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RESEARCH NOTE

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# Local lateral connectivity of inhibitory clutch cells in layer 4 of cat visual cortex (area 17)

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**Abstract** To characterise spatially a major component of the anatomical basis of local lateral inhibition in layer 4 of cat visual cortex (area 17), we analysed the lateral distribution of neuronal somata postsynaptic to electrophysiologically characterised GABAergic clutch (basket) cell axons (CC1 and CC2). We report two main results. First, the clutch cell axons appear to show isotropic lateral connectivity near their cell body (less than 50 µm radius), but beyond this core region they show anisotropic lateral connectivity, preferring particular angular sectors around their cell body. Second, we estimated the probability of lateral connection for each axon arbor as a function of radial distance from the parent soma. We found that this radial function has a brief rising phase, to a peak at 30–45 µm, and a longer, exponential decaying phase, with a space constant of around 50 µm. The shape of the radial connection probability function suggests that most lateral inhibitory connections of clutch cells are formed with neurons in nearest-neighbour cortical columns. Taken together, the results suggest that these individual layer-4 clutch cell axons may inhibit all (isotropic) nearest-neighbour cortical columns with a relatively high probability of connection, but outside this core region may provide a type of anisotropic lateral inhibition of cortical columns with a radially decreasing probability of connection.

**Keywords** GABA · Receptive-field · Lateral network · Basket cell · Neuroinformatics · Cat

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## Introduction

Lateral inhibition modulates the spatiotemporal response of visual cortical neurons to sensory stimulation (see Eysel 1992; Fitzpatrick 2000). The nature of this modulation depends on the spatial pattern of connections of individual GABAergic inhibitory neurons as well as their physiological effect on postsynaptic cells. In general, most inhibitory synaptic inputs to a cortical neuron are believed to originate locally (≤250–500 µm radius; Kisvárday et al. 1997; Crook et al. 1998; Roerig and Kao 1999) and from a variety of GABAergic cell types (see Somogyi 1989; Tamás et al. 1997; Gupta et al. 2000). In the cat visual cortex, layer 4 is the main termination zone for dorsal lateral geniculate nucleus (dLGN) afferent axons (see Peters and Payne 1993) and represents the first stage of cortical processing, where many of the receptive field (RF) characteristics have been shown to depend on GABAergic inhibition (for reviews, see: Sillito 1984; Vidyasagar et al. 1996; Sompolinsky and Shapley 1997). However, there has been no quantitative analysis of the lateral distribution of connections of any type of GABAergic cell axon in layer 4. This information is essential in order to understand the purpose of lateral inhibition in the early cortical processing of visual information.

The aim of this study was to characterise local lateral inhibitory interactions in layer 4 of cat visual cortex through a quantitative analysis of the lateral connections of individual clutch cell (a type of basket cell) axons obtained in vivo. This type of cell, which may represent up to a third of all GABAergic neurons in layer 4 of cat area 17 (Budd 2000), preferentially synapses with both the cell body and proximal dendrites of a cortical neuron (see Kisvárday 1992). Importantly, perisomatic inhibition has been shown to control the firing time of postsynaptic spiny cells (Cobb et al. 1995; Tarczy-Hornoch et al. 1998). Inferred from this, the somatic contacts by basket cells may control the output firing response of layer-4 simple cells. On the other hand, their proximal dendritic contacts may regulate geniculate-based excitation to the same spiny cells (Kisvárday et al. 1985; So-

mogyi 1989; Kisvárday 1992; Budd 2000). Thus, clutch cell lateral inhibition may control spatiotemporally both the input stimulus signal to and the output firing response of layer-4 simple cells.

We examined only the somatic connections of clutch cell axons, because these precisely locate the position of the postsynaptic cell body. This approach is more accurate than an analysis of the bouton distribution alone, because many basket cell synapses are formed with dendrites (see Somogyi 1989; Kisvárday 1992) and these contacts may be up to a hundred or more microns away from the postsynaptic cell body. Preliminary results have been presented previously in abstract form (Budd and Kisvárday 2000).

## Materials and methods

We analysed two layer-4 clutch cell axons previously obtained from two adult cats in the binocular region of cat area 17. The morphology and synaptology of these horseradish peroxidase (HRP)-labelled cells, designated CC1 and CC2, have been reported previously (Kisvárday et al. 1985). In the original study, the spatial position of each presumed postsynaptic soma, based on light microscopy of labelled boutons apposed to somata, was recorded as a three-dimensional vector co-ordinate relative to the clutch cell soma (origin). Electron microscopy of a random sample of 29 somata revealed that of two to five labelled boutons apposed to each soma at least one formed a synapse (see Kisvárday et al. 1985). We have therefore assumed that all somata apposed to boutons of clutch cell axons are postsynaptic targets (i.e. receive at least one synapse).

To study lateral connectivity, we projected the three-dimensional (3D) distribution of postsynaptic somata contacted by each clutch cell axon onto a 2D plane parallel to the cortical surface (see Fig. 1). For each layer-4 postsynaptic soma, we measured (1) the vector angle (in degrees of arc) relative to anteroposterior axis, and (2) the 2D Euclidean distance relative to the clutch cell soma. We used this information to examine the spatial structure of clutch cell lateral connectivity in layer 4.

We estimated the probability of connection as a function of radius for each clutch cell in the following way. Viewed from the cortical surface, we considered a series of concentric circles, centred on a clutch cell body, whose radius, $r$, increased at a constant rate, $\Delta r$. Each 2D circle represents the cross-section of a 3D cylinder of neuropil encompassing the entire depth of layer 4. The raw distance distribution was binned at a width $\Delta r$ to give the number of postsynaptic cells per shell. The total mean number of neurons per shell was estimated from the volume of the shell multiplied by the mean neuronal density of layer 4 in the binocular region of cat area 17, $\bar{N}_V = 54000\,\text{cells}\,\text{mm}^{-3}$ (Beaulieu and Colonnier 1983). The volume of each shell, $V_{\text{shell}}$, was found from the difference between the volume of consecutive cylinders: $V_{\text{shell}}=V_{\text{cylinder}}(r+\Delta r)-V_{\text{cylinder}}(r)$, where $V_{\text{cylinder}}=2\pi r^2 h$, and the mean thickness of layer 4 of cat area 17, $h$=0.5 mm (Beaulieu and Colonnier 1983). Note these anatomical parameter values are subject to verification by unbiased stereological methods. The probability of connection by a clutch cell axon per shell, $p_i$, was estimated using the equation (see Abeles 1991):

$$p_i = n_i/N_i \tag{1}$$

where $n_i$ is the number of postsynaptic somata in shell i, and $N_i$ is the mean total number of neurons in shell i, $N_i = V^i_{\text{shell}}\bar{N}_V$. This calculation assumes that every soma per shell has an equal chance of contact by the axon and that the local neuronal density within each shell is close to the value of $\bar{N}_V$.

The mean probability of connection by a clutch cell axon, $\bar{p}$, was found using the formula (see Abeles 1991):

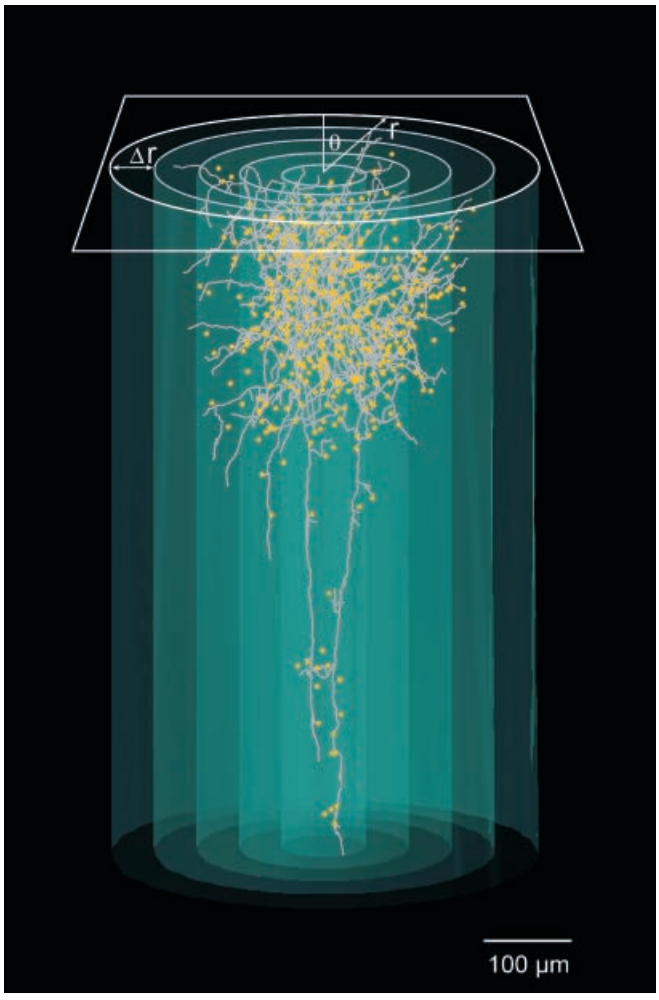


**Fig. 1** A three-dimensional reconstruction of a clutch cell CC1 axon viewed close to the frontal plane (see Kisvárday et al. 1985) shows the cortical surface angle, θ, and radius, $r$, measurements used to analyse the spatial distribution of postsynaptic somata (*yellow dots*). Concentric cylinders centred on the clutch cell body create a series of cylindrical shells each of width $\Delta r$. The raw distance distribution as a function of radius is obtained from the number of postsynaptic somata per shell

$$\bar{p} = n_{\text{total}}/(V_{\text{total}}\bar{N}_V) \tag{2}$$

where $n_{\text{total}}$ is the total number of postsynaptic somata per arbor (CC1, $n_{\text{total}}$=434; CC2, $n_{\text{total}}$=311), and $V_{\text{total}}\bar{N}_V$ is the total number of neurons within the maximum volume of the arbor ($V_{\text{total}}$), which was taken as the cylinder with a radius given by the most distant postsynaptic soma. Note that the overall mean across $p_i$ need not equal $\bar{p}$, as different sample spaces are involved.

Curve fitting, and principal component and regression statistical analyses were performed using the Matlab Statistical Toolbox (Mathworks, Natick, Mass., USA).

## Results

### Cortical surface distribution

To understand the lateral connectivity of clutch cells, we examined the spatial distribution of postsynaptic somata

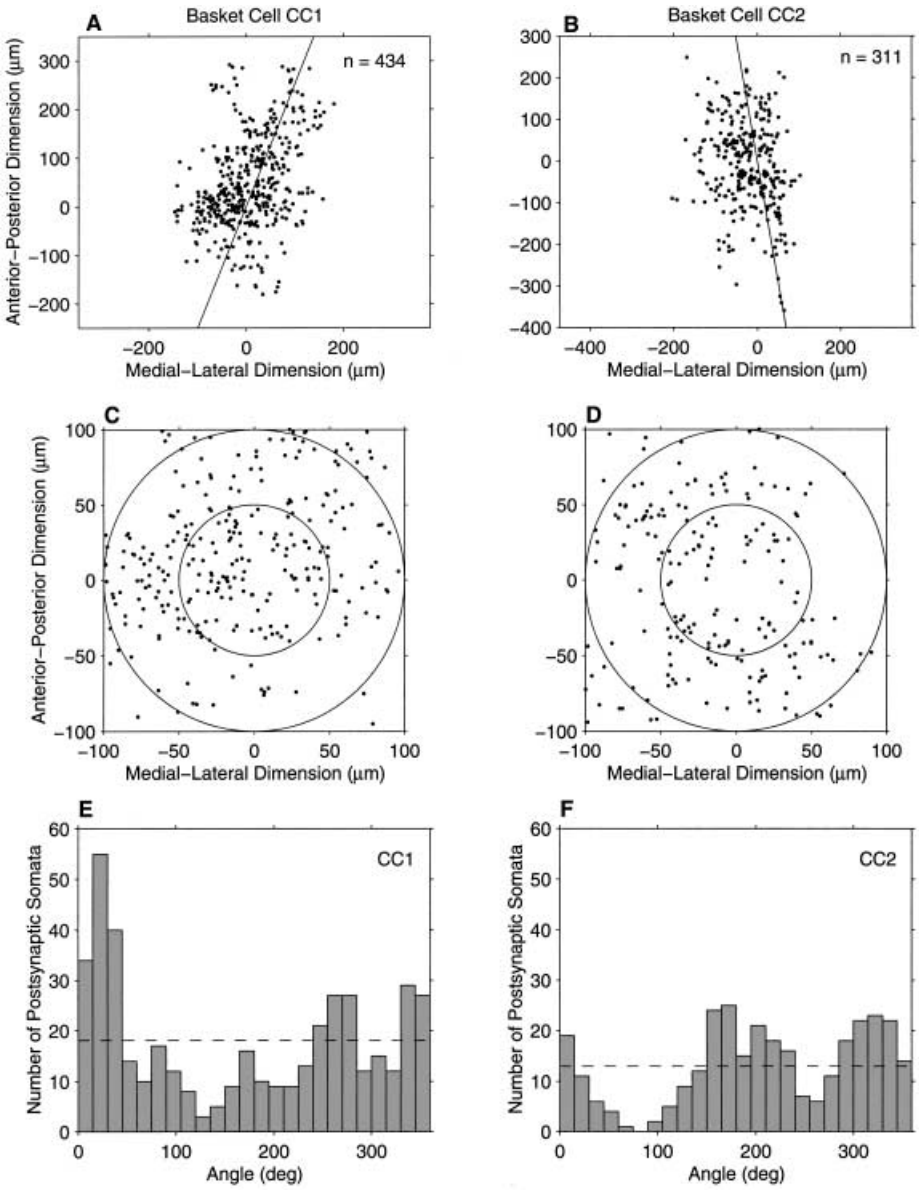


**Fig. 2A–F** Lateral connectivity of clutch (basket) cell axons as viewed from the cortical surface. **A, B** Surface distribution of postsynaptic somata (*filled dots*) relative to clutch cell body (at origin) for each clutch cell axon: **A** CC1; **B** CC2. Both axons show an oriented distribution (*line* indicates the principal axis of variance with respect to saggital plane for CC1=21° and CC2=170° relative to A-P axis). **C, D** Magnified local surface distribution of postsynaptic somata around each clutch cell body (inner circle 50 µm radius, outer circle 100 µm radius): **C** CC1; **D** CC2. **E, F** Overall angular distribution of postsynaptic somata for clutch cell axons: **E** CC1 and **F** CC2 relative to anterior-posterior axis. Both distributions are multimodal. The *dashed line* shows the mean number of somata per angular sector. Bin size 15°

as viewed from the cortical surface (Fig. 2). Principal component analysis (PCA) of the 2D spatial distribution quantified earlier qualitative observations that the connectivity pattern of both cells was oriented (Kisvárday et al. 1985): the principal axis of variance with respect to sagittal plane for cell CC1 was 21° (accounts for 75% of total variance), and cell CC2 was 170° (79% of total variance; Fig. 2A, B). For both clutch cells, most postsynaptic somata were located within a 100-µm radius of the parent soma (see Fig. 2C, D). Proximal to the clutch cell body (0–50 µm radius), the angular distribution of postsynaptic somata (number of postsynaptic somata as a function of angle) for both cells was consistent with an isotropic distribution (CC1: $\chi^2_{obs}$=16.86, *df*=11, *P*>0.1; CC2: $\chi^2_{obs}$=9.20, *df*=7, *P*>0.1), but in the next-nearest shell (50–100 µm radius), the angular distribution was significantly different from an isotropic distribution (CC1: $\chi^2_{obs}$=36.33, *df*=11, *P*<0.01; CC2: $\chi^2_{obs}$=36.48, *df*=7, *P*<0.01). In addition, the overall angular distribution was clearly anisotropic for both cells (Fig. 2E, F): for cell CC1, a large peak around 20°, with a smaller secondary peak around 260° (Fig. 2E), and, for cell CC2, peaks near 170° and 330°, and a strong dip to zero at about 80° (Fig. 2F). These results suggest a difference in the degree of specificity between the local (isotropic) and more distant (anisotropic) patterns of lateral inhibitory connections made by these individual layer-4 clutch cell axons.

## Probability of connection estimates

The raw number of postsynaptic somata per clutch cell axon as a function of radius from the presynaptic cell body (origin) showed a similar qualitative profile for both cells: a relatively short, early rising phase reaching

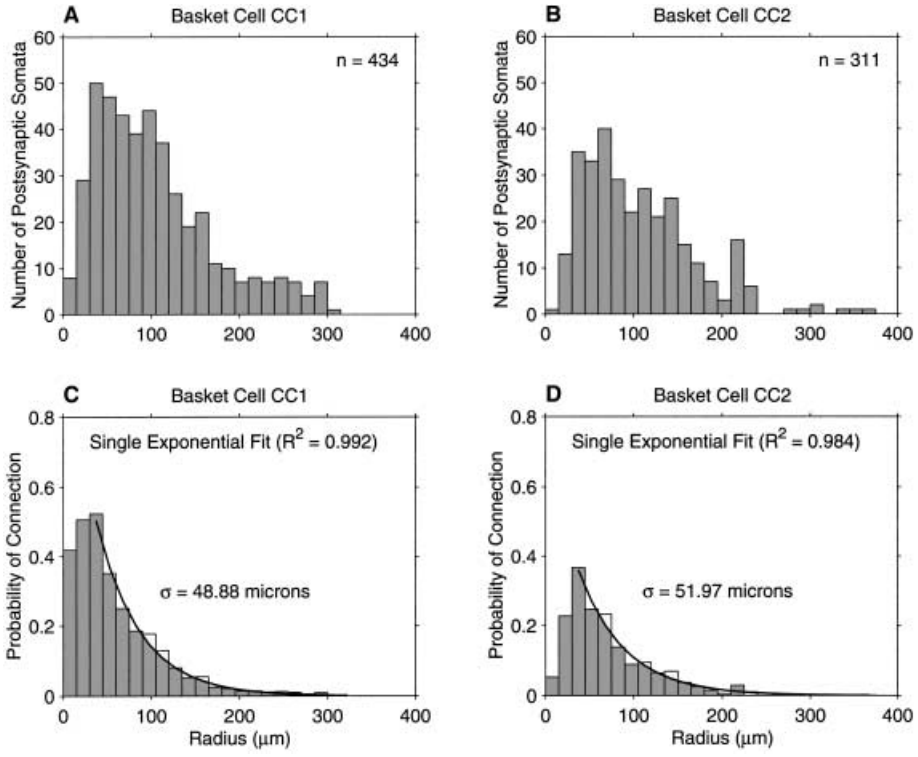


**Fig. 3A–D** Probability of connections estimates for clutch cell axons as a function of radius. (**A, B**) Raw number of postsynaptic somata (Δ*r*=15 μm): **A** CC1; **B** CC2. **C, D** Probability of connection estimates for: **C** CC1, and **D** CC2, which is clearly decreases exponentially (*fitted line*) from peak value (CC1 and CC2, 30–45 μm radius) with a space constant σ(CC1)=48.88 μm and σ(CC2)=51.97 μm

a peak followed by a longer, irregular decaying phase (Fig. 3A, B). For both clutch cells, the magnitude of connection probability estimated per shell close to the presynaptic cell body was high (Fig. 3C, D), especially within an ~100-μm radius. This radius, equivalent to the width of around two minicolumns in cat area 17 (50 μm diam.; Peters and Yilmaz 1993), suggests that the highest probability of connection is mostly for nearest-neighbour or next-nearest-neighbour minicolumns. The position of the peak connection probability was the same for the two cells at 30–45 μm radius for CC1 ($p$=0.52) and CC2 ($p$=0.37). The magnitude difference is because cell CC2 contacted fewer proximal postsynaptic somata than cell CC1. Over the full extent of the axon arbor, we obtained a much lower mean probability of connection (using Eq. 2), $\bar{p}(CC2) = 0.039$ and $\bar{p}(CC2) = 0.039$. That is, a layer-4 clutch cell axon contacted on average around 4–5% of all cells within its arbor. But when only cells at, for example, a radius of 15–30 μm were considered, a clutch cell was estimated to contact on average around 23% (CC2) to 51% (CC1) of all available cells, roughly an order of magnitude greater than the mean estimate.

The probability of connection for each axon decreased from a peak value as an exponential function of radius from the clutch cell body (Fig. 3B). For a mono-exponential function of the form $y(r)=k\ \exp(-r/\sigma)$, the rate of decrease was similar for both cells, with a space constant of σ~50 μm (Δ$r$=15 μm: CC1, σ=48.88 μm, $R^2$=0.992; CC2, σ=51.98 μm, $R^2$=0.984). The peak position and degree of fit of the decaying phase of the distribution from the peak value were relatively insensitive across a range of shell (bin) widths.

## Discussion

The sample analysed here represents two-sevenths of all reconstructed cat striate visual cortex clutch cells (Kisvárday et al. 1985; Gabbott et al. 1988; Ahmed et al. 1997). At present, 3D distributions of postsynaptic somata are only available for these two clutch cells, as the remaining cells have been sectioned for electron microscopy. Although we have only examined two layer-4 clutch (basket) cell axons, the qualitatively similar characteristics of their lateral connectivity suggest the results may be used to draw some preliminary conclusions until more clutch cells are recovered. First, the clutch cell axons appear to display isotropic lateral connectivity near their cell body (less than 50 μm radius), but beyond this local region and over the entire extent of their arbor show anisotropic lateral connectivity, preferring specific angular sectors around their cell body (Fig. 2). Superficial- and deep-layer large basket cells with long, horizontal collateral axons (more than 1 mm) also appear to contact specific angular or "wedge-shaped" sectors (Kisvárday and Eysel 1993; Kisvárday et al. 1994), suggesting that overall anisotropic connectivity may be a common organising principle for single basket cell axons. However, it is not known whether large basket cells have a local region of isotropic lateral connectivity. Second, after a brief rising phase, the estimated probability of connection from a peak value decreases exponentially as a function of radius (Fig. 3C, D). The overall shape of the radial connection probability distribution is reminiscent of the inhibitory portion of the difference-of-gaussians (DoG) kernel function used to describe lateral inhibitory interactions in cor-

tical models (Sabatini and Solari 1999). The estimated peak connection probability (30–45 µm radius) coincides with the isotropic lateral connectivity region of the clutch cell axons (less than 50 µm radius). Taken together, these results suggest that these individual layer-4 clutch cell axons may inhibit all (isotropic) nearest-neighbour cortical columns with a relatively high probability of connection, but outside this local region may provide a type of anisotropic lateral inhibition of cortical columns with a radially decreasing probability of connection.

### Differences between clutch cells

Although the differences in the radial connection probability profile between cell CC1 and CC2 axons may well be due to interanimal variability, they may also be at least partly explained by experimental factors. For cell CC2, only the axon was recovered and neither the cell body nor dendrites were filled with HRP (see Kisvárday et al. 1985). There was also some tissue bulging following the withdrawal of the recording pipette, but this affected chiefly the superficial layers, where only a few axon collaterals were located, with the distortion mainly along the dorsoventral axis. The axon branch tips were strongly filled with HRP, but it is possible that some minor proximal axon collaterals emerging from near the cell body may not have been filled. The cortical surface position of the cell body of CC2 was estimated from the clear radial orientation of the main axon projected onto the cortical surface (see Fig. 3 by Kisvárday et al., 1985), assuming its cell body lies within the same narrow column of cells as the main axon. However, the tilting angles used for the axon reconstructions of CC1 and CC2 to be viewed from the cortical surface could not be determined unambiguously. These angles were calculated on the basis of nearby apical dendrites that were visible in the osmicated sections and assumed to run perpendicular to the pial surface. Either the loss of a few minor proximal axon collaterals for CC2 or differences in tilting angles may explain why cell CC2 accounts for fewer proximal postsynaptic somata than cell CC1. Nevertheless, there was a clear similarity in the qualitative spatial connectivity characteristics of these two cells taken from different animals and under in vivo conditions where the whole cell can be recovered.

A preliminary analysis of the postsynaptic somatic targets of a nearly fully recovered area 18 clutch cell axon (Buzás et al. 2001) supports the main qualitative results reported here regarding arbor anisotropy and the exponential decay in connection probability as a function of radius, but it was not included here due to the obvious incompleteness of its axon.

### Functional implications

The specific spatial pattern of lateral connectivity of a clutch cell axon may relate to its specific function within the local cortical circuitry. Mainly through local columnar interactions, the pattern of connections is likely to subserve a number of different receptive field effects, including subfield antagonism, orientation and direction selectivity (see Sillito 1984). Both layer-4 clutch cell axons share a similar-shaped probability of connection distribution as a function of radius, but this distribution is anisotropic except within a 50-µm radius of the parent cell body. The simplest interpretation of these observations is that, while the general shape of the radial profile may be cell type-specific and RF invariant, outside 50 µm radius the spatial distribution of connections within each shell may be function-specific so that particular sectors are more strongly targeted (e.g. iso-orientations). Unfortunately, we do not have functional maps with which to test this hypothesis or to determine whether any relationship exists between the axon arbor orientation and stimulus orientation preference maps. However, most physiological evidence suggests that orientation tuning involves isotropic lateral inhibition with a bias towards iso-orientations (Hata et al. 1988; Bonds 1989; Douglas et al. 1991; Das and Gilbert 1999), though there is evidence for anisotropic lateral inhibition in direction selectivity (Crook et al. 1998; Roerig and Kao 1999). Within the isotropic region of lateral inhibitory connections, the high connection probability of clutch cell axons may lead to mutual suppression between adjacent cortical columns. Similar to the retina, this arrangement of recurrent lateral inhibition could, at a high resolution, enhance the spatial and temporal contrast between visual features (e.g. lines or edges) in order to improve the discrimination of complex stimulus patterns (Nothdurft et al. 1999).

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