Orientation-specific Relationship Between Populations of Excitatory and Inhibitory Lateral Connections in the Visual Cortex of the Cat

The topography of lateral excitatory and lateral inhibitory connections was studied in relation to orientation maps obtained in areas 17 and 18. Small iontophoretic injections of biocytin were delivered to the superficial layers in regions where orientation selectivity had been mapped using electrode recordings of single- and multi-unit activity from various cortical depths. Biocytin revealed extensive patchy axonal projections of up to 3.5 mm in both areas while labelled somata occurred chiefly at the injection site, indicating that the labelling was primarily anterograde. Two types of boutons could be clearly distinguished: (i) putative excitatory boutons either en passant or having a short stalk and (ii) inhibitory boutons which were invariably of the basket-type. Three-dimensional reconstructions of all labelled boutons showed that the excitatory and the inhibitory networks had a distinctively different relationship to orientation maps. The overall distribution of connections showed that 53–59% of excitatory and 46–48% of inhibitory connections were at iso-orientation, ±30°; oblique-orientation, ±(30–60)°, was shown by 30% of excitatory and 28–39% of inhibitory connections; cross-orientation was shown by 11–17% of excitatory and 15–24% of inhibitory connections. Although excitatory patches occupied mainly iso-orientation locations, interpatch regions representing chiefly non-iso-orientations (oblique + cross orientation) were also innervated. There was considerable overlap between the excitatory and inhibitory network. Nonetheless, inhibitory connections were more common than excitatory connections with non-iso-orientation locations. There was no significant difference between the orientation topography of area 17 and area 18 projections. The results suggest that in general the lateral connectivity system is not orientation specific, but shows a moderate iso-orientation preference for excitation and an even weaker iso-orientation preference for inhibition. The broad orientation spectrum of lateral connections could provide the basis for mechanisms that require different orientations, as for example in detecting orientation discontinuities.

Introduction
Recent data suggest that corticocortical connections play an important role in mechanisms ranging from simple binding of coherent stimuli to those involved in higher cognitive function (for reviews see Singer and Gray, 1993; Salin and Bullier, 1995). Although the exact cellular mechanisms are still unclear, recent simulation (Lyton and Sejnowski, 1991; Bush and Sejnowski, 1996) and electrophysiological data (Traub et al., 1996) predict a strong effect arising from lateral excitation and lateral inhibition. However, there is still controversy in the literature as to the specificity of corticocortical networks in relation to the topography of even the best known of physiological attributes, namely orientation selectivity.

In the visual cortex, orientation selectivity is the most prominent modular feature; neurons of the same orientation column share similar stimulus orientations and neurons of neighbouring orientation columns prefer increasingly different stimulus orientations (Hubel and Wiesel, 1962, 1965). When the distribution of orientation selectivity in the tangential plane is inspected, one sees an orientation map of periodically arranged columns within which, at every 1–1.5 mm (hypercolumn distance), the same orientation is encountered. Anatomical studies have demonstrated that lateral connections of pyramidal and spiny stellate neurons are also arranged in a periodic manner (Rockland and Lund, 1982; Gilbert and Wiesel, 1983; Martin and Whitteridge, 1984; Kisvárday and Eysel, 1992).

The strong similarity between the periodic patchy distribution of orientation selectivity and the spatial characteristics of patchy horizontal connections has given rise to the suggestion that they may be linked (Mitchison and Crick, 1982). Indeed, experiments using the cross-correlation technique (Michalski et al., 1983; Nelson and Frost, 1985; Ts’o et al., 1986) and combined anatomical approaches (Gilbert and Wiesel, 1989) in area 17 of the cat showed that lateral excitatory connections are selective for similar orientations, a result which casts doubt on previous results obtained in the neighbouring area 18 (Matsubara et al., 1985, 1987). There are, however, some problems in interpreting the former. First, cross-correlation studies most often reveal common excitatory input, while inhibitory interactions are often missed. In addition they could be biased to reveal iso-orientation interactions. Secondly, the data published by Gilbert and Wiesel (1989) mainly concerned iso-orientation connections though they also provided numerous examples of non-iso-orientation connections. Thirdly, the large dye injections used in the study by Matsubara et al. (1985, 1987) make it difficult to interpret the orientation distribution of the labelling. Furthermore, neither the contribution of excitatory and inhibitory connections to orientation selectivity, nor their relationship, were addressed in these studies.

To clarify these issues we have studied the functional-topographic relationship between the lateral excitatory and the lateral inhibitory systems in the cat visual cortex. We obtained orientation maps in areas 17 and 18 using electrophysiological recordings from systematically sampled locations and labelled corticocortical connections with biocytin. The labelled structures were divided into excitatory and inhibitory elements on the basis of morphological criteria including bouton size and shape, branching pattern of axon segments and soma type when the latter could be traced. Our quantitative comparisons revealed that areas 17 and 18 follow the same organization inasmuch as they showed that excitatory connections were more biased to similar orientations than inhibitory connections. Our findings are compatible with a view of intracortical mechanisms which allows information transfer between dissimilar as well as similar functional compartments.

Materials and Methods
The data presented here were obtained from two out of 19 adult cats used for a combination of electrophysiological mapping of orientation selectivity and subsequent anatomical tracing of intracortical...
connections. The small number of animals used in the present study was partly due to the fact that the labelling was often insufficient for quantitative analysis, and partly because the recording electrode often damaged a capillary, the resulting bleeding making further histological reconstruction impossible. Satisfactory physiological and anatomical data were obtained from two animals. No major differences in either the domain-like layout of the electrophysiological maps or in the type of the anatomical structures were observed in the remaining 17 animals in which anatomical or electrophysiological data were incomplete.

Preparation and Surgical Procedures

Anaesthesia was induced with a mixture of ketamine hydrochloride (25 mg/kg, Ketanest, Parke-Davis, Berlin, Germany) and xylazine (3 mg/kg, Rompun, Bayer, Leverkusen, Germany), i.m., and then maintained throughout the experiments with a mixture of 0.2–0.5% halothane (Eurim-Pharm Arzneimittel, Piding, Germany) and N₂O/O₂ (70%/30%) for artificial ventilation. The physiological condition of the animal was continuously monitored by registering heart rate, blood pressure, body temperature, end tidal CO₂ and EEG.

Figure 1. (A) Surface image of the lateral gyrus through protective cellophane showing a portion of area 18 approximately at Horsley–Clarke coordinates A2–A6 and L0.5–L3.5. The centre of each solid bar corresponds to a recording site where the preferred stimulus orientation of cell clusters was determined using computer-controlled stimuli and data analysis. The preferred orientations are indicated by the orientation of the bars. The white bar shows the preferred orientation of a site where biocytin was iontophoretically microinjected (asterisk). Question marks indicate penetrations where the preferred orientation of cells could not be determined. (B) Interpolated orientation map generated from the discontinuous orientation map framed in (A). Notice that the preferred orientations change smoothly although at some locations that might correspond to orientation centres they change abruptly.
Craniotomy was made between Horsley–Clarke coordinates A2–P8 and L1–L8 for studying area 17, and A10–A0 and L1–L8 for studying area 18. Since large regions of the cortex were exposed brain movement due to heart beat and artificial respiration had to be reduced to enable us to record single- and multi-unit activity. Therefore a chamber whose bottom was covered with thin plastic foil was lowered until the plastic foil gently touched the cortical surface. The recording electrodes (5–10 µm tip size) could be readily driven through the plastic foil without breaking their tips.

Tracer Injection and Physiological Mapping
To allow sufficient time for neuronal transport, iontophoretic delivery of the tracer biocytin preceded physiological mapping. Accordingly a glass pipette (inner tip size 7–10 µm) containing 5% biocytin (Sigma, Deisenhofen, Germany) in 0.5 M sodium acetate was placed at the centre of the exposed cortical region and an anodal current of 0.5–0.9 µA was applied at 1 Hz with a 0.5 s on/off duty cycle for 20 min. Multi-unit activity was subsequently recorded via the same pipette to obtain the visuotopic location, the preferred orientation and direction preferences at the injection site. In some cases additional recordings were made 50–100 µm above and below the injection site.

Orientation maps were obtained with recordings of multi-unit, or occasionally single-unit, activity in layers II–V via glass pipettes (inner tip diameter 5–10 µm) filled with 0.5 M sodium acetate. All penetrations were made perpendicular to the cortical surface. When recordings from a single penetration were made from more than one depth there was no appreciable difference in the preferred orientation of the cells encountered, indicating that the neurons were sampled from the same orientation column. Subsequent penetrations were made systematically in 50–300 µm lateral steps using an xy stereotaxic microdrive. This enabled us to screen a rectangular cortical area of ~1.3–3.6 mm². The exposed cortical area was photographed and the location of each penetration was marked on an enlarged printout using blood vessels as landmarks (Fig. 1).

Electrical responses of single cells and multi-unit clusters were recorded for moving light bars whose optimal length and orientation was determined with a hand-held projector. Computer-controlled (Cambridge Electronic Design, CED-1401 laboratory interface) stimuli were then generated with a ‘Picasso’ image synthesizer (Innisfree, Cambridge, MA, USA) and presented on a Tektronix 608 monitor 28.5 cm in front of the animal’s eye. The stimulus orientation was varied in pseudo-random sequence by multiples of 22.5° and drifted with optimal velocity along the orthogonal axis of the orientation across the receptive field area. Peristimulus time histograms were generated from five stimulus sweeps in opposite directions at each orientation.

After completion of the physiological mapping the animals were killed with an overdose of anaesthetic and perfused through the heart with oxygenated Tyrode’s solution followed by fixative containing 1–2% paraformaldehyde (E. Merck, Darmstadt, Germany) and 0.5–1% glutaraldehyde (Merck) in 0.1 M phosphate buffer (PB, pH 7.4).

Histological Procedures
Large blocks comprising the entire cortical thickness were dissected from regions used for physiological mapping, and sectioned on a vibratome (70–80 µm) in a plane parallel to the cortical surface. The sections were kept in sequence. After thorough wash in PB and Tris buffer (TBS, pH 7.6) they were incubated with avidin–biotin-complexed horseradish peroxidase (ABC, Vector Laboratories, Burlingame, CA, USA) in TBS containing 0.05% Triton X-100 at 4°C overnight. The enzymatic reaction was revealed with 0.005% 3,3′ diaminobenzidine–4–HCl (Sigma) in TBS supplemented with GoO₂ intensification (20 min). The reaction was completed by adding 5–10 µl of 1% H₂O₂ solution to every millilitre of incubating medium. Finally, the sections were rinsed in Tris buffer and PB and postfixed in 1% OsO₄ in PB for 20–30 min, dehydrated and embedded in Durcupan ACM (Fluka, Neu-Ulm, Germany) resin on slides.

Computer-assisted Reconstruction

Electrode Tracks
A critical issue in the present study was obtaining an accurate alignment of the physiological and the corresponding anatomical maps. Therefore it was essential to determine the position of each recording electrode in relation to the biocytin-labelled structures. Since details of these procedures have been given previously (Kisvárday and Eysel, 1993) only a brief description is provided here. Electrode tracks were identified by the presence of local tissue damage and the presence of darkly stained macrophages along their course. The complete layout of the electrode tracks was determined in each section and their xy coordinates were digitized using a personal computer and NeuroLucida software (MicroBrightField, USA).

Labelled Boutons
In addition to the electrode tracks the distribution of boutons given off by biocytin-labelled axons were reconstructed using a 50× oil objective and the same reconstruction software referred to above. For each section the focal depth was manually controlled, such that only the x and y coordinates of the labelled boutons were computer monitored, resulting in a two-dimensional boutonal distribution map. Then all the reconstructed elements of a single section were assigned an appropriate z-value, which gave the radial distance of the particular section from the cortical surface, and neighbouring sections were merged into a single data file. In this way, a semi-three-dimensional distribution of the labelled elements and of the electrode tracks was obtained. Because the electrode tracks and the biocytin-labelled structures were reconstructed from the same sections, the resulting physiological and anatomical maps could be directly compared.

Analysis of the Data

Interpolation of Orientation Maps
In order to calculate a continuous map of orientation values from a set of irregularly spaced extracellular recordings the x,y position of each recording location was triangulated (Delaunay triangulation) and then interpolated on a regular grid (50 µm grid-constant) using Akima’s quintic polynomials and IDL software (Research Systems, Inc., Boulder, CO, USA).

Tissue Shrinkage
Electrode penetration marks of known lateral separation allowed us to determine the shrinkage factor along the tangential plane. Accordingly, we determined the distance between penetration marks from the histological reconstruction and devided the resulting value with the corresponding distance that had been measured in vivo using stereotaxic coordinates. In the following, all numerical values and scale bars of figures have been accommodated to the corresponding shrinkage factors: in cat 1 (area 18): 0.969, in cat 2 (area 17): 0.894.

Cortical Layers
Laminar borders were conventionally determined from sections cut perpendicular to the surface of the cortex. Since we analysed the labelling in tangential sections laminar boundaries could be defined with less certainty. Nevertheless, landmarks such as the presence of layer-specific cell types, the density of neuronal cell bodies, e.g. large pyramidal cells of lower layer III/upper IV and of layer Vb, and the density of myelinated axons were used. In addition to these morphological features the biocytin-labelled structures themselves and their distance from the cortical surface were also helpful.

Results

General Topography of Orientation Maps
As illustrated in Figure 1A, orientation selectivity changed gradually parallel with the cortical surface in accordance with previous findings (Hubel and Wiesel, 1963; Albus, 1975; Swindale et al., 1987). This change was occasionally abrupt with large jumps over short distances that possibly correspond to the locations of orientation centres previously described on the basis of optical imaging (Bonhoeffer and Grinvald, 1991, 1993). Obviously, the exact position of orientation centres could not be determined with our technique because mapping with
electrodes compromised the spatial resolution. Because our discontinuous orientation maps had a spatial resolution of 50–300 µm (Fig. 1A) we applied an interpolation procedure to improve the resolution, resulting in a smoother orientation map (Fig. 1B). We used the same procedure for areas 17 and 18 and displayed the preferred orientations with a resolution of 50 µm, closely matching the resolution of optical images. For the sake of simplicity, orientation preferences are represented by bars in Figure 1B instead of colour coding. Notice that the interpolated orientation map in Figure 1B contains regions where the orientation gradient is low and these regions are likely to represent orientation domains; at a few locations one can see the simultaneous occurrence of many orientations and these sites resemble orientation centres (Bonhoeffer and Grinvald, 1991, 1993). In the following, we use these interpolated orientation maps in evaluating the orientation topography of the labelled structures.

**Pattern of Biocytin Labelling**

In area 17, biocytin was injected into the lower division of layer III comprising the uppermost part of layer IV at a depth of 300–600 µm from the cortical surface (Fig. 2A). In area 18, the injection site occupied the middle of layer III at a depth of 300–500 µm from the cortical surface (Fig. 4A). The core regions had a lateral extent of 150 µm in area 17, and 80 µm in area 18. In each case, the halo zone had a short extent around the core region; hence, the labelled structures were readily recognized in the close vicinity of the injection site. Only in the core was the labelling obscured by the dark deposit of the reaction end-product.
In general, biocytin labelled neurons and fibres up to several millimetres from the centre of the injections. The majority of labelled somata were found within the central zone of the injection site with dendrites overlapping with the core region. These cells, some of which are shown in Figure 4A, probably became labelled directly via their soma or dendrites. In remote regions, only a few retrogradely labelled cell bodies were found, many of them showing medium to weak labelling. Weakly labelled somata could not be identified unequivocally as to neuron type (Fig. 4C). Of the few strongly labelled somata, virtually all had spiny dendrites and were considered to be pyramidal-type cells (Figs 2A, B and 4B). No distinction was made between pyramidal, star-pyramidal and spiny stellates cells in layer IV. In addition to spiny cells a small number of labelled neurons possessed smooth dendrites and invariably showed characteristics of basket cells (Fig. 2C, D).

The density and the strength of labelling of axons originating from the injection sites exceeded that of retrogradely labelled somata, indicating that biocytin was transported mainly anterogradely. That labelling of damaged axons made little, if any, contribution to the overall pattern is suggested by the absence of any significant number of faintly labelled somata in remote locations.

Qualitative inspection of the labelled axons revealed that they formed patches or clusters at quasi-regular intervals with a patch-to-patch distance of ~900 µm in area 17 and ~1200 µm in area 18. In Figure 2A, an example is shown for area 17 where the region delineated by arrowheads contains a labelled axon cluster at ~800 µm from the centre of the injection site.

**Morphological Distinction Between Excitatory and Inhibitory Axons**

To determine whether lateral excitatory and lateral inhibitory connections have a similar organization in areas 17 and 18 we established a set of morphological criteria by which we were able to divide the labelled structures into two populations. During this analysis we utilized the peculiar nature of extracellularly applied biocytin, namely that it marks chiefly large basket cells among the GABAergic cell types (Fig. 2C, D). Because in the cat visual cortex large basket cells represent the neuronal substrate for providing long-range lateral inhibitory connections, we had a tool whereby the topographical relationship between lateral excitatory and lateral inhibitory connections could be analysed in the same specimen. Accordingly, excitatory and inhibitory axons were distinguished using morphological characteristics of labelled axons as follows. Putative excitatory axons had sparsely distributed boutons along their course, the boutons were often small in size and frequently

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**Figure 3.** Morphological differences between putative excitatory and putative inhibitory axons in area 17. All pictures were taken from sections cut parallel with the surface of the cortex. In (A–C), axons and boutons (arrows) of large basket cells are shown. Characteristically, basket axons emit bursts of en passant boutonal swellings, many of which contact the somata of unlabelled neurons. Two such somata (asterisks) receiving multiple input from basket boutons are visible in (B) and (C). Representative examples for neuropil labelling in regions 2 mm laterally from the injection site (D) and in the vicinity of the injections site (E). In (D), the region contains exclusively excitatory axons that give off a mixture of en passant (arrows) and club-like boutons (arrowheads). In (E), the region contains excitatory and inhibitory (basket, arrows) axon types. Scale bars: (A, D, E) 20 µm; (B, C) 10 µm.
connected to the axon stem via a short stalk; finally, unlike inhibitory basket boutons, they did not establish multiple pericellular contacts with other neurons. Some of these features are illustrated in Figures 3D and 4D. On the other hand, axons of large basket cells gave off ‘bursts’ of boutons, typically 4–6, and often formed the typical basket-like multiple contact pattern with neighbouring neuronal somata (Figs 3A–C and 4E). Basket cell axons seldom gave off boutons connected via a short stalk with the parent axon. The boutons were often large and bulbous (1–1.5 µm, e.g. in Fig. 3B, C). During reconstruction all these micro-anatomical features were taken into account, together with the global appearance of the labelled axons and parent soma if the latter was available. Obviously, it was difficult to distinguish between excitatory and inhibitory axons in the neighbourhood of the injection site where a dense mixture of the two axon categories was found (Fig. 3E). Hence in the
immediate vicinity of the injection site our morphological criteria could not be unequivocally employed although the neuron type giving rise to labelled somata could still be determined.

**Topography of Lateral Excitatory and Inhibitory Connections**

In order to examine the topographic relationship between excitatory and inhibitory connections, all labelled boutons were taken into account and reconstructed from the entire depth of the cortex. In this way the synaptic destination of axons originating from the injection site could be determined accurately. The overall distributions of the labelled excitatory and inhibitory boutons are shown in Figure 5 for area 17, and in Figure 6 for area 18; they show a longitudinal extent of ∼6.5 mm for both regions. The patchy character of the labelling is readily seen in both areas. At least 20 distinct boutonal patches could be distinguished in area 17 and ∼15 in area 18. Qualitatively, there was no difference in the average patch-size (diameter 500–600 µm) between the two areas. However, there was great variation between individual patches within each area, the diameter ranging from 200 to 1000 µm. Moreover, the average patch-to-patch distance was smaller in area 17 (0.9 mm) than in area 18 (1.2 mm).

To examine the topographic relationship between the labelled excitatory and inhibitory boutons their distributions were displayed separately and are shown in Figures 7 and 8. The upper panels illustrate excitatory connections, and the lower panels inhibitory connections, within the same regions. It should be noted that many of the inhibitory boutons distal to the injection site were provided by a single basket cell in area 18 (lower panel in Fig. 8). For a better comparison, we used crossbars marking the centre of some of the excitatory boutonal patches and the same set of crossbars was superimposed onto the distribution of inhibitory boutons. The distributions of excitatory and inhibitory projections reveal three important features: (i) the excitatory network is 2–3 times larger in extent than the inhibitory network; (ii) the location of boutonal density maxima for the two systems differs, notably the distribution of inhibitory terminals does not entirely overlap with that of the excitatory terminals, inhibitory terminals tend to interleave and surround excitatory clusters; (iii) these features do not appreciably differ for the two areas 17 and 18. Another important point is that the accumulation of excitatory and inhibitory terminals was most dense at the injection site. According to the colour-coded logarithmic scales in Figures 7 and 8, the injection sites and their immediate neighbourhood contain at least 10 times more boutons per unit area than the most dense regions of any remote cluster; this is suggestive of powerful local processing. The relatively low density of inhibitory boutons at the very core of the injection site arises from the fact that here all labelled boutons were placed into the excitatory category, i.e. our morphological criteria for differentiating between excitatory and inhibitory boutons could not be used (see also above, and Figs 9 and 10). At the core the number of excitatory terminals is probably somewhat overestimated.

**Comparison Between Orientation Maps and Anatomical Density Maps**

Using the interpolated orientation maps and the digitized data on the distribution of excitatory and inhibitory terminals we made a quantitative calculation of their topographic relationship. Each orientation map covered approximately the central one-third of the respective anatomical map. They comprised 56% of all labelled boutons in area 17 and 73% of all labelled boutons in area 18 including the injection sites and surrounding zones up to 1.5 hypercolumn distances. Because of the more restricted lateral spread of inhibitory boutons a higher proportion (72.8 and 89.4% of all inhibitory boutons in areas 17 and 18 respectively) could be taken into account for the orientation analysis than was possible for excitatory counterparts (53 and 69.7% of all excitatory boutons in areas 17 and 18 respectively).

In the quantitative analysis, we first determined the overall orientation distribution of the terminals using a simple procedure: the entire orientation map was dissected into three orientation zones according to iso- (±30°), oblique- (±30–60°) and cross-orientations (±60–90°) with respect to the orientation preference at the injection site. The number of terminals in each zone was determined and the results are summarized in Table 1. In area 17, 53% of the excitatory boutons occupied...
iso-orientation sites, 30% were located at oblique-orientation sites and cross-orientation was represented by 17%. Inhibitory terminals showed a not very different distribution: 48% were found at iso-orientation sites, 28% occupied oblique-orientation sites and 24% were at cross-orientation sites. A similar trend was seen for the orientation distribution of terminals in area 18 (Table 1). This means that the orientation distribution of inhibitory boutons is slightly shifted towards non-iso-orientation values compared with excitatory boutons. To test whether these distribution values simply reflect the situation at the injection site (where the density of terminals is overwhelming) we carried out a calculation of the orientation distributions in which the injection site and its immediate neighbourhood — 500 µm radius around the injection centres (Table 1) — were omitted. Most values show only a small change in areas 17 and 18. The orientation distributions of remote excitatory and inhibitory boutons have a very similar tendency to that of more proximal projections, i.e. inhibitory boutons more frequently encounter non-iso-orientation sites than excitatory boutons. The only exception was found for remote excitatory boutons of area 17 which showed a 16% decrease in the iso-orientation category. We think that this is largely because in this area the biocytin injection was larger than in area 18, so that non-iso-orientation sites at interpatch zones were also densely innervated (see Figs 7 and 9).

Orientation maps have an inherent structure so that the preferred orientations gradually shift a full cycle for each hypercolumn distance. We asked with what frequency the labelled excitatory and inhibitory projections encounter different orientations along their course. This kind of relationship can be interpreted as the ‘impact’ of connections on different orientations as a function of lateral distance. We used a previously established procedure (Kisvárday et al., 1994) whereby concentric rings of 100 µm width were centred onto the injection site and the number of boutons was counted in each ring according to iso-, oblique- and cross-orientations. The results are shown in Figures 9 and 10 for areas 17 and 18 respectively. It is quite evident that iso-orientation excitation and iso-orientation inhibition is strongest in the vicinity of the injection site (leftmost graphs) and in the case of excitatory

Figure 7. Density distribution of presumed excitatory (upper panel) and presumed inhibitory boutons of basket cells (lower panel) in area 17 also shown in Figure 5. In both panels, crossbars indicate sites of boutonal density maxima of the patchy excitatory system. It is evident that inhibitory boutons tend to avoid excitatory patch centres, rather they occupy the rim of the excitatory patches as well interpatch zones.
connections in area 18 at one hypercolumn distance, which corresponds to the distance of proximal bouton patches. With respect to these bouton patches in area 17 it should be noted that the relatively large injection made in this area resulted in an increased occurrence of remote boutons in the oblique-orientation category (middle histogram for excitatory boutons at 800–900 µm lateral distances in Fig. 9). Oblique-orientation excitation and oblique-orientation inhibition is strongest primarily close to the injection site (middle graphs). Cross-orientation excitation is the weakest of all three excitatory categories and cross-orientation inhibition is strongest at half a hypercolumn distance (400–600 µm in area 17 and 500–700 µm in area 18).

**Precision of Orientation Specificity of the Patches**

To assess the precision with which the orientation specificity of patches was determined, two clusters from area 17 and two clusters from area 18 were chosen which had a complete coverage by their respective orientation map. The orientation preferences were then computed for every pixel in the interpolated orientation maps over a radius of 200 µm centred onto the highest boutonal density location within each cluster. It was found that the clusters had an average orientation range of ±20.0° with respect to the orientation preferences at the injection sites. According to our criteria these values represent iso-orientations. However, it should be noted that for an individual neuron iso-orientation means a range of orientations that evokes an increase in the discharge rate of the cell. Thus, while for broadly orientation-tuned cells (complex cells) an input representing 20° offset from its preferred orientation could still be considered as part of the optimal stimulus for sharply tuned or orientation-selective neurons: in some simple cells in layer IV such an offset could represent a non-optimal stimulus. In this respect the patchy excitatory network, even at the ‘heart’ of the patches, could mediate iso- as well as non iso-orientation effects.
Discussion
The focus of interest of the present study is whether lateral connections link regions representing similar or dissimilar orientations. In addition to this we wanted to know the topographic relationship between excitatory and inhibitory components of the connections. Therefore we used the neuronal tracer biocytin, which has the peculiar characteristic that it reveals inhibitory basket cells which provide the most extensive lateral axon arborizations (for review see Kisvárday, 1992) and excitatory pyramidal and spiny stellate cells. We carried out a direct comparison of the orientation topography between lateral inhibitory and lateral excitatory connections. The main finding is that the entire network, containing patchy excitatory connections up to 3–4 mm and non-patchy inhibitory
connections up to 1.5–2 mm, is less specific for orientation than has been thought (Mitchison and Crick, 1982; Ts'o et al., 1986; Gilbert and Wiesel, 1989). Quantitative assessments along the orientation map revealed that lateral excitatory and lateral inhibitory connections could communicate almost freely between regions possessing different orientation preferences.

Methodological Considerations

Biocytin as Antero-retrograde Tracer

In this study anterogradely labelled corticocortical connections were analysed. However, because of the presence of retrogradely labelled cells some axon terminals could have been labelled via retrograde rather than anterograde transport. Since retrogradely labelled neurons \( n = 49 \) in area 17 and \( n = 13 \) in area 18) with dendritic fields not overlapping with the injection sites represented only one-third of all labelled neurons \( n = 143 \) in area 17 and \( n = 69 \) in area 18) and because most of these neurons showed weaker labelling than most boutons, it is reasonable to assume that the bulk of the labelled boutons were indeed of anterograde origin. It is unlikely that the weakly labelled cells accounted for the strong bouton labelling.

Analysis of Patchy Labelling

A common procedure for outlining labelled patches is to draw their borders by hand using low-magnification microscopic images and a drawing tube. While this approach provides a relatively rapid evaluation of the labelling pattern, it is entirely based on subjective criteria as to assessing the border of single patches. We think that such a procedure provides a picture which overestimates the significance of patchy connections by introducing a threshold that underestimates the interpatch connectivity. The reason for this is the following. Retrograde tracers like fluorescent dextrans and WGA-HRP often result in weak soma labelling that could be readily overlooked in interpatch regions. On the other hand, using antergrade tracers such as biocytin also takes into account thick axons, although they emit no, or only a few, synaptic boutons, and so do not faithfully represent the synaptic weight of the projection. To overcome these difficulties we decided to reconstruct the position of each bouton emitted by labelled axons since they are distinct structures and represent the genuine site of synaptic transmission. There is, however, still the question as to how to identify labelled patches, where to draw their borders. Clearly, if we were to determine quantitatively the spatial extent of the patches we would have to set a baseline level for the density of the labelling. Once this is done the evaluation becomes quite simple, locations showing labelling densities higher than the baseline would correspond to patch regions and those below the baseline would correspond to interpatch regions. Unfortunately, no objective baseline exists and, even if it did, it would be likely to change from location to location since the average labelling density for the most part gradually decreases with distance from the injection site.

Previous Attempts

Other studies have tried to employ some kind of baseline level function. In area 17 of the cat cortex, Lübke and Albus (1992) and Albus and Wahle (1994) used a semi-quantitative threshold function for the density of labelled cells per unit area whereby discrete patches could be delineated. In their study, an arbitrary threshold function was chosen to provide the patches. In area 18 of the cat, Matsubara et al. (1987) used a simpler, though in principle similar, approach. In the above studies, slight changes in threshold levels would have resulted in different patch sizes. In another study, Malach et al. (1995) used digitized and filtered images taken from photographs of histological sections of monkey V1 that were evaluated with a boundary detection algorithm for delineating the borders of the patches. Yoshioka et al. (1996) employed a similar procedure in monkey V1; they determined presumptive boundaries for delineating cytochrome oxidase (CO) blob and interblob regions on the basis of average greyscale values. Again the threshold for detecting boundaries was arbitrarily chosen. A further uncertainty in these studies stems from the fact that because signals (greyscale intensity) deriving from labelled somata, dendrites and axons were pooled, their individual contributions remain unknown.

Our Own Attempt

We calculated the density of the bouton labelling on a pixel-by-pixel (50 × 50 µm) basis for excitatory and inhibitory projections over the entire range of orientations. In this way, we obtained a quantitative measure of the orientation specificity of the entire network irrespective of whether it contained orientation-specific patches. To make this analysis as simple as possible we dissected the orientation map into three equal zones — iso-, oblique- and cross-orientations with respect to the orientation preferences at the injection sites.

Pitfalls

One potential problem is that in our reconstruction of the entire cortical depth patches belonging to supragranular and
infragranular layers might be out of register. This could occur where orientation columns possess an oblique course, especially at convoluted regions or at sites which were mechanically damaged, resulting in a smearing in the overall labelling pattern. To eliminate any such problems only flat cortical regions were analysed. When we dissected the labelled projections according to supra- and infragranular segments (not shown) we found that they occupied the same anatomical regions in accordance with previous observations (Gilbert and Wiesel, 1983; Kisvárday et al., 1986; Matsubara et al., 1987). Another potential problem that could lead to errors in estimating the proportion of excitatory but not inhibitory connections to orientation categories derives from the anatomical observation that pyramidal cell axons terminate most frequently on spines of small dendrites (Kisvárday et al., 1986; Gabbott et al., 1987). Because small dendrites could be as far as 200–300 µm laterally from the parent soma the location of the presynaptic boutons reconstructed here might not truly represent the orientation preference of the contacted cells. To resolve this problem future studies will need to identify simultaneously excitatory projections with somata and dendrites of the target cells.

Lateral Extent of Excitatory and Inhibitory Projections
The extent of excitatory projections in areas 17 and 18 did not appreciably differ, the most distant patches being found at ~3 hypercolumns (~2.5 mm in area 17 and ~3.5 mm in area 18) from the centre of the injection. This is consistent with earlier observations made in areas 17 (Gilbert and Wiesel, 1989) and 18 (Matsubara et al., 1987; LeVay, 1988; Boyd and Matsubara, 1991). The lateral spread of inhibitory projections (~1.5 mm), however, is smaller than that reported by Albus and Wahle (1994) for area 17 and by Matsubara and Boyd (1992) for area 18. Both groups reported that retrogradely labelled inhibitory neurons could be encountered up to 3 mm in the adult cat, whereas in the present study these projections were typically no longer than 1.5–2 mm (Kisvárday and Eysel, 1993; Kisvárday et al., 1994; however, see our single-axon reconstructions that go for 2.5 mm as well in Kisvárday et al., 1993). Anatomical data from other species also indicate that the lateral spread of direct inhibition does not exceed the distance observed here (rat visual cortex area 17: 1.2 mm in McDonald and Burkhalter, 1993; monkey V1, V2: 1.5–1.7 mm in Kritzer et al., 1992). In this respect, a reasonable explanation for the discrepancy between our findings and those of Albus and Wahle (1994) and Matsubara and Boyd (1992) is that they used much larger injections, 800–1000 µm core diameter, giving rise to more widespread labelling.

Is the Lateral Network Specific for Orientation?
Interestingly, there is consensus in the literature that the patchy system in the visual cortex favours regions of similar orientation despite the fact that the evidence to support this is based entirely on qualitative and semi-quantitative observations. Here we have attempted to give a quantitative measure of the precision of lateral connections in orientation. Our results clearly demonstrate that the population of lateral excitatory connections is not as specific for iso-orientation as previously suggested (Mitchison and Crick, 1982; Gilbert and Wiesel, 1989). This is partly explained by the well-known, but still often ignored, fact that the projections are not restricted to patches but occupy virtually the entire area within their reach. That is, interpatch regions are also heavily innervated, predominantly at distances <1 mm or one hypercolumn (see Figs 5 and 6). Another indication for the weakly orientation-selective nature of the lateral network derives from optical imaging studies in the cat (Bonhoeffer and Grinvald, 1991, 1993), namely the lateral dimensions of orientation domains are often not commensurate with those of the anatomically labelled patches. While orientation domains usually do not exceed 400–600 µm in diameter, the size of anatomically detected patches could vary from 200 to 1000 µm. As a consequence a range of orientations, ±20–90°, might be represented within an individual patch.

Comparison with Previous Data
Our data on the orientation specificity of connections support the conclusions reached by Matsubara et al. (1985, 1987), though they are not as clear cut. They found that in area 18 of the cat patchy connections selectively link columns representing non-iso-orientations including cross-orientations. Our data from the same area show that this is not entirely the case because the excitatory network revealed here is biased towards iso-orientations, and non-iso-orientations are represented to a smaller, though significant, extent. With regard to lateral inhibitory connections, in both areas 17 and 18, they broadly follow the orientation distribution of the excitatory network, though with a consistently weaker commitment to iso-orientations.

Our findings demonstrating weak orientation specificity of lateral connections are supported by data obtained in primates. In a recent anatomical study, Malach et al. (1993, 1994) showed results comparable to ours using optical imaging and subsequent bulk injections of biocytin. Although their evaluation procedure differed from ours, as noted above, they estimated that half of the patchy connections were at non-optimal orientations (see Fig. 5D in Malach et al., 1993). In the present study, a very similar proportion of lateral connections was identified at non-iso-orientations (see values of oblique-orientation + cross-orientation in Table 1). The weak iso-orientation bias shown here and those values reported by Malach et al. (1993, 1994) are in conflict with electrophysiological cross-correlation results which emphasize strict iso-orientation connectivity (Michalski, 1983; Ts' o et al., 1986). However, the cross-correlation measurements carried out by Hata et al. (1991) are more in accordance with our iso-orientation-biased lateral connections. In Figure 8 of their report, about one-quarter of the excitatory linkages were detected between cell pairs having differences in their optimal orientations of >30°. This figure would be expected given the broad orientation coverage of the connections presented here. In addition, they found that inhibitory interactions between pairs of cells were most frequent when their orientation preferences slightly differed up to 45°. Again, this is consistent with our data showing that the majority of inhibitory connections has a larger share from non-iso-orientations than do excitatory connections. This difference between excitatory and inhibitory links is quite obvious in the density distributions shown in Figures 7 and 8 and could be important for the function(s) of the network.

Finally, the present findings raise the question as to whether ‘low specificity’ of lateral connections exclusively concerns orientation selectivity maps? In the light of available data it seems unlikely that any other attributes would utilize highly selective lateral projections. Well-known systems, like ocular dominance columns and CO blobs/interblobs, are linked by connections exhibiting only a moderate specificity. For example, in the primate V1, Yoshioka et al. (1996) found that 46% of connections originating from ocular dominance bands of one eye are linked to ocular dominance bands of the other eye. The
same study revealed a similar trend for the compartments of the CO system, suggesting a less strict segregation than previously thought (Livingstone and Hubel, 1984). Further experimental evidence for ‘low specificity’ of lateral connections was provided by Levitt et al. (1994). They studied the connectivity rules between CO compartments in monkey V2 and found 75% specificity between connections from CO-rich zones to other CO-rich zones while the CO-poor zones projected to other CO-poor zones randomly. These results are consistent with our findings in areas 17 and 18 of the cat.

**Functional Considerations**

**Orientation Selectivity**
The most important features of the networks revealed here are that lateral excitatory connections possess only a moderate bias to similar orientations, and that inhibitory connections – within their limited extent – provide more input to non-iso-orientations than do excitatory connections. A plausible interpretation of these virtually pan-oriented excitatory and inhibitory connections might be that they are not involved in mechanisms of orientation selectivity. We think that this is unlikely. First, lateral excitatory connections show a bias to similar orientations which could reinforce the orientation bias carried by the incoming thalamic afferents. Furthermore, inhibitory connections with even less orientation selectivity could serve for sharpening orientation tuning via controlling the contrast gain of the thalamic input (Bonds, 1989; Eysel and Shevelev, 1994). In conjunction with the above data, recent simulations on horizontal network properties indicated that populations of neurons with ‘low anatomical specificity’ could indeed give rise to ‘high functional specificity’ (Wörgötter et al., 1991). Secondly, experiments using pharmacological manipulation of lateral connections – e.g. selectively turning inhibitory mechanisms on and off – demonstrated the presence of orientation-selective influences between remote locations (Eysel et al., 1990; Crook and Eysel, 1992; Crook et al., 1997).

Further implications for the possible functional role of the iso-orientation-biased horizontal network derive from experiments using complex visual stimuli. Shevelev et al. (1995) reported that in the cat visual cortex about one-third of cells increase their responses to the presentation of specific cross-like figures. In another study, they found visual cortical units displaying double-orientation tuning (Shevelev et al., 1994). These findings were interpreted as excitatory convergence of orthogonally oriented neurons for which the network presented here for areas 17 and 18 provides numerous examples.

**Possible Effects from Beyond the ‘Classical’ Receptive Field**
While these various effects represent interactions within the neurons’ classical receptive fields, recent optical imaging data indicate interactions at a much larger scale. For example, small, point-like stimuli activate cortical regions that are up to 10 times larger than that defined by spiking activity of neurons for the same stimuli (Grinvald et al., 1994; Das and Gilbert, 1995). The extent of such subthreshold responses corresponds well with the overall extent of horizontal connections. Functionally, they may become unmasked following damage to the visual pathway (Gilbert and Wiesel, 1992; Das and Gilbert, 1995) or in complex stimulus situations when the stimulus simultaneously affects regions outside the neurons’ receptive fields. Interestingly, the orientation specificity of these long-range effects suggests a close relationship with the orientation specificity of our networks in areas 17 and 18 of the cat. In the study of Gilbert and Wiesel (1990), a conspicuously large proportion of cells showed increased responses to surround stimuli which represented non-optimal orientations up to 45° with respect to the receptive field centre of the same cells. Results from primate V1 confirm and extend the cat data in that long-range excitatory interactions are not restricted to columns of similar orientations. A plausible interpretation of the results is that non-iso-orientation connections are likely to be involved in mechanisms for representing orientation discontinuities such as corners or junctions across visual space (Sillito et al., 1995). Again a simple link between such electrophysiological findings and our data is that the former could have been mediated by a network of many different orientations described here.

**Conclusions**
In the present study we have analysed the topographic relationship of lateral excitatory and lateral inhibitory connections with reference to the topography of orientation selectivity in visual cortical areas 17 and 18 of the cat. Direct comparison between the two connectivity systems and the orientation maps revealed that the topography of excitatory connections slightly differs from the topography of inhibitory connections in a manner related to distance. This leaves room for considerable cross-talk between different functional compartments, or in the case of orientation selectivity maps, between regions of different orientations.

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**References**


