically prevails in simple cells and lateral excitation in complex cells.

9. Among the inhibitory and excitatory mechanisms affected by lateral GABA inactivation, inhibition is organized with a higher topographic specificity. It is suggested that the inhibitory elements involved in direction selectivity are spatially directed and located in or activated via the upper cortical layers.

INTRODUCTION

Direction specificity is one of the earliest described characteristic properties of visual cortical cells (Hubel & Wiesel, 1962). Such cells display a strong preference for the direction of motion of an optimally oriented stimulus. This property is absent or only weakly present amongst geniculocortical afferents, but is strongly expressed by cortical cells in layers receiving direct geniculate input (layers IV and VI). Therefore, directionality must be generated at an early stage of intracortical processing.

Postsynaptic inhibition has been suggested as the underlying mechanism for direction selectivity of visual cortical cells (Benevento, Creutzfeldt & Kuhnt, 1972; Innocenti & Fiore, 1974). This has been supported by the evidence that blockade of GABAergic inhibition abolished direction specificity in many cells (Sillito, 1977). Several models based on intracortical inhibition have been suggested (Sillito, 1979; Barlow, 1981; Ganz & Felder, 1984; Koch & Poggio, 1985) but excitatory convergence has also been proposed as a source of direction selectivity in complex cells (Movshon, Thompson & Tolhurst, 1978).

It has been shown that direction selectivity is not dependent on the spatial arrangement of 'on' and 'off' subregions (Ganz & Felder, 1984; Peterhans, Bishop & Camarda, 1985). Detailed physiological studies (Goodwin, Henry & Bishop, 1975; Emerson & Gerstein, 1977; Ganz & Felder, 1984) have shown inhibitory effects in direction selectivity and imputed the presence of a mechanism within the static discharge region with a small lateral spread of between 0·125 and 0·5 deg in receptive fields within 5–10 deg of the visual axis. Incidental observations indicate the possibility of suppressive regions outside the classical discharge region of a direction-selective cell (Glezer, Tsherbach, Gauselman & Bondarko, 1982).

The intracortical mechanisms and pathways involved in direction selectivity are far from clear. Detailed analyses have provided information about correlations between cell types and cellular morphology (Gilbert & Wiesel, 1979; Martin & Whitteridge, 1984) and vertical and horizontal excitatory connections have been demonstrated with anatomical and physiological methods (Ferster & Lindström, 1983; Gilbert, 1983, 1985; Ts'o, Gilbert & Wiesel, 1986). Seven different types of inhibitory, GABAergic interneurones have meanwhile been distinguished in the cat's visual cortex (Somogyi, Kisvarday, Martin & Whitteridge, 1983; Somogyi, 1986) and the functional significance of GABAergic inhibition in the cortex has been clearly demonstrated (Sillito, 1975, 1977, 1979). However, experimental evidence directly assessing topography and function of connections between visual cortical neurones is rare. With local GABA inactivation of layer VI Bolz & Gilbert (1986) have shown

loss of end-inhibition in layer IV cells, which might be related to inhibitory connections demonstrated between layer VI and layer IV cells (McGuire, Hornung, Gilbert & Wiesel, 1984). Direction selectivity and orientation specificity were apparently not mediated by this pathway. In our first approach to studying intracortical mechanisms of direction selectivity, we have applied local heat lesions to the upper cortical layers and abolished direction selectivity in single area 17 simple cells by inactivation of lateral inhibition (Eysel & Wörgötter, 1986).

The present experiments have applied GABA microionophoresis in the upper layers of area 17, in order reversibly to induce local cortical inactivation posterior or anterior to the recording sites and thereby to silence the cortical elements surrounding the injection site. Our aims have been to investigate the contributions of topographically specified excitatory and inhibitory lateral cortical interactions to the direction specificity of simple and complex cells, to differentiate and quantitatively relate these interactions to the two cell types, and finally to gain some insight into the topography of cortical interactions, in an attempt to identify the underlying, intracortical pathways involved in direction selectivity.

METHODS

Preparation and maintenance of animals

Sixteen adult cats (2·5–5·0 kg) were used. The animals were initially anaesthetized with ketamine hydrochloride (20–25 mg kg^{-1} I.M.). The head was fixed in a stereotaxic head-holder with xylocaine cream applied to all pressure points. The skull was opened to allow access to area 17 of the visual cortex in both hemispheres between Horsley–Clarke co-ordinates P0–P6 and L0·5–L3·5. The femoral artery was cannulated for continuous measurement of arterial blood pressure and heart rate. Following the initial dose of ketamine hydrochloride, the anaesthesia was maintained by artificial respiration with $N_2O:O_2$ (70:30) with the addition of 0·2–0·5% Fluothane to ensure adequate anaesthesia. The blood pressure, heart rate and EEG were used to monitor the depth of anaesthesia, which could be adjusted by the level of added Fluothane. A continuous infusion of *d*-tubocurarine (0·3 mg kg^{-1} h^{-1}) and gallamine triethiodide (4·0 mg kg^{-1} h^{-1}) in a glucose (1·25%) and Ringer solution was given throughout the 2–3 day experiments. The mean arterial blood pressure remained constant at approximately 100 mmHg, no sudden rises in heart rate occurred and the synchronized EEG displayed δ-waves. The end-expired CO_2 was held at 3·8%. Rectal temperature was kept constant at 38·5 °C. Atropine sulphate (1%) was applied to the eyes for mydriasis, and phenylephrine hydrochloride (5%) for retraction of the nictitating membranes and eyelids. The corneae were covered with zero-power contact lenses with vertical slit pupils. Refraction was assessed with a refractoscope (Heine) and corrected with lenses for a viewing distance of 0·25 m.

Recordings and microionophoresis

Glass-coated tungsten microelectrodes were used for recording from layers III–VI of the cortex. The electrodes had a tip diameter of approximately 1 μm, and the impedance was adjusted to 10 MΩ by reopening the tip with hydrofluoric acid during measurement with a microelectrode admittance meter (Corti, Switzerland). Four barrels of a multibarrel micropipette were used for microionophoresis of GABA (0·5 M, pH 3·0); the remaining barrel was filled with a solution of 2% Pontamine Sky Blue in 0·5 M-sodium acetate. The tip of each barrel had a diameter of 2–3 μm to prevent clogging during long recording sessions. These large tips, however, made single-cell recordings with the pipette impossible. Retaining and ejection currents were controlled with a Neurophore-2 (Medical Systems Corp., U.S.A.). Ejection currents were increased, as necessary, for periods of up to 1 h, starting at 20 nA. Total currents were kept below 400 nA to avoid tissue damage due to electrolytic lesions.

Prior to recordings, in thirteen experiments the microionophoresis pipette was placed in area 17, posterior to the recording site on the apex of the gyrus, at a depth of approximately 400–600 μm.

The pipette was kept in place until the end of the recording session. Recordings were made from area 17 cells with receptive fields (RFs) within 5 deg of the area centralis. The recording sites were between 500 and 2500 μm anterior to the microionophoresis injection site, as measured across the cortical surface. In two control experiments the arrangement of injection and recording sites was inverted, placing the pipette similar distances anterior to the recording electrode.

The end of individual recording tracks was marked by small electrolytic lesions (2–4 μA, 10 s, electrode negative) and the position of the pipette was labelled with Pontamine Sky Blue (4 μA, 8 min, electrode negative) after each recording session.

Visual stimulation

Visual stimuli were generated by a cathode ray tube image generator ('Picasso', Innisfree, U.S.A.) and presented on an oscilloscope 0·25 m in front of the cat's eyes. For stimulation of visual cortical cells light and dark bars moving back and forth across the RF with constant velocities were adjusted in width and length and orientation to evoke optimal responses. Background illumination and dark bars had a luminance of 0·25 cd m^{-2}; luminances of light bars could be varied between 1·0 and 12·5 cd m^{-2}. A pseudo-random sequence was used to change the stimulus orientation by multiples of 30 deg. Velocity and contrast were individually adjusted to obtain maximum responses.

Classification of cells

Cells were classified as belonging to the S-group (simple) or C-group (complex), respectively, according to schemes proposed by Kato, Bishop & Orban (1978), and Henry, Harvey & Lund (1979). Intermediate classes A and B (Henry *et al.* 1979) were not distinguished. S-cells were classified by their small RFs (width $< 1·5$ deg) with non-overlapping 'on' and 'off' regions. In addition, the criteria of low spontaneous activity and strong orientational tuning were used. Preliminary classification was performed by hand-held stimuli on a plotting board in front of the cat and confirmed by quantitative analysis of the discharge regions with moving light and dark bars as seen in peristimulus time histograms (PSTH). In cells with end-inhibition, stimulus length was adjusted to obtain maximal responses.

Data analysis

The initialization of the stimulus sweeps, data acquisition and analysis were performed on-line by a digital computer (LSI-11/23, Digital Equipment Corp., U.S.A.) via laboratory interface (Cambridge Electronic Design, U.K.). Peristimulus time histograms were computed for at least five sweeps (back and forth movement) for each orientation. Peak response rates derived from each half of the histograms were used to generate polar plots (Fig. 2*B*) in which the impulse rate per second was plotted as vector length, and the direction of stimulus movement as vector angle, in the polar co-ordinate system.

Histological verification

All animals were fixed by intra-arterial vascular perfusion with 4% phosphate-buffered paraformaldehyde (pH 7·4) at the end of the experiment, under deep halothane anaesthesia. Pontamine Sky Blue injections and electrocoagulations were identified in frozen sections of 50 μm, counter-stained with Cresyl Violet.

RESULTS

Retinotopic relationship between recording and microionophoresis sites

To simplify the interpretation of the results described in the following sections Fig. 1 shows an outline of the topographical transformations of a visual stimulus performed by area 17 of the visual cortex. The visual field (Fig. 1*A*) is projected retinotopically in a continuous way onto area 17 (Fig. 1*B*; Bilge, Bingle, Seneviratne & Whitteridge, 1967; Tusa, Palmer & Rosenquist, 1978) so that a stimulus moving downward in the visual field on its trajectory (S, Fig. 1*A*) elicits a response wave travelling from posterior to anterior in the cortex (R, Fig. 1*C*). Assuming that the

recorded cell (C, Fig. 1*C*) responds optimally to horizontal stimulus orientation and vertical movement but shows direction asymmetry, the directional tuning of the cell could arise either from excitatory or inhibitory intracortical influences involving neuronal elements along the trajectory of the elicited response wave (R). With an electrode configuration as schematized in Fig. 1*C* (recording electrode anterior to GABA pipette) inactivation due to GABA microionophoresis affects cells posterior to

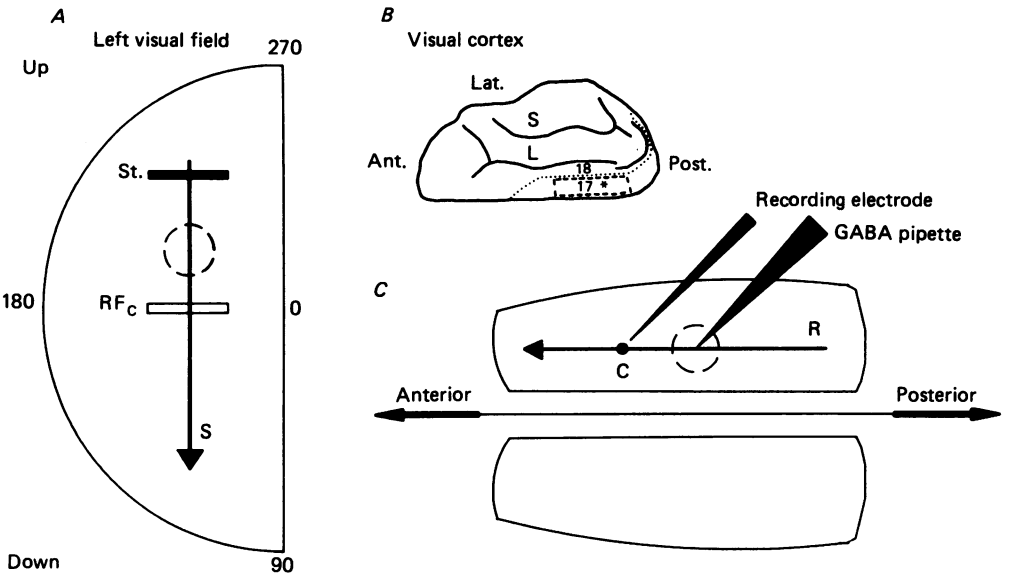

Fig. 1. Topographical transformations of a visual stimulus by area 17 of the visual cortex and juxtaposition of recording and inactivation sites. *A*, left visual field. St. = stimulus bar, S = stimulus trajectory, RF_C = receptive field of a cell C, circle = retinotopic representation of the region inactivated by GABA microionophoresis. *B*, dorsal view of the right hemisphere of cat cortex. S = suprasylvian sulcus, L = lateral sulcus. The border between area 17 and area 18 is indicated by the dotted line. The area in the small rectangle was exposed during the experiments (asterisk = representation of the area centralis). *C*, schematic drawing of area 17 with the electrode arrangement for microionophoresis and recording used in most of the experiments. C = recorded cell, R = response wave in the cortex elicited by stimulus movement along S, circle = region inactivated during GABA application.

the recorded cell. Retransformed into the visual field the affected region represents receptive fields situated above the receptive field RF_C of cell C (circles).

This arrangement, in principle, allows one to investigate excitatory and inhibitory intracortical interactions related to directional tuning under the following conditions. If cell C displays a direction preference for downward movement (90 deg), this could be based on a predominance of intracortical *excitation* from regions retinotopically above RF_C. GABA-induced inactivation of the corresponding cortical region could then reduce lateral excitation and the response to stimulus St. moving along S should *decrease* leading to diminished direction selectivity. If, on the other hand, cell C shows a direction preference for 270 deg (upward movement) this could be due to *inhibitory* influences during downward movement from cortical regions posterior to

cell C. In this case GABA microionophoresis could inactivate inhibitory elements acting at cell C, leading to a loss of directional selectivity by an *increase* of the response to stimulus St. Any influences from elements anterior to cell C should not be affected.

In thirteen experiments the electrode configuration was as shown in Fig. 1. In two experiments we inverted the configuration.

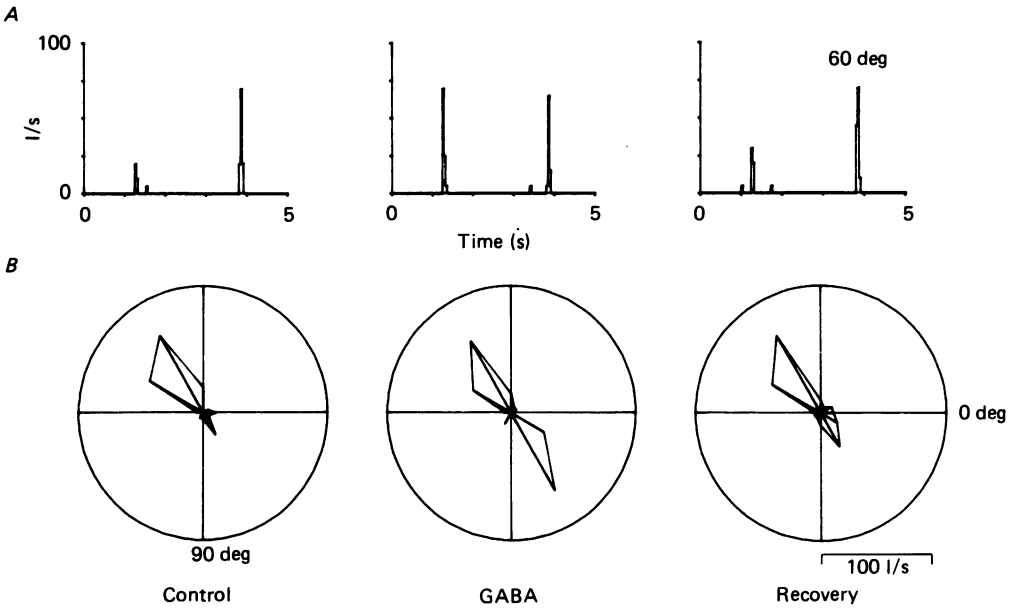

Fig 2. Change in directional tuning of a layer IV S-cell during GABA microionophoresis (GABA pipette in layer II/III, 700 μm posterior to recording site; GABA applied with 60 nA ejection current 12 min after control; recovery 8 min). *A*, PSTHs (bin width 20 ms, 5 sweeps) of the responses to stimulus movement back-and-forth along the 60 deg movement axis. GABA exclusively affected stimulus-evoked responses, with no change in background activity. *B*, polar plots displaying the peak response rates in impulses per second (I/s), computed from the PSTHs for different directions of movement in 30 deg steps. GABA disinhibited the response along the 60 deg movement axis, but orientation tuning was otherwise not altered.

Experimental details

A total of 301 cells was sampled. In fifty cells, spontaneous changes without GABA application were studied for control. In the remaining cells, GABA ejection currents were increased for between 5 min and 1 h, until the directional tuning had obviously changed or until non-specific effects of GABA inhibition were apparent. Recovery periods of up to 1 h were allowed, to show reversibility of the effects. With this time-consuming procedure we were able to completely test 143 cells.

In all experiments the GABA pipette was placed in layers II or III, as subsequently verified histologically. In general, the distances between GABA pipette and recording electrode were measured along the cortical surface during the experiments and histologically confirmed later on.

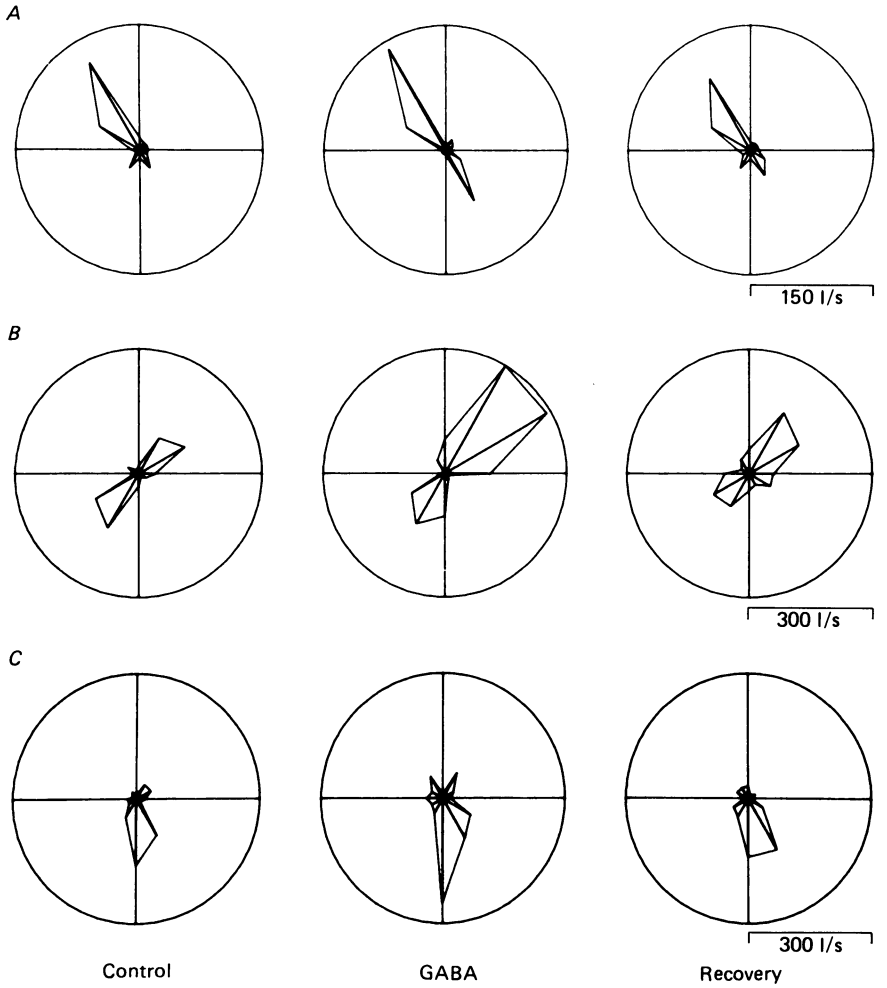

Fig. 3. Change in the directional bias of several layer IV S-cells with different initial directionality and using different relative arrangements of recording and GABA electrodes. *A*, cell with upward preferred direction developing a response to downward movement and thereby losing directionality (GABA pipette in layer II/III, 700 μm posterior; GABA with 160 nA ejection current 4 min after control; recovery 4 min). *B*, cell with no direction preference developing increased response to upward movement during GABA microionophoresis (note inverted electrode arrangement: GABA pipette in layer II/III, anterior, distance 1400 μm; GABA injection current 80 nA 16 min after control; recovery 20 min). *C*, preferred response to downward movement is increased in this cell during posterior GABA inactivation. (GABA pipette in layer II/III, 1900 μm posterior; GABA application with 40 nA ejection current 4 min after control; recovery 12 min.)

Disinhibitory effects of local lateral inactivation by GABA microionophoresis

Layer IV S-cells. In all cases we compared the resulting polar plots with the PSTHs used for computation. This enabled us to distinguish between specific changes in directionality and non-specific effects such as changes of activity not related to the visual stimulus.

Figure 2 shows an example of a layer IV S-cell, direction selective for movement obliquely upwards. The GABA pipette was placed posterior to the recording site, thus influencing cells activated before the recorded cell when the stimulus moved in the non-preferred direction. The cell displayed a clear loss of directionality during GABA microionophoresis, with an increased response to motion in the non-preferred direction (Fig. 2*B*; 60 deg). This change of the response characteristic was limited to the stimulus-evoked discharge (Fig. 2*A*). No increase in spontaneous activity or response to other directions of movement was observed (Fig. 2*B*). The effect was

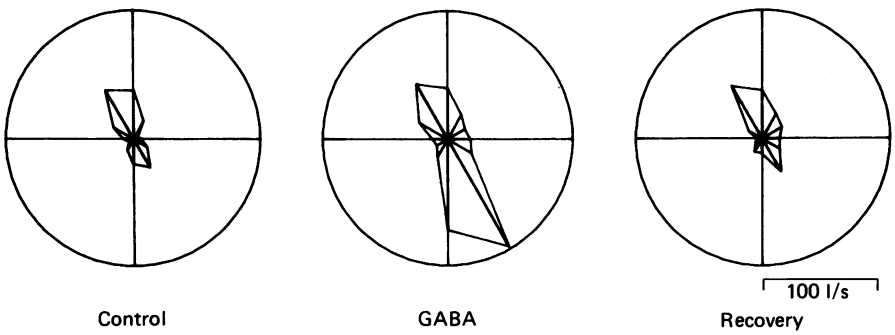

Fig. 4. GABA effects in layer VI. A direction-insensitive layer VI S-cell developed a directional preference for 60 deg during GABA microionophoresis (GABA pipette in layer II/III, 2350 μm posterior; GABA (60 nA) 40 min after control; recovery 40 min).

obtained with an ejection current of 60 nA 12 min after the control recording. The time necessary for complete recovery after GABA application was 8 min.

The specific response of this cell seems to rely on two main contributions: an orientationally tuned excitation with optimal orientation perpendicular to the 60–240 deg axis of movement and an effective inhibitory input from the more posterior cortex that suppresses the response for downward movement (60 deg direction). The inhibition is removed by local GABA inactivation posterior to the cell, releasing a response for 60 deg equal to that for 240 deg.

Another example of a layer IV S-cell (from the same penetration as before) is shown in Fig. 3*A*. Again the strong directional preference for 240 deg initially present is reduced, due to an increased response in the non-preferred direction (60 deg) during the application of GABA (Fig. 3*A*). The cell shown in Fig. 3*B* was investigated with the inverted electrode configuration (GABA pipette anterior). This cell initially displayed almost no directional preference, but developed a marked preference for approximately 300 deg, as was to be expected if inhibition from elements anterior to the cell had been abolished. Already existing direction preferences could even be accentuated by local GABA-induced inactivation. In a cell with a direction preference for 90 deg (Fig. 3*C*, GABA pipette posterior), the already strong response to downward motion was enhanced during GABA application.

Such disinhibitory effects due to inactivation of inhibitory elements by GABA microionophoresis could be demonstrated in many cells with a vertical axis of preferred movement. Regardless of their initial directionality (90 or 270 deg) and strength of directional tuning it was always the downward direction of motion that

was disinhibited if the GABA pipette was posterior to the recorded cell (Figs 2*A* and 3*A* and *C*). On the other hand, the upward direction of motion was disinhibited if the inactivation was anterior to the cell (Fig. 3*B*).

Layer VI S-cells. As all other layers – directly or indirectly – receive inputs from layer IV, one might expect that the disinhibitory effects described above would be at least to some extent reflected in the cells of other layers.

A layer VI S-cell (GABA pipette 2350 μm posterior, Fig. 4) was affected in the same way as the layer IV S-cells in the previous examples. The ejection current was

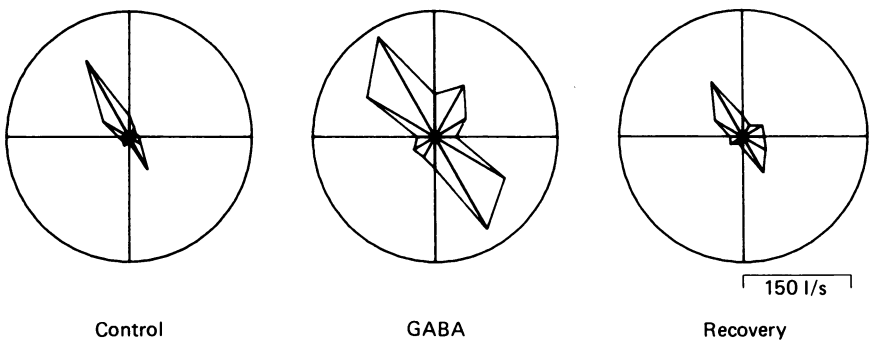

Fig. 5. GABA effects in a C-cell. Directionally tuned layer III C-cell with loss of directionality and increased response to 60 deg stimulus movement following GABA application. The overall responsiveness of the cell was also increased, leading to reduced orientation tuning. (GABA pipette in layer II/III, 1000 μm posterior; GABA (40 nA) 16 min after control; recovery 8 min.)

increased up to 60 nA over a period of 40 min. The weak direction preference for upward movement was reversed by GABA application and, during recovery, the original response returned. The long time necessary for the GABA effect to develop in this case may be due to the large distance between recorded cell and GABA pipette. Diffusion of GABA over a certain distance might be necessary to reach the relevant inhibitory elements. Irrespective of this observation, layer VI cells were generally less easily influenced and often exhibited only minor effects compared with layer IV cells.

C-cells. Figure 5 shows a layer III C-cell. This cell responded with a clear preference for upward movement. During GABA application (GABA pipette posterior), direction selectivity was abolished, with an increase of response in the originally non-preferred direction of motion. In addition, this C-cell displayed a significant increase in overall responsiveness. Both effects proved reversible after cessation of GABA microionophoresis. In most of the tested C-cells, unspecific effects were mixed with specific changes in directional tuning. Overall, disinhibition leading to an increase in spontaneous activity was observed almost as frequently as an overall reduction of responsiveness.

The depressant influence of lateral GABA microionophoresis

In the previous sections we have described the disinhibitory actions of lateral GABA inactivation on cells with preference for vertical movement, independent of

their original directionality. In such cells we observed depressant effects, most likely due to direct GABA-induced inhibition at the recorded cells, almost as frequently as disinhibition (Fig. 6*A*; layer VI S-cell; GABA pipette posterior). Purely depressant actions of GABA were mainly observed in cells preferring horizontal movement (Fig. 6*B*; layer IV S-cell; GABA pipette posterior). This direct GABA-induced inhibition was often so effective that the response of the cell was completely abolished.

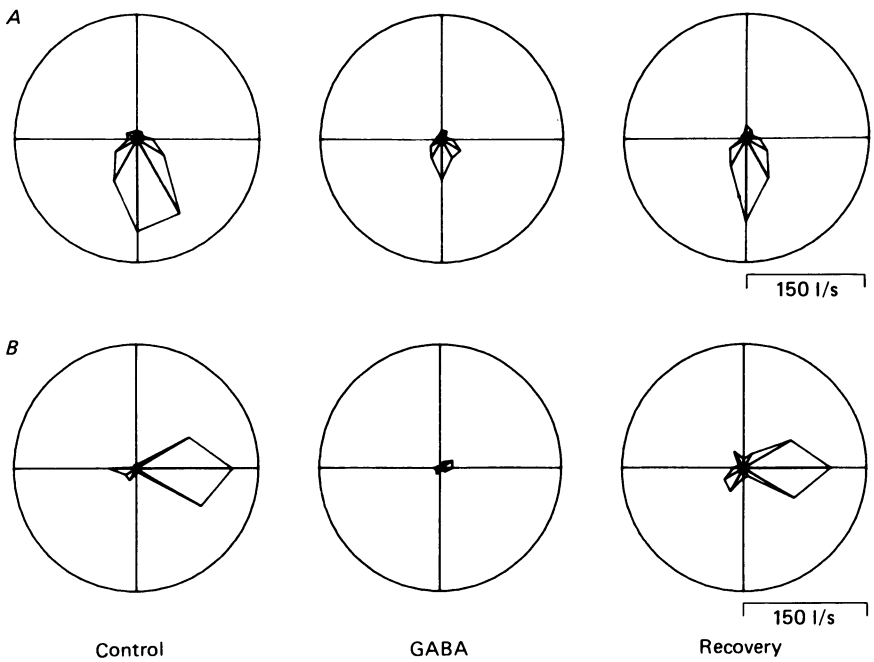

Fig. 6. Depressant action of distant GABA microionophoresis. *A*, layer VI S-cell (GABA pipette in layer II/III, 1000 μm posterior; GABA application 8 min after control with 80 nA ejection current; recovery 12 min). *B*, layer IV S-cell (GABA pipette in layer II/III, 2150 μm posterior; GABA applied with 40 nA 20 min after control; recovery 24 min). Responses are reduced by GABA to all directions of movement but the preferred direction is most affected.

In many cases we obtained sequences of initial disinhibition followed by strongly reduced responsiveness when increasing the current strength and duration of GABA microionophoresis (Fig. 7). This could be explained by the increasing radius of the region affected by GABA with increasing strength and duration of microionophoresis. After initially silencing elements close to the pipette tip, GABA inhibition eventually reaches synapses on the dendrites and somata of the recorded cells. Recovery after cessation of GABA application was also complete in the cases of severe GABA inhibition (Figs 6 and 7). The depressant GABA effects were more pronounced when the distance between pipette and recording site was small, but with longer durations of GABA application one could compensate for larger distances.

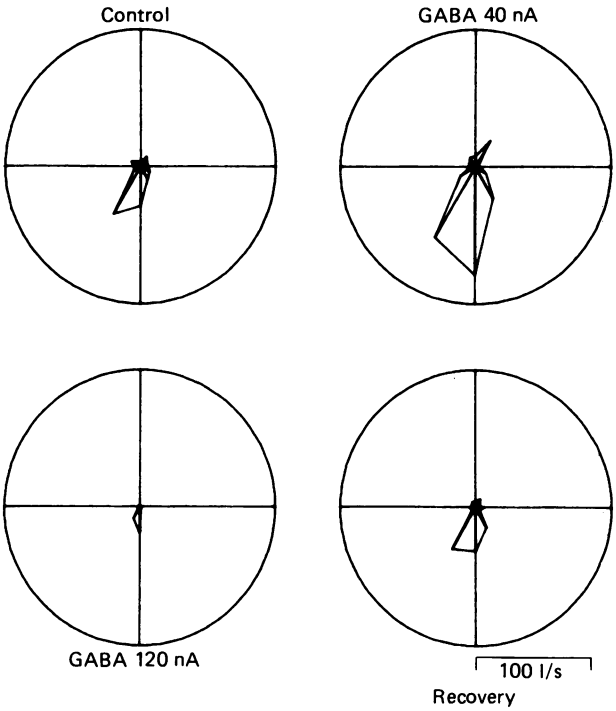

Fig. 7. Effect of long-lasting GABA microionophoresis in a layer IV S-cell. First, after 8 min and with 40 nA ejection current the response in the preferred direction (90 deg) was enhanced. After an additional 20 min and with an increased ejection current (120 nA), direct GABA inhibition was seen. Complete recovery occurred 28 min later. (GABA pipette in layer II/III, 1450 μm posterior to recording electrode.)

Vertical range of GABA effects

To gain an impression of the radius of diffusion and the vertical range of GABA effects during long-lasting microionophoresis we performed one control experiment with recording tracks passing obliquely below the GABA pipette. Histology established the location of the GABA pipette in layer III, and the recording electrode vertically below in layer VI with a distance of 1120 μm from tip to tip. Despite high ejection currents (maximum 100 nA) and prolonged GABA application (40 min) only minor direct inhibitory effects were seen in some of the recorded layer VI C-cells. In layer IV, on the other hand, with a lateral distance of 800–900 μm between GABA pipette and recording electrode tip, we were able to record an S-cell during the same penetration that clearly changed directionality in the way as described above.

Statistical analysis

Two effects of lateral GABA inactivation in cat area 17 have been shown in this study: increased responses, probably based on inactivation of inhibitory elements, and depressant actions, either due to direct inhibition of the recorded cell or to inactivation of excitatory interneurones. In many of the tested cells both effects were present and for differentiation of cell classes it was necessary to decide from the polar

plots which effect predominated in a given cell. Cell groups were distinguished according to the preferred direction of motion and change of mean peak response rate (average of all response rates represented in a polar plot) during GABA application. Four categories of direction preference (PD groups) were defined: PD-00, PD-90, PD-180, PD-270, each including cells with preferred directions in a sector of ±30 deg around the PD group value. The sign of the change of mean peak response rates in each group was used to further separate the cells into those exhibiting depression (decrease) or disinhibition (increase) of firing rates (Table 1). In addition, a sample of cells without GABA application was treated in a comparable way and served as control for the range of spontaneous changes.

TABLE 1. Cells grouped according to their preferred direction of motion and increase or decrease of mean peak response rate (averaged over all directions) during GABA application

	Depressant action		Disinhibition		
PD group	No. of cells	Change of peak rates	No. of cells	Change of peak rates	Total number
0	26 (*a*)	−7·12	3 (*h*)	+4·56	29
90	20 (*b*)	−10·79	28 (*f*)	+10·10	48
180	12 (*c*)	−8·09	7 (*g*)	+10·42	19
270	24 (*d*)	−7·64	23 (*e*)	+12·96	47
Σ	82		61		143
Control without GABA	24	−3·34	26	+6·12	50

The letters in parentheses refer to the curves in Fig. 8.

Depressant actions of GABA were observed in 57·3% of the cells distributed over all PD groups (Table 1). Disinhibition was strongly expressed in the PD-270 group (twenty-three cells, change of mean peak response rate +12·96 spike/s), as might be expected if direction selectivity involves pronounced inhibition topographically arranged along the path of the response wave during stimulus movement in the non-preferred (90 deg) direction. Similarly, in the PD-90 group, inhibition could be removed by GABA inactivation (twenty-eight cells, change of +10·1 spike/s). Disinhibition only seldom occurred in cells preferentially responding to horizontal movement (20·8%), whereas it was seen in 53·6% of the cells preferring vertical stimulus movement.

The separation into cell groups (Table 1) gave a useful first impression of the GABA effects. Now it became possible to further quantify the behaviour of the different cell groups. The disinhibited and the depressed cells of each PD group were treated separately, and the mean absolute changes of peak rates (peak rates during GABA application minus control peak rates) in response to all directions of stimulus movement were computed (Fig. 8, curves *a–h*).

For the eighty-two cells that, in general, were depressed during GABA application, we found in all PD groups maxima of depression in the response to motion in the preferred direction (Fig. 8, negative curves with peaks *a–d*). Among the sixty-one cells with increased mean response rates, cells showing disinhibitory maxima due to

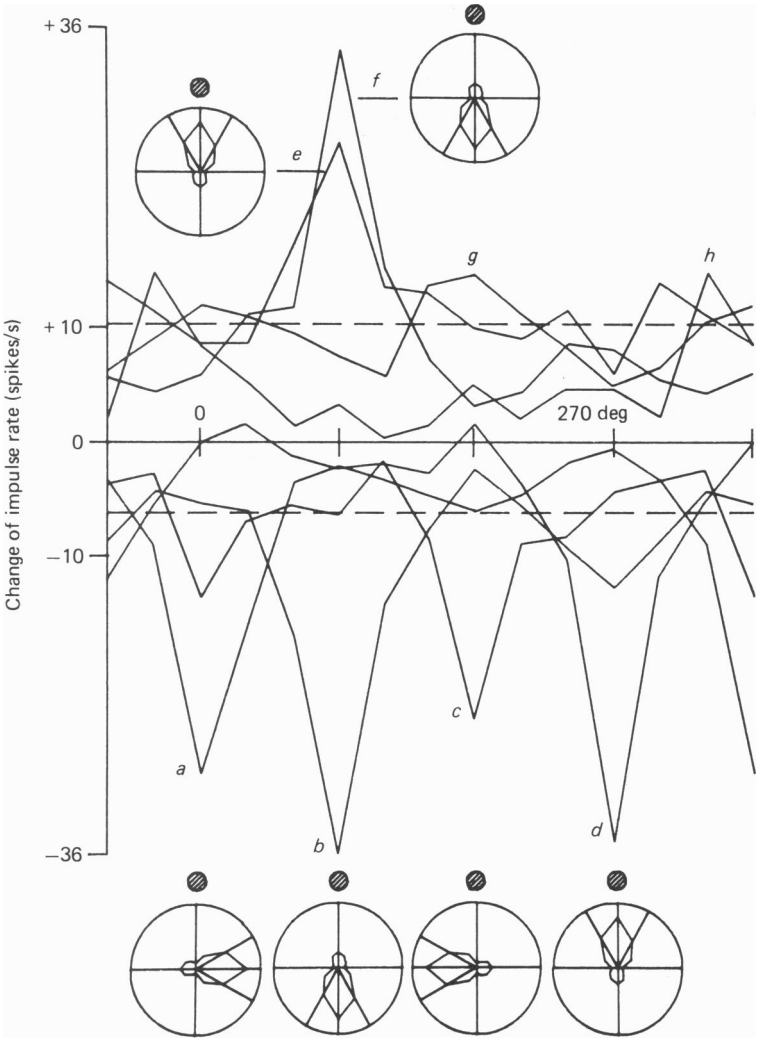

Fig. 8. Mean absolute changes of peak response rates during GABA application (ordinate) plotted with respect to direction of stimulus movement (abscissa) for the cell groups determined in Table 1. The small insets of schematic polar plots indicate the respective PD groups and the relative location of the GABA pipette (hatched circles). Depressant GABA effects influencing the preferred responses were present in all PD groups (negative peaks *a–d*), whereas disinhibition was exclusively observed for the 90 deg response in the vertical PD groups (PD-90 and PD-270, positive peaks *e* and *f*). The disinhibited groups with horizontal preferred direction (PD-0 and PD-180, curves marked *g* and *h*) displayed no significant changes. The dashed lines indicate the maximal spontaneously occurring changes averaged from control cells without GABA.

GABA application were only found in the PD-270 and PD-90 groups (Fig. 8, peaks *e* and *f*; fifty-one cells) where, in both cases, maximal disinhibitions occurred in the 90 deg direction. The remaining ten cells with horizontal preferred directions (PD-00 and PD-180) that showed increased mean peak response rates (Table 1) did not show any significant maximal changes during GABA application (Fig. 8, curves *g* and *h*).

For all the other cases, the maximal GABA-induced depressant as well as disinhibitory effects (Fig. 8, *a–f*) were significantly different (*t* test, $P < 0.001$) from the maximal spontaneously occurring changes without GABA, averaged from the control group of fifty cells (+10·2 and −6·0 spikes/s in Fig. 8, dashed lines).

Differences between the S- and C-cell groups

The question arose whether the disinhibitory and depressant effects were differentially distributed among S- and C-cells, and whether quantitative differences of cell properties were present in different cortical layers. Therefore we tested the S- and C-cells of the PD-90 and PD-270 groups with respect to the different GABA effects, and also compared the frequency of disinhibited and depressed S-cells within layers IV and VI.

TABLE 2. Distribution of disinhibitory and depressant effects amongst S- and C-cells (A) and numbers of S-cells with disinhibition and depression observed in layers IV and VI (B).

A

Cell type	Depressant action	Disinhibition
S-cells	26	40
C-cells	18	11

B

S-cells	Depressant action	Disinhibition
Layer IV	11	19
Layer VI	11	18

Table 2*A* shows that most S-cells are disinhibited ($n = 40$ out of 66), whereas for the C-cells the reverse is true, with depressant actions in eighteen of twenty-seven cells. This is significant with $\chi^2 = 4{\cdot}166$; $P < 0{\cdot}05$. On the other hand, no differences could be detected between S-cells in the input layers IV and VI of the visual cortex (Table 2*B*).

DISCUSSION

GABA microionophoresis has been used in the present study to inactivate visual cortical regions in a way similar to that recently applied by Bolz & Gilbert (1986). GABA acts as an inhibitory transmitter in the cat's visual cortex (Sillito, 1977) and it is assumed that practically all visual cortical cells – whether excitatory or inhibitory – are silenced by GABA, whilst the afferent input to the cortex is not affected.

Inactivations centred some distance from the recorded cells abolished direction selectivity in certain cells and produced it in others, depending on the relative topography of recording and inactivation sites and on the properties of the investigated cells before local inactivation of nearby regions. These results raise questions about lateral signal processing in general and its possible contributions to direction selectivity of cells in the cat's visual cortex.

Two main effects have been observed during local inactivation of the upper cortical layers anterior or posterior to the investigated cells: most specifically, an increased response due to loss of inhibition. This was obtained when the GABA pipette was located in a cortical region activated before the recorded cell during movement of the stimulus in the non-preferred direction, suggesting a high

topographical specificity of involved inhibitory interactions in the cat's visual cortex similar to those suggested first for directional cells in the rabbit's retina (Barlow & Levick, 1965). Less specifically, in many cells inhibitory GABA effects were observed irrespective of the electrode arrangement. Such inhibitory effects could be due to silencing of excitatory cells converging on the investigated cell or might be based on direct inhibitory actions of GABA on the cell itself. In a number of cases this led to a loss of directionality, indicating an excitatory convergence involved in determining the direction sensitivity of these cells. More frequently, however, the inhibitory effects led to an overall reduction of responsiveness, not accompanied by any changes of the recorded cell's specificity.

In general, our results are consistent with the view that direction specificity involves intracortical inhibition (Benevento *et al.* 1972; Innocenti & Fiore, 1974; Sillito, 1975, 1977, 1979; Matsubara, Cynader, Swindale & Stryker, 1985). Our method does not allow us to assess the possible contributions of excitatory convergence from subcortical inputs as suggested by Ferster (1986).

The fact that it proved possible by removal of inhibition from one side of a cell, to abolish or even invert direction selectivity in cells with a certain preferred direction (Figs 2, 3*A*, 4 and 5), to produce direction selectivity in non-directional cells (Fig. 3*B*), and to increase the directionality in cells with the opposite preferred direction (Figs 3*C* and 7), suggests a model with inhibition acting from both sides of a visual cortical cell along the common axis of the preferred and non-preferred stimulus motion (Fig. 8, *e* and *f*). An imbalance of this inhibition seems to determine the sign and the strength of direction preference. The direction preference results from a stronger inhibition from one side, not from the exclusive existence of inhibition only from that side. The reversal of the direction selectivity of a cell by removal of lateral inhibition further implies that after local inactivation of the underlying inhibitory elements the inhibition originally effective during movement in the non-preferred direction must now be weaker than that acting during movement in the direction formerly preferred.

The results presented further suggest that inhibitory elements possibly involved in orientational tuning (Sillito, 1975, 1979; Matsubara *et al.* 1985) are different from those involved in direction selectivity (Hammond, 1978). This is supported by our evidence that direction selectivity could be changed without influencing orientation specificity. One can speculate that elements involved in orientation tuning must be closer to the recorded cell (and more distant from the GABA pipette) than those elements involved in directionality that are closer to the distant GABA pipette and accordingly inactivated first.

In the present study, GABA inactivation primarily affected the supragranular cortical layers but the localization of the GABA micropipette in layer II/III is only of significance if GABA does indeed not inactivate deep layers of the cortex simultaneously. Possibly there is diffusion of GABA over considerable distances, since effects of local inactivation were produced over distances between 0·5 and nearly 2·5 mm. On the other hand, Bolz & Gilbert (1986) assume that local inactivation by GABA injected into layer VI does not directly affect cells recorded above them in layer IV, the distance involved in this case being of the order of 0·5 mm. In our own control experiments there was, indeed, practically no inhibitory

effect observed in layer VI during long-lasting microionophoresis with high ejection currents directly above them in layer III. Accordingly, the type of inhibition related to direction selectivity that was inactivated in the present study seems to involve the upper cortical layers. This is not contradicted by the findings of Bolz & Gilbert (1986) who simply described changes in end-inhibition but not of direction and orientation specificity after inactivation of layer VI.

In a recently published study, Schwark, Malpeli, Weyand & Lee (1986) reported no changes in direction selectivity of cells in the deep layers of the visual cortex during superficial cortical cooling. There are, however, important methodological differences between that study and our approach. In the cited investigation, cortical cooling affected the whole cortical surface above the cells under study, i.e. symmetrical rather than asymmetrical inactivation with respect to the recording site. Furthermore, the barbiturate anaesthesia used by Schwark *et al.* (1986) might have silenced the kind of cells involved in the present study. Finally, very different topographical regions of area 17 were studied: peripheral regions in Schwark's research as opposed to the projection of the central visual field in the present study. Any of these differences might explain the discrepancies between the two investigations.

Several neurophysiological observations show that direction selectivity can be elicited by stimuli within the excitatory discharge region of a cortical cell (Goodwin *et al.* 1975; Emerson & Gerstein, 1977; Ganz & Felder, 1984). This does not necessarily imply that regions outside the classical RF are not involved. In our experiments GABA inactivation was centred at distances between 0·5 and 2·5 mm from the recorded cells, i.e. about 0·3–1·5 deg away from the RF centre, in the vicinity of the area centralis. The observed long-range effects can be interpreted in several ways. On the one hand, GABA might diffuse horizontally and reach elements closer to the recorded cells. On the other hand, more distant lateral effects, not revealed by tests with single-spot stimuli, might be transmitted in a cascade-like fashion and build in strength during movement towards the cell. In this case, continuity in space and critical time factors would be important. The velocity dependence of directionality (Orban, Kennedy & Maes, 1981) supports this interpretation.

In fact, the existence of lateral inhibitory interactions (in part over longer distances) in striate cortex is predicted by a number of observations (Jones, 1970; Benevento *et al.* 1972; Bishop, Coombs & Henry, 1973; Innocenti & Fiore, 1974; Glezer *et al.* 1982; Duysens, 1987; see also Allman, Miezin & McGuiness, 1985) and supported by local stimulation experiments and the present inactivation study. Lateral cortical excitation with L-glutamate (Hess, Negishi & Creutzfeldt, 1975) evoked inhibition over about 400 μm in the visual cortex, and electrical microstimulation of the motor cortex (Asanuma & Rosen, 1973) yielded lateral inhibition up to 1000 μm. Long-ranging horizontal connections have been demonstrated by horseradish peroxidase injections in the visual cortex (Rockland & Lund, 1983; Gilbert, 1985), but experimental evidence suggests that these interactions are excitatory in nature (Ts'o *et al.* 1986).

Unlike the relatively orderly pattern of preferred orientations in the cat's visual cortex (Hubel & Wiesel, 1962, 1963), direction selectivity displays a patchy

distribution (Payne, Berman & Murphy, 1980) in so far as cells recorded close together tend to show comparable direction preferences. Our experience suggests that direction selectivity can reverse abruptly during a given penetration while the orientation specificity remains unchanged at the same time. This suggests that different cells share the same source of direction selectivity independent of their orientational tuning. This source might be individual inhibitory interneurones serving a cluster of cells in the visual cortex.

Hubel & Wiesel (1962) were the first to suggest that complex-cell receptive fields might be generated by excitatory convergence of simple cells. In fact, one of the main differences between complex and simple cells in our study was the relatively higher percentage of cells with depressant GABA effects among the C-cell group (62%) as opposed to 39% in the S-cell group. While 61% of the S-cells showed effects due to loss of inhibition, this was only seen in 38% of the C-cells. This might be an indication of a more pronounced inhibitory modulation of S-cells as opposed to a preponderance of excitatory convergence in C-cells (Movshon *et al.* 1978). The property of direction selectivity might be generated in S-cells and then transmitted excitatorily to C-cells (Goodwin & Henry, 1975). Nevertheless, inhibition was removed by GABA inactivation in about one-third of the C-cells, indicating the additional possibility of influences from inhibitory interneurones in this class of cells.

About 20% of the neurones in the cat's visual cortex are GABAergic inhibitory interneurones with more than 50% located within lower layer III and layer IV (Gabbott & Somogyi, 1986). Matsubara, Nance & Cynader (1987) have shown numerous tangentially oriented GABA-immunoreactive fibres in area 18 of the cat. These fibres enable inhibitory connections between functional columns. Seven types of GABAergic interneurones have so far been individually identified (Somogyi *et al.* 1983; Kisvarday, Martin, Whitteridge & Somogyi, 1985; Somogyi, 1986). These include two types of large basket cells, one typically situated close to the border between layers III and IV (Somogyi *et al.* 1983), the other found in layers V and VI (Kisvarday, Martin, Friedlander & Somogyi, 1987). Both have horizontal axons up to 2 mm in length and one could speculate that these large basket cells may be involved in the directionality effects observed in our present study.

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REFERENCES

ALLMAN, J., MIEZIN, F. & MCGUINESS, E. (1985). Stimulus specific responses from beyond the classical receptive field: neurophysiological mechanisms for local–global comparisons in visual neurons. *Annual Reviews of Neuroscience* **8**, 407–430.

ASANUMA, H. & ROSEN, I. (1973). Spread of mono- and polysynaptic connections within cat's motor cortex. *Experimental Brain Research* **16**, 507–520.

BARLOW, H. B. (1981). Critical limiting factors in the design of the eye and visual cortex. *Proceedings of the Royal Society* B **212**, 1–34.

BARLOW, H. B. & LEVICK, W. R. (1965). The mechanism of directionally selective units in the rabbit's retina. *Journal of Physiology* **178**, 477–504.

BENEVENTO, L. A., CREUTZFELDT, O. D. & KUHNT, U. (1972). Significance of intracortical inhibition in the visual cortex. *Nature* **238**, 124–126.

BILGE, M., BINGLE, A., SENEVIRATNE, K. N. & WHITTERIDGE, D. (1967). A map of the visual cortex in the cat. *Journal of Physiology* **191**, 116–118*P*.

BISHOP, P. O., COOMBS, J. S. & HENRY, G. H. (1973). Receptive fields of simple cells in the cat striate cortex. *Journal of Physiology* **231**, 31–60.

BOLZ, J. & GILBERT, C. D. (1986). Generation of end-inhibition in the visual cortex via interlaminar connections. *Nature* **320**, 362–365.

DUYSENS, J. (1987). Is direction selectivity of cat area 17 cells always independent of contrast and dependent on short-distance interactions? *Experimental Brain Research* **67**, 663–666.

EMERSON, R. C. & GERSTEIN, G. L. (1977). Simple striate neurons in the cat. II. Mechanisms underlying directional asymmetry and directional selectivity. *Journal of Neurophysiology* **40**, 136–155.

EYSEL, U. TH. & WÖRGÖTTER, F. (1986). Specific cortical lesions abolish direction selectivity of visual cortical cells in the cat. *Society for Neuroscience Abstracts* **12**, 583.

FERSTER, D. (1986). Orientation selectivity of postsynaptic potentials in neurons of cat primary visual cortex. *Journal of Neuroscience* **6**, 1284–1301.

FERSTER, D. & LINDSTRÖM, S. (1983). An intracellular analysis of geniculo-cortical connectivity in area 17 of the cat. *Journal of Physiology* **342**, 181–215.

GABBOTT, P. L. A. & SOMOGYI, P. (1986). Quantitative distribution of GABA-immuoreactive neurons in the visual cortex (area 17) of the cat. *Experimental Brain Research* **61**, 323–331.

GANZ, L. & FELDER, R. (1984). Mechanism of directional selectivity in simple neurons of the cat's visual cortex analyzed with stationary flash sequences. *Journal of Neurophysiology* **51**, 294–324.

GILBERT, C. D. (1983). Microcircuitry of the visual cortex. *Annual Reviews of Neuroscience* **6**, 217–247.

GILBERT, C. D. (1985). Horizontal integration in the neocortex. *Trends in Neurosciences* **8**, 160–165.

GILBERT, C. D. & WIESEL, T. N. (1979). Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex. *Nature* **280**, 120–125.

GLEZER, V. D., TSHERBACH, T. A., GAUSELMAN, V. E. & BONDARKO, V. M. (1982). Spatio-temporal organization of receptive fields of the cat striate cortex. *Biological Cybernetics* **43**, 35–49.

GOODWIN, A. W. & HENRY, G. H. (1975). Direction selectivity of complex cells in a comparison with simple cells. *Journal of Neurophysiology* **38**, 1524–1540.

GOODWIN, A. W., HENRY, G. H. & BISHOP, P. O. (1975). Direction selectivity of simple striate cells: properties and mechanism. *Journal of Neurophysiology* **38**, 1500–1523.

HAMMOND, P. (1978). Directional tuning of complex cells in area 17 of the feline visual cortex. *Journal of Physiology* **285**, 479–491.

HENRY, G. H., HARVEY, A. R. & LUND, J. S. (1979). The afferent connections and laminar distribution of cells in the cat striate cortex. *Journal of Comparative Neurology* **187**, 725–744.

HESS, R., NEGISHI, K. & CREUTZFELDT, O. (1975). The horizontal spread of intracortical inhibition in the visual cortex. *Experimental Brain Research* **22**, 415–419.

HUBEL, D. H. & WIESEL, T. N. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *Journal of Physiology* **160**, 106–154.

HUBEL, D. H. & WIESEL, T. N. (1963). Shape and arrangement of columns in cat's striate cortex. *Journal of Physiology* **165**, 559–568.

INNOCENTI, G. M. & FIORE, L. (1974). Post-synaptic inhibitory components of the responses to moving stimuli in area 17. *Brain Research* **80**, 122–126.

JONES, B. H. (1970). Responses of single neurons in cat visual cortex to a simple and more complex stimulus. *American Journal of Physiology* **218**, 1102–1107.

KATO, H., BISHOP, P. O. & ORBAN, G. A. (1978). Hypercomplex and simple/complex cell classification in cat striate cortex. *Journal of Neurophysiology* **41**, 1071–1095.

KISVARDAY, Z. F., MARTIN, K. A. C., FRIEDLANDER, M. J. & SOMOGYI, P. (1987). Evidence for interlaminar inhibitory circuits in the striate cortex of the cat. *Journal of Comparative Neurology* **260**, 1–19.

KISVARDAY, Z. F., MARTIN, K. A. C., WHITTERIDGE, D. & SOMOGYI, P. (1985). Synaptic connections of intracellularly filled clutch cells: a type of small basket cell in the visual cortex of the cat. *Journal of Comparative Neurology* **242**, 111–137.

KOCH, C. & POGGIO, T. (1985). The synaptic veto mechanism: does it underlie direction and orientation selectivity in the visual cortex? In *Models of the Visual Cortex*, ed. ROSE, D. & DOBSON, V. G., pp. 408. Chichester, New York: John Wiley & Sons.

MCGUIRE, B. A., HORNUNG, J.-P., GILBERT, C. D. & WIESEL, T. N. (1984). Patterns of synaptic input to layer 4 of cat striate cortex. *Journal of Neuroscience* **4**, 3021–3033.

MARTIN, K. A. C. & WHITTERIDGE, D. (1984). Form, function and intracortical projections of spiny neurones in the striate visual cortex of the cat. *Journal of Physiology* **353**, 463–504.

MATSUBARA, J., CYNADER, M., SWINDALE, N. V. & STRYKER, M. P. (1985). Intrinsic projections within visual cortex: Evidence for orientation-specific local connections. *Proceedings of the National Academy of Sciences of the U.S.A.* **82**, 935–939.

MATSUBARA, J., NANCE, D. M. & CYNADER, M. S. (1987). Laminar distribution of GABA-immunoreactive neurons and processes in area 18 of the cat. *Brain Research Bulletin* **18**, 121–126.

MOVSHON, J. A., THOMPSON, I. D. & TOLHURST, D. J. (1978). Receptive field organization of complex cells in the cat's striate cortex. *Journal of Physiology* **283**, 79–99.

ORBAN, G. A., KENNEDY, H. & MAES, H. (1981). Response to movement of neurons in area 17 and 18 of the cat: velocity sensitivity. *Journal of Neurophysiology* **45**, 1043–1058.

PAYNE, B. R., BERMAN, N. & MURPHY, E. H. (1980). Organization of direction preferences in cat visual cortex. *Brain Research* **211**, 445–450.

PETERHANS, E., BISHOP, P. O. & CAMARDA, R. M. (1985). Direction selectivity of simple cells in cat striate cortex to moving light bars. *Experimental Brain Research* **57**, 512–522.

ROCKLAND, K. S. & LUND, J. S. (1983). Intrinsic laminar lattice connections in primate visual cortex. *Journal of Comparative Neurology* **216**, 303–318.

SCHWARK, H. D., MALPELI, J. G., WEYAND, T. G. & LEE, C. (1986). Cat Area 17. II. Response properties of infragranular layer neurons in the absence of supragranular layer activity. *Journal of Neurophysiology* **56**, 1074–1087.

SILLITO, A. M. (1975). The contribution of inhibitory mechanisms to the receptive field properties of neurones in the striate cortex of the cat. *Journal of Physiology* **250**, 305–329.

SILLITO, A. M. (1977). Inhibitory processes underlying the directional specificity of simple, complex and hypercomplex cells in the cat's visual cortex. *Journal of Physiology* **271**, 699–720.

SILLITO, A. M. (1979). Inhibitory mechanisms influencing complex cell orientation selectivity and their modification at high resting discharge levels. *Journal of Physiology* **289**, 33–53.

SOMOGYI, P. (1986). Seven distinct types of GABA-immunoreactive neuron in the visual cortex of cat. *Society for Neuroscience Abstracts* **12**, 583.

SOMOGYI, P., KISVARDAY, Z. F., MARTIN, K. A. C. & WHITTERIDGE, D. (1983). Synaptic connections of morphologically identified and physiologically characterized large basket cells in the striate cortex of cat. *Neuroscience* **10**, 261–294.

TS'O, D. Y., GILBERT, C. D. & WIESEL, T. N. (1986). Relationships between horizontal interactions and functional architecture in cat striate cortex as revealed by cross-correlation analysis. *Journal of Neuroscience* **6**, 1160–1170.

TUSA, R. J., PALMER, L. A. & ROSENQUIST, A. C. (1978). The retinotopic organization of area 17 (striate cortex) in the cat. *Journal of Comparative Neurology* **177**, 213–236.