Local Potential Connectivity in Cat Primary Visual Cortex

Time invariant description of synaptic connectivity in cortical circuits may be precluded by the ongoing growth and retraction of dendritic spines accompanied by the formation and elimination of synapses. On the other hand, the spatial arrangement of axonal and dendritic branches appears stable. This suggests that an invariant description of connectivity can be cast in terms of potential synapses, which are locations in the neuropil where an axon branch of one neuron is proximal to a dendritic branch of another neuron. In this paper, we attempt to reconstruct the potential connectivity in local cortical circuits of the cat primary visual cortex (V1). Based on multiple single-neuron reconstructions of axonal and dendritic arbors in 3 dimensions, we evaluate the expected number of potential synapses and the probability of potential connectivity among excitatory (pyramidal and spiny stellate) neurons and inhibitory basket cells. The results provide a quantitative description of structural organization of local cortical circuits. For excitatory neurons from different cortical layers, we compute local domains, which contain their potentially pre- and postsynaptic excitatory partners. These domains have columnar shapes with laminar specific radii and are roughly of the size of the ocular dominance column. Therefore, connections between most excitatory neurons in the ocular dominance column can be implemented by local synaptogenesis. Structural connectivity involving inhibitory basket cells is generally weaker than excitatory connectivity. Here, only nearby neurons are capable of establishing more than one potential synapse, implying that within the ocular dominance column these connections have more limited potential for circuit remodeling.

Keywords: excitatory, inhibitory, interlaminar connectivity, morphology, neurogeometry

Introduction

Complete understanding of cortical function seems impossible without a comprehensive account of synaptic connectivity in cortical circuits. Although synaptic connectivity in smaller invertebrate circuits can be fully determined electrophysiologically (Harris-Warrick 1992) or electron microscopically (White et al. 1986; Chen et al. 2006), these techniques lack the capacity to reconstruct connectivity on the scale of the cortical thickness (but for a promising approach, see Denk and Horstmann 2004). An alternative and long recognized approach, which we call neurogeometry, is to use the geometry of neuronal arbors reconstructed by light microscopy to calculate the number of touches between axonal and dendritic branches of 2 neurons, which are necessary to establish synapses between them (Ramón y Cajal 1891; Sholl 1956; Szentágothai 1975; Gilbert and Wiesel 1981; Gilbert 1983; White and Keller 1989; Abeles 1991; Braitenberg and Schüz 1998; Kalisman et al. 2003, 2005; Lübke et al. 2003; Binzegger et al. 2004; Amirikian 2005; Shepherd et al. 2005) (for review, see Chklovskii et al. 2004; Stepanyants and Chklovskii 2005).

Using neurogeometry to describe connectivity may be particularly suitable for vertebrate and, especially, cortical circuits because the resulting description is statistical (or probabilistic) rather than deterministic. The statistical nature of this method is due to the fact that touching axons and dendrites (as judged by light microscopy) do not necessarily make functional synapses and that only a few neurons can be reconstructed in one preparation, requiring combining data from different animals. Coincidentally, a statistical description of connectivity seems appropriate for vertebrate and, especially, cortical circuits. Indeed, cortical neurons are not identifiable individually from animal to animal, and connectivity in the cortex is likely to vary among different animals. Furthermore, synaptic connectivity within one animal may vary over time due to growth and retraction of dendritic spines (Lendvai et al. 2000; Trachtenberg et al. 2002; Holtmaat et al. 2005), precluding time invariant deterministic description of connectivity at the level of single synaptic connections.

We base a geometric description of synaptic connectivity on the concept of a potential synapse (Stepanyants et al. 2002). A potential synapse is a location in the neuropil where an axon of one neuron passes within distance s of a dendrite of another neuron so that an actual synapse can be formed. Distance s depends on the type of synapses that can exist between given neurons. For synapses on spines, this distance is on the order of the sum of the average spine length and axonal and dendritic radii (typically 2 μm [Peters and Kaiserman-Abramof 1970; Spacek and Hartmann 1983; Anderson et al. 1994; Harris 1999]). For shaft synapses and gap junctions, it is roughly equal to the sum of the dendritic and/or axonal radii (0.4–1.0 μm [Peters et al. 1991; Braitenberg and Schüz 1998]). For synapses made on terminal boutons, their average length determines the value of distance s. Because the expected number of potential synapses between neurons scales linearly with distance s (Stepanyants et al. 2002), it can be recalculated if necessary for different numerical values of this parameter.
What is the relationship between potential and actual synapses? Clearly, a potential synapse is a necessary although not a sufficient condition for an actual synapse. Therefore, if no potential synapses are expected between a pair of neurons, there can be no actual synapses. Generally, the number of actual synapses is a fraction of the number of potential synapses. The value of this fraction for a given projection can be found from other considerations and used to predict the number of actual synapses (Stepanyants et al. 2002). Finally, predicted potential connectivity for several intracortical projections correlates strongly with functional connectivity measured by laser scanning photostimulation (Shepherd et al. 2005).

In this paper, we find that, although potential synaptic connectivity exhibits strong laminar specificity, most excitatory neurons separated laterally by a few hundred microns are potentially connected (share potential synapses). Hence, potentially pre- or postsynaptic partners of a given excitatory neuron belong to a few hundred micron columnar domain of potential connectivity spanning the thickness of the cortex. In the cat, the scale of a few hundred microns is related to the size of cortical ocular dominance (Kaschube et al. 2003) or iso-orientation (Rao et al. 1997; Kaschube et al. 2002) columns. Therefore, potential connectivity in an ocular dominance or iso-orientation column is nearly all-to-all, and most excitatory neurons in these columns can establish a synaptic connection by local spine growth. Then, the number of achievable local circuits among excitatory neurons is limited only by the space available to form new synapses (Chklovskii et al. 2002).

Domains of potential connectivity between axons of inhibitory basket cells and dendrites of excitatory neurons are smaller in size and often do not extend throughout the cortical thickness. The expected numbers of potential synapses received by inhibitory basket cells from other excitatory neurons or basket cells are typically less than one. These connections have small round (or completely lack) domains of potential connectivity. Hence, connections with inhibitory basket cell dendrites have a limited potential for structural synaptic remodeling. Presynaptic neurons and postsynaptic basket cells can form a limited number of circuits by structural synaptic reorganization and only if located within about a 100 μm from each other.

Materials and Methods

Brief Outline of the Methods

1. Neurons were filled in vivo either intracellularly or retrogradely (Fig. 1) and reconstructed in 3 dimensions (3Ds) with Neurolucida system (MicroBrightField Inc., Colchester, VT).
2. Soma position and cortical laminar borders were marked in each preparation.
3. All reconstructions were corrected for tissue shrinkage.
4. Common cortical template was determined.
5. All reconstructed neurons were piecewise-linearly conformed to the common cortical template.
6. The distributions of potential synapses between all neuron pairs were generated by using Monte Carlo algorithm.
7. The expected numbers of potential synapses and the probabilities of potential connectivity were obtained from these distributions.
8. Calculations were performed for each neuron pair at different lateral separations between somata, that is, one of the arbors was translated along the cortical surface from 0 to 500 μm in 25 μm increments.
9. The results were interpolated on a 10-μm grid and smoothed to produce maps of potential connectivity.
10. For each potential connectivity map, reliable pixels were determined as pixels where the effective numbers of reconstructed potentially pre- and postsynaptic neurons are greater than or equal to 2.
11. Standard error to the mean and coefficient of variation at each pixel in the maps were calculated from bootstrap resampling with replacement procedure.

Experimental Procedures

Experimental procedures differed between the J.A.H. and the Z.F.K. laboratories. Anesthetized (J.A.H. laboratory: ketamine [10 mg/kg, intramuscularly] followed by thiopental sodium [20 mg/kg, intravenously [IV]] or a mixture of diprivan [Propofol] and sufentanil citrate [Sufenta] [5 mg + 1 mg/kg, IV]. Z.F.K. laboratory: 0.5–0.7% Halothane in 70% N2O and 30% O2) adult cats were prepared for experiments as described earlier (Buzás et al. 1998; Hirsch, Alonso, et al. 1998). In the J.A.H. laboratory, cells were intracellularly injected with biocytin, and in the Z.F.K. laboratory, the majority of cells were labeled using extracellular iontophoresis of biotinylated dextran amine or biocytin (Fig. 1). Only a few of pyramidal cells were injected intracellularly. All the cells were drawn using a computerized 3D reconstruction system Neuron-lucida (MicroBrightField Inc.) from multiple tissue sections, which were cut perpendicular (J.A.H.) or parallel to the surface of the cortex (Z.F.K.). Because neurons were labeled in vivo, their axonal branches are preserved in their entirety. In the J.A.H. laboratory, laminar boundaries were visualized by using standard cytoarchitectural criteria in Nissl-stained sections or with differential interference contrast. Laminar boundaries in the Z.F.K. laboratory were determined based on changes in neuron densities, presence of large layer-specific neurons, density of myelinated axons, and distances from pia and white matter. All reconstructions were corrected for tissue shrinkage in 3Ds. The overall tissue shrinkage in the J.A.H. laboratory from fixation in 3–4% paraformaldehyde and cryoprotection with 30% sucrose before sectioning was estimated to be 20%. This estimate was based on average measurements of distance between edges of the cortex exposed by the craniotomy in situ and after fixation and cryopreservation. Frozen sections that are 100-μm thick were mounted on gelatin-coated slides and air dried. These sections do not shrink appreciably in the xy plane. Shrinkage in depth was determined from comparison of the original section thickness to the average dried and coverslipped section thickness. Both corrections were applied to all the Neurolucida drawings. Because in the Z.F.K. laboratory osmicated tissue sections were imbedded in resin, shrinkage was equal in all dimensions, and a single correction factor was applied to x, y, and z dimensions (Buzás et al. 2003). Examples of the labeled and reconstructed excitatory neurons and inhibitory basket cells are shown in Figures 1 and 2. Our goal is to analyze potential connectivity in the local cortical circuits, and we limit all reconstructed arbor to a cylinder 1 mm in radius centered at the soma (dotted rectangles in Fig. 2). Analysis of long-range horizontal projections is hindered by large variability in the shapes of long-range axons.

Common Cortical Template

Individual neurons were reconstructed from cortical serial sections from different animals, with differing laminar thicknesses. In addition, the reconstructions were performed in 2 different laboratories with different experimental protocols. Because our analysis relies on combining the data from multiple animals, it is important to eliminate all the anatomical and technical differences. This was done by conforming all the reconstructed axonal and dendritic arbors to a single common template of the cat V1. The common template represents a slice through the cat V1 in the area where the cortical surface is relatively flat. Neurons reconstructed near the V1/V2 border where the cortical surface is significantly curved on a 500-μm scale often had divided axonal arbors, one part in V1 and another in V2. Such neurons were excluded from the analysis. The common template for a flat part of V1 was chosen in a way that minimizes the mean square distortion arising from piecewise-linear confomation of tissue sections from individual animals to this template. To calculate the common template, we first linearly scaled all sections to the mean cortical thickness. Next, individual laminar thicknesses were linearly scaled to the corresponding average laminar thicknesses. This procedure expanded some laminar...
while compressing others, resulting in a ~15% root-mean-square distortion of the tissue. The resulting common cortical template and laminar positions of all reconstructed neurons are shown in Figure 3. Same piecewise-linear transformations were applied to the reconstructed neurons conforming them to the common template. Images of all conformed neurons analyzed in this study are shown in Supplementary Figures S1 and S2.

Expected Number of Potential Synapses and Probability of Potential Connectivity
To determine the expected number of potential synapses, \( N_p(z_{\text{pre}}, z_{\text{post}}, \rho) \), and the probability of potential connectivity, \( P_p(z_{\text{pre}}, z_{\text{post}}, \rho) \), (from neuron \( i \) to neuron \( j \)) as function of neurons' depths in the template, \( z_{\text{pre}} \) and \( z_{\text{post}} \), and their relative lateral position, \( \rho \), we utilize axonal and dendritic arbors of pyramidal, spiny stellate, and basket cells reconstructed from different cortical layers of different animals and conformed to the common cortical template. To calculate the number of potential synapses between a given pair of neurons with particular positions in the common template, we use a computer search algorithm, which looks for proximal axonal and dendritic branches according to the definition of the potential synapse. Because the number of potential synapses is highly sensitive to the exact relative arbor positions and locations of individual axonal and dendritic branches within the arbors, it has to be described in terms of a distribution. We generate the distribution of potential synapses by using the following Monte Carlo procedure: the positions of neurons'
Interpolation and Gaussian Smoothing

Expected numbers of potential synapses, $N_e(z_{pre}, z_{post}, \rho)$, between all reconstructed neuron pairs were linearly interpolated onto a 10-µm grid along lateral dimension, $\rho$. Due to the nonuniform distribution of depths of reconstructed neurons (see Fig. 3), we chose to apply the following Gaussian smoothing procedure to the laminar dimensions, $z_{pre}$ and $z_{post}$:

$$N_e(z_{pre}, z_{post}, \rho) = \sum_{i=1}^{m_{pre}} \sum_{j=1}^{m_{post}} N_e(z_{pre}, z_{post}, \rho) \exp \left( -\frac{(z_{pre} - z_{pre, i})^2 + (z_{post} - z_{post, j})^2}{2\sigma^2} \right)$$

$$N_{pre, post}(z) = \sum_{i=1}^{m_{pre}} \exp \left( -\frac{(z - z_{pre, i})^2}{2\sigma^2} \right)$$

Here, $m_{pre}$ and $m_{post}$ are the numbers of reconstructed axonal and dendritic arbors, $z_{pre}$ and $z_{post}$ are the somata depths of $i$th reconstructed axon and $j$th reconstructed dendrite, and $z_{pre}$ and $z_{post}$ are the pixel positions on the 10-µm interpolation grid. The normalization factors in the denominator of the top equation, $N_{pre, post}(z)$, represent the effective numbers of potentially pre- and postsynaptic neurons contributing to the maps at depth $z$. Note that if all the neurons were at the same depth $z$, $N_{pre, post}(z)$ would simply equal the number of neurons. The choice of smoothing parameter $\sigma$ is dictated by the numbers of reconstructed cells. This parameter has to be larger than the typical distance (in depth) between cells to achieve averaging. In our sample, this distance is 66 µm for excitatory neurons and 90 µm for inhibitory basket cells. Hence, we chose $\sigma = 100$ µm. It is important to remember that our maps smooth out all the details on the smaller scale (see Supplementary Fig. S3). For pixels located within tight (less than $\sigma$ in size) clusters of reconstructed cells (e.g., $z = 600$ µm), Gaussian smoothing returns the average value for that cluster, as all the exponential weight factors for the neurons in the cluster reduce to one and outside the cluster to zero. For pixels located far (few $\sigma$ away) from the reconstructed cells, the above smoothing extrapolates the values of the nearest neuron cluster. The same interpolation and smoothing procedures are applied to probability of potential connectivity, $P_c(z_{pre}, z_{post}; \rho)$.

Estimation of Error Bars

The main source of variability in the numbers of potential synapses is the variability in the morphology of neurons belonging to the same class with similar (within scale $\sigma$) laminar positions in the template. This variability is especially striking for the excitatory neuron axons. To estimate the impact of this variability on our results, we use bootstrap resampling with replacement procedure (Mooney and Duval 1993) and calculate standard errors and coefficients of variation for all the results at every pixel on the interpolation grid. To do this, we create 1000 new bootstrap samples of the same size as the original data set of reconstructed neurons by randomly sampling neurons from it with replacement. As a result, the given bootstrap sample will not contain some of the neuron reconstructions, whereas other reconstructions will appear multiple times. Subsequently, standard errors and coefficients of variation for all the results in this study are calculated based on these bootstrap samples. The isolines of coefficients of variation or standard errors are shown in all maps of potential connectivity. In addition, in generating maps of potential connectivity, we disregard pixels (gray pixels in all maps) with a small effective number of contributing potentially pre- and postsynaptic neurons, $N_{pre, post}/2$ (see Supplementary Fig. S3). The above interpolation, Gaussian smoothing, and bootstrap procedures were applied to the numbers of potential synapses and the probability of potential connectivity for all 4 types of potential connections among excitatory neurons and inhibitory basket cells.

Results

To calculate potential connectivity, we used reconstructions of 41 neurons from the cat primary visual cortex (V1). Single neurons were labeled with biocytin or biotinylated dextran amin in vivo (Fig. 1) in order to maximally preserve axonal and dendritic arbors and reconstructed in 3D (Hirsch, Gallagher, et al. 1998; Kisvárday et al. 2002) (Fig. 2, Supplementary Figs S1 and S2) with the help of the Neurulcida system (MicroBright-Field Inc.), see Materials and Methods. These single-neuron reconstructions were combined in silico to determine potential connectivity. This approach possesses a combinatorial advantage as every pair of neurons contributed to the statistical description of connectivity. As brains differ from animal to animal, reconstructions had to be morphed to a common template of the cat V1 (Fig. 3, Supplementary Figs S1 and S2).

Our method relies on several assumptions and approximations:

1. We assume that reconstructed arbor shapes are representative of other neurons of the same class and laminar position (position perpendicular to cortical surface).
2. By combining neurons from different brains, we treat positions of axonal and dendritic branches as independent. The assumption that no short-range correlations exist between spatial positions of axonal and dendritic branches has been validated in the rat somatosensory cortex for pyramidal cell axons (Stepanyants et al. 2004). Yet, this assumption could be incorrect for inhibitory basket cell axons, in which case the results must be rescaled (Stepanyants et al. 2004).

3. When morphing neurons to a common template, we used only layer boundaries as landmarks, without taking into account possible dependence of dendritic and local (lateral distance from soma <500 μm) axonal arbor shapes on neurons’ functional properties or on their position along the cortex. This approximation seems reasonable for the cat V1 (Anderson et al. 1999) yet would be incorrect when strong bias in the shapes exists, for example, as in the rat barrel cortex (Shepherd et al. 2005).

4. We did not keep track of arbor orientation around the z axis passing through its soma perpendicular to the cortical surface, assuming that average dendritic (Anderson et al. 1999) and local axonal (Stettler et al. 2002) arbor shapes are isotropic around z. Keeping track of arbor orientations would be important for long-range horizontal connections, which were left out of this study due to our focus on local circuits (see Fig. 2A).

The choice of parameter s in the calculation of potential connectivity depended on the connection type. Reconstructed neurons from layers 2–6 (L2–6). Figure 3, were classified into excitatory neurons (Supplementary Fig. S1), including spiny stellate (n = 4) and pyramidal cells (n = 20), and inhibitory basket cells (n = 17, Supplementary Fig. S2). Such classification resulted in 4 types of potential connections: excitatory to excitatory (e → e), excitatory to inhibitory basket (e → i), inhibitory basket to excitatory (i → e), and inhibitory basket to inhibitory basket (i → i).

Because e → e connections are realized primarily through synapses on spines (Kisvárdy et al. 1986), we set parameter s = 2 μm (Peters and Kaiserman-Abramof 1970; Spacik and Hartmann 1983; Anderson et al. 1994; Harris 1999) for this potential connection type. An important exception to this rule is the L6 to L4 projection for which about 70% of synapses are made onto dendritic shafts of spiny stellate neurons (Ahmed et al. 1994; Anderson et al. 2002). The majority of these shaft synapses are mediated by boutons terminaux, club-like structures extending 5–10 μm away from main axon branches (Martin and Whitteridge 1984; Katz 1987). As there is no reliable information on the fraction of terminal boutons and the average length of protrusions they are connected to, it is not entirely clear what the appropriate value of the parameter s should be. We used s = 2 μm for this projection as well keeping in mind that the expected number of potential synapses is proportional to the parameter s (Stepanyants et al. 2002, 2004), and one could easily rescale the results once more precise estimates of this parameter become available.

For e → i and i → i connection types, we use s = 0.5 μm (Peters et al. 1991; Braitenberg and Schüz 1998), as these types are mediated mainly by shaft synapses (Braitenberg and Schüz 1998). In contrast, i → e connections can be accomplished through shaft and spine synapses. In this paper, we only consider potential axon-dendritic shaft synapses and hence use s = 0.5 μm for this connection type. Because inhibitory spine synapses are commonly formed on spine neck, many such synapses can approximated with axodendritic shaft synapses.

As a result, the numbers of potential synapses we obtain may only slightly underestimate the overall (on shafts and dendritic spines) potential synaptic connectivity for the i → e connection type. Finally, although it is possible to extend the potential synapse formalism to describe i → i dendrodendritic gap junctions and synapses on soma (Fig. 1F), in the following, we consider axodendritic synapses only.

As the number of potential synapses between 2 neurons depends strongly on their exact relative position and positions of individual axonal and dendritic branches, we characterized potential synaptic connectivity with a probability distribution. To obtain the probability distribution for a given pair of neurons at a particular lateral separation (distance ρ along cortical surface ranging from 0 to 500 μm), we performed the following Monte Carlo procedure, justified by the assumptions stated above. We randomly shifted each neuron’s soma position within a 25-μm cube centered at the neuron’s original position and, at the same time, randomly rotated both arbors around their z axes. For each combination of positions and orientations, we counted the number of potential synapses by searching for axonal and dendritic branches located closer than the scale s, Figure 4.

As displaying the probability distribution for different lateral separations and laminar positions of neurons is difficult, we chose 2 distribution parameters with transparent biological meanings: expected number of potential synapses (mean of the distribution) and probability of potential connectivity (probability of having at least one potential synapse). Although these parameters have been calculated only for laminar positions of reconstructed neurons, we interpolated them onto a 10-μm grid to produce smooth maps of potential connectivity. For each point on the grid, the standard error and coefficient of variation were calculated with bootstrap resampling with replacement procedure (Mooney and Duval 1993). Points with small numbers of contributing reconstructed neurons were excluded from the analysis (see Materials and Methods for details).

**Expected Number of Potential Synapses between Pairs of Neurons**

As a result of the described procedure (also see Materials and Methods), we obtained the expected number of potential synapses $N_p(\mathbf{z}_{\text{pre}}, \mathbf{z}_{\text{post}}, \rho)$ for 4 types of potential connections among excitatory neurons and inhibitory basket cells. For each connection type, the expected number of potential synapses is a function of 3 variables: the laminar positions of potentially pre- and postsynaptic neurons in the common cortical template, $\mathbf{z}_{\text{pre}}$ and $\mathbf{z}_{\text{post}}$, and their relative lateral displacement, $\rho$ (Fig. 4). To display a result of such calculations on a 2D plot, we fix one of the variables.

If the laminar position of the potentially presynaptic neuron, $\mathbf{z}_{\text{pre}}$, is fixed, we get a geometric output map. Figure 5A shows an example of e → e output map for a L2/3 pyramidal cell at $\mathbf{z}_{\text{pre}} = 400 \mu m$ (reference neuron whose soma position is shown with a triangle, representative axon is in black). The color of each pixel reflects the number of potential synapses made by the reference neuron onto the excitatory neuron at that pixel. For example, a L4 spiny stellate cell at $\mathbf{z}_{\text{post}} = 720 \mu m$ (soma position is marked with a square, representative dendrite is gray), which is displaced laterally by $\rho = 70 \mu m$, receives on...
average 2 potential synapses from the reference neuron. We also show e → e geometric output maps for L4 spiny stellate (z_{pre} = 700 µm, Fig. 5B) and L5 pyramidal (z_{pre} = 1000 µm, Fig. 5C) cells. Bold white contours in the output maps demarcate domains where reference neurons make at least one potential synapse. Then, all neurons belonging to these domains can become postsynaptic to the corresponding reference neuron by means of local synaptogenesis. Gray pixels in the maps of potential connectivity correspond to cortical depths with insufficient numbers of reconstructed neurons (see Materials and Methods for details). Thin white contours, which are the isolines of the coefficient of variation, are superimposed on the output maps to illustrate the error bars to the calculated average numbers of potential synapses.

By plotting the expected number of potential synapses \( N_e(z_{pre}, z_{post}, \rho) \) for a fixed laminar position of the postsynaptic neuron, \( z_{post} \), we obtain geometric input maps showing numbers of potential synapses received by the reference neuron from neighboring neurons. Figure 5D-F shows e → e geometric input maps for the same reference neurons as those in Figure 5A-C. Geometric input/output maps for different values of \( \rho \) for all 4 types of potential connections among excitatory neurons and inhibitory basket cells can be found at http://www.neurogeometry.net (see MatLab Demo).

The examination of \( e \leftrightarrow e \) geometric input and output maps leads to 2 observations. First, the number of potential synapses is highest (5–6 for neurons in Fig. 5) for neurons directly on top of each other (zero lateral separation) and decays monotonically with increasing lateral displacement between neurons. Second, in spite of large differences in the shapes of individual maps, the domains of potential connectivity of excitatory neurons based on input/output maps are roughly columnar with laminar specific radii on the order of 100–300 µm.

The 3 remaining connection types among excitatory neurons and inhibitory basket cells result in smaller numbers of potential synapses primarily due to the smaller value of the parameter \( s (s = 0.5 \, \mu m) \) used for these connection types (see MatLab Demo at http://www.neurogeometry.net). Figure 6 shows sample maps of potential output, \( e \rightarrow i \) (Fig. 6A), \( i \rightarrow e \) (Fig. 6B), and \( i \rightarrow i \) (Fig. 6C), and input, \( e \leftrightarrow i \) (Fig. 6D), \( i \leftrightarrow e \) (Fig. 6E), and \( i \leftrightarrow i \) (Fig. 6F), for reference neurons located 800-µm deep in the column. Unlike connections made between excitatory cells, these types of connections typically have domains of potential connectivity that are both small and round—the domains rarely cross layers to form columns. For some cortical depths, domains of potential connectivity vanish completely (Fig. 6A,E) as the expected numbers of potential synapses drop below one. The compact shape of the domains that include inhibitory connections is not surprising. It is a result of smaller \( s \) and more compact morphology of basket cell dendrites and axons in comparison with excitatory arbors that can span multiple layers.

### Probability of Potential Connection between Pairs of Neurons

Another way to characterize potential connectivity between 2 locations in the common cortical template is by calculating the probability for 2 neurons at these locations to have at least one potential synapse, \( P_e(z_{pre}, z_{post}, \rho) \) (see Materials and Methods for details of calculation). Again, by holding one of the variables constant, we can plot the results on a 2D color map. Several probability maps for potential \( e \leftrightarrow e \) connections are shown in Figure 7 for the reference neurons with the same positions in the template as in Figure 5. The color of a given pixel in Figure 7A-C indicates the probability that an excitatory neuron at that pixel is potentially postsynaptic to the reference neuron (probability of potential output). Figure 7D-F shows maps of the probability of potential input for the same neurons.

Maps of probabilities of potential input and output for all 4 types of potential connections among excitatory neurons and inhibitory basket cells can be found at http://www.neurogeometry.net (see MatLab Demo). One can define the domains of potential input/output by drawing a line at 50% probability (white contours in Fig. 7). Note that domains based on the probability of potential connection (Fig. 7) are similar in shape and size to the corresponding domains based on the expected number of potential synapses (Fig. 5). Because potential synapse is a necessary condition for an actual synapse, the potential connection probability maps provide upper bounds for the probabilities of finding synaptically connected pairs of neurons.

### Size of Potential Connectivity Domains of Excitatory Neurons

To characterize the lateral extent of \( e \rightarrow e \) potential connectivity domains (e.g., laminar specific radii of columnar domains
in Figs 5 and 7), we introduce a new map \( p(z_{\text{pre}}, z_{\text{post}}) \) that is a function of laminar positions of potentially pre- and postsynaptic excitatory neurons. In Figure 8A, we show the radii of potential connectivity domains based on the expected number of potential synapses for \( e \rightarrow e \) connection type. The color of each pixel indicates the lateral displacement between a potentially presynaptic neuron (laminar position is given by the vertical axis) and a potentially postsynaptic neuron (laminar position is given by the horizontal axis) at which the expected number of potential synapses \( N_p(z_{\text{pre}}, z_{\text{post}}; \rho) = 1 \).

Figure 8B shows a similar map calculated based on the probability of potential connection being \( P_p(z_{\text{pre}}, z_{\text{post}}; \rho) = 0.5 \). Maps in Figure 8A,B illustrate the asymmetry in the projections of excitatory neurons. This asymmetry arises from the large fan out of L4 and L5 excitatory neuron axons projecting to the supragranular layers. Similar maps for the remaining 3 types of projections involving inhibitory basket cells are not shown because for these connection types, domains of potential connectivity often vanish as the expected numbers of potential synapse drops below one.

**Local Potential Convergence and Divergence of Excitatory Neurons**

Based on the expected number of potential synapses between pairs of excitatory neurons, \( N_p(z_{\text{pre}}, z_{\text{post}}; \rho) \), we can estimate the overall number of potential synapses received (potential convergence) and sent (potential divergence) by excitatory neurons located at different depths. For example, local potential \( e \rightarrow e \) divergence, \( D \), for a neuron with laminar position \( z_{\text{pre}} \) is estimated as a sum of expected numbers of potential synapses made by that neuron onto all other excitatory neurons within reach of its local axonal arbor. This definition does not account for long-range projections and as a result underestimates the overall potential divergence. Local potential convergence, \( C \), is estimated in a similar way:

\[
D(z) = \int N_p(z, z_{\text{post}}; \rho) \, n_e(z_{\text{post}}) 2\pi \rho \, d\rho \, dz_{\text{post}}
\]

\[
C(z) = \int N_p(z_{\text{pre}}, z; \rho) \, n_e(z_{\text{pre}}) 2\pi \rho \, d\rho \, dz_{\text{pre}}
\]

(1)

In these equations, \( n_e \) is the density of excitatory neurons, which is estimated in the following way (Binzegger et al. 2004): the total number of neurons per 1 mm\(^3\) in layers 1, 2/3, 4, 5, and 6 of the cat area 17 are 7300, 58,000, 59,500, 41,900, and 63,500, respectively (Beaulieu and Colonnier 1983). Of these numbers, \( \gamma \)-aminobutyric acidergic (GABAergic) cells comprise the fractions of 0.97, 0.22, 0.20, 0.18, and 0.17 (Gabott and Somogyi 1986). We estimate densities of excitatory neurons as the difference between overall density of neurons and density of GABAergic cells 220, 45240, 47600, 34360, and 52710 mm\(^3\).

Figure 9A shows local convergence and divergence as functions of cortical depth calculated according to equation
Figure 6. Example of expected numbers of potential synapses for connection types involving inhibitory basket cells. (A) Expected potential output of a layer 4 spiny stellate cell (located 800 μm deep, white square) onto the dendrites of neighboring inhibitory basket cells, e → i (see Fig. 5A for the key). (B, C) Similar output maps for i → e and i → i potential connectivity. Input maps for reference neurons at the same depth: (D) e → i, (E) i → e, and (F) i → i. Squares represent spiny stellate cells, circles, basket cells. White contours demarcate the regions where the expected number of potential synapses is greater than one. Thin white contours are isolines for the coefficient of variation of the expected number of potential synapses. Gray pixels indicate positions where confidence in the estimate was low due to small number of reconstructed neurons (see Materials and Methods). The bottom and right axes show the scale in micrometers. Left axis shows cortical layers.

Figure 7. Probability of potential connectivity between excitatory neurons. Probability of potential connectivity is the probability that axonal and dendritic arbors of 2 neurons have at least one potential synapse. (A, B, C) Probability of potential output (see Fig. 5A for the key) for the same neurons as in Figure 5. White triangles and a square show soma positions of potentially presynaptic neurons. White contours demarcate the regions where the probability of potential connectivity is greater than 50%. (D, E, F) Same for the probability of potential input. White triangles and a square show soma positions of potentially postsynaptic neurons. Thin white contours are isolines for the coefficient of variation of the probability of potential connectivity. Gray pixels indicate positions where confidence in the estimate was low due to the small number of reconstructed neurons (see Materials and Methods). Bottom and right axes show the scale in micrometers. Left axis shows cortical layers.
Expected Number of Potential Synapses for Neurons Directly on Top of Each Other

Next, we describe the maximum potential connectivity between potentially pre- and postsynaptic laminar locations, $z_{\text{pre}}$ and $z_{\text{post}}$. For all laminar locations, maximum potential connectivity was attained by neurons located directly on top of each other (zero lateral displacement), that is, $\max(\tilde{N}_p(z_{\text{pre}}, z_{\text{post}}, \rho)) = N_p(z_{\text{pre}}, z_{\text{post}}, 0)$. Figure 10 shows maximum number of potential synapses for each of the 4 potential connection types between excitatory neurons and inhibitory basket cells (note the difference in color bar scale). For the $e \rightarrow e$ connection type (Fig. 10A), the number of potential synapses is highest for the L2 intralayer projection (8 potential synapses). This number is followed by a (interlayer) projection from L3/4 boundary to layers 2/3 (6 potential synapses). For the remaining 3 connection types, the numbers of potential synapses for neurons on top of each other are significantly lower primarily because $s$ is smaller.

Differences among maps in Figure 10 can be traced to the morphology of axonal arbors. Strong interlayer projections observed in $e \rightarrow e$ and $e \rightarrow i$ connection types, Figure 10A,B, result from the flame-like shape of excitatory axons. At the same time, relative isotropy of inhibitory axons accounts for the absence of strong inhibitory interlayer projections, Figure 10C,D.

Structural Projection Strength

In order to describe the overall strength of anatomical projection from neurons at cortical depths $z_{\text{pre}}$ to neurons at depth $z_{\text{post}}$, we introduce the structural projection strength, $G^{\ell, t}(z_{\text{pre}}, z_{\text{post}})$. This function provides the total number of potential synapses in the cortical region (e.g., cat V1) between potentially pre- and postsynaptic neurons of different types ($t$ and $\ell$) with somata centers confined to 1-μm strips at depths $z_{\text{pre}}$ and $z_{\text{post}}$ divided by the surface area of the considered cortical region. The structural strength is measured in potential synapses per micrometer$^2$ and takes into account variations in cell densities, $n_p$ in different laminae as illustrated by the following expression:

$$
G^{\ell, t}(z_{\text{pre}}, z_{\text{post}}) = n_t(z_{\text{pre}})n_\ell(z_{\text{post}}) \int \tilde{N}_p(z_{\text{pre}}, z_{\text{post}}, \rho) 2\pi \rho d\rho.
$$

In this expression, indexes $t$ and $\ell'$ denote the classes of potentially pre- and postsynaptic neurons ($e$ and $i$) and $G^{\ell, t}(z_{\text{pre}}, z_{\text{post}})$ describes 4 types of potential connections. In evaluating equation (2), we assumed that basket cells in layers 2/3, 4, and 5 account for 0.42, 0.78, and 0.42, respectively, of all GABAergic cells (Binzegger et al. 2004) (see, however, Budd [2000] where this estimate in L4 is 25–35%), resulting in the following densities of basket cells in these layers 5359, 9282, and 3168 mm$^{-3}$. Next, the densities of excitatory neurons and basket cells are used to produce maps of structural projection strengths, $G^{\ell, t}(z_{\text{pre}}, z_{\text{post}})$, among these cell classes, Figure 11 (note the difference in color bar scales).

As expected, among the 4 considered connection types, $e \rightarrow e$ is the strongest, Figure 11A. This connection type is dominated by structural projections within L2/3, closely followed by the L4 to L2/3 projection. Structural strength of $i \rightarrow e$ connection type is weaker by a factor of 20, Figure 11C, due to

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Figure 8. Potential connectivity domain radius of excitatory neurons. (A) Maximum lateral separation (in micrometers) between potentially pre- and postsynaptic excitatory neurons at which they establish one potential synapse. Left and top axes correspond to laminar positions of potentially pre- and postsynaptic neurons within the common cortical template. (B) Maximum lateral separation between excitatory neurons corresponding to 50% probability of potential connectivity. Thin white contours are isolines for the coefficient of variation of the domain radius. Gray pixels indicate positions where confidence in the estimate was low due to small number of reconstructed neurons (see Materials and Methods). Left and top axes show cortical layers. Bottom axis provides the scale in micrometers.

(1). Results are shown only for the depths where the numbers of reconstructed neurons were sufficient (see Materials and Methods for details). As expected, potential convergence in supragranular layers is higher than divergence, or the ratio of divergence to convergence is less than one, Figure 9B. In the granular and infragranular layers, the situation is reversed, with the largest divergence/convergence ratio (factor of 2) in the lower half of L4. An implication of the latter is that the output of L4 must have a widespread influence on L2/3, whereas L4 cells are generally less dependent on other cortical layers. Remarkably, the transition from convergent to divergent potential connectivity occurs precisely at the boundary between L3 and L4. On the other hand, in L5/6 cells, divergence exceeds convergence moderately. In contrast to excitatory projections, where the divergence/convergence ratio changes in depth by a factor of 4, for projections between inhibitory basket cells, this ratio is not significantly different from one, Figure 9B. The functional significance of such a balanced connectivity scheme remains unclear.
number of potential synapses received by a postsynaptic neuron at depth \( z_{\text{pre}} \) from all its potentially presynaptic partners with somata centers confined to 1-\( \mu \)m strip at depth \( z_{\text{post}} \) is equal to \( G_{p}^{f,r}(z_{\text{pre}}, z_{\text{post}}) / n(z_{\text{pre}}) \). This ratio, integrated over the domain of postsynaptic neurons, will result in the total potential convergence from that domain. As an example, we calculate the local potential convergence onto an excitatory L2/3 neuron located 400-\( \mu \)m deep in the template from all potentially presynaptic excitatory neurons in L4. From Figure 11A, the average structural strength of projections from L4 to the location 400-\( \mu \)m deep in L2/3 is \( 1.5 \times 10^{-7} \) \( \mu \)m\(^{-2} \). Hence, the total potential convergence onto the L2/3 neuron from L4 is equal to \( 1.5 \times 10^{-7} \) \( \mu \)m\(^{-2} \) divided by the density of excitatory neurons in L2/3 (4.52 \( \times \) \( 10^{-5} \) \( \mu \)m\(^{-2} \)) and multiplied by the width of L4 (335 \( \mu \)m). This results in 1110 potential synapses, which is roughly one-third of all potential synapses received by this L2/3 cell (Fig. 9A).

**Directionality of Structural Projections**

We characterize the asymmetry in the structural projection strengths within the classes of excitatory neurons (Fig. 11A) and inhibitory basket cells (Fig. 11D) by introducing the following function:

\[
D_{p}^{f}(z_{\text{pre}}, z_{\text{post}}) = \frac{G_{p}^{f,r}(z_{\text{pre}}, z_{\text{post}}) - G_{p}^{f,r}(z_{\text{post}}, z_{\text{pre}})}{G_{p}^{f,r}(z_{\text{pre}}, z_{\text{post}}) + G_{p}^{f,r}(z_{\text{post}}, z_{\text{pre}})}. \tag{3}
\]

Defined this way, the asymmetry function, \( D_{p}^{f}(z_{\text{pre}}, z_{\text{post}}) \), is independent of the neuron densities, the values of parameter \( s \) and confined to the [−1, 1] interval. The value of 1 indicates that there is direct, but no reciprocal potential projection between depths \( z_{\text{pre}} \) and \( z_{\text{post}} \). The value of −1 indicates that only a reciprocal projection is present, and the value of 0 indicates balanced potential input/output. Maps of directionality for \( e \rightarrow e \) and \( i \rightarrow i \) potential connection types are shown in Figure 12.

As expected, potential connections mediated by the excitatory neuron axons, Figure 12A, have higher directionality than the ones established by inhibitory basket cell axons, Figure 12B (note 10-fold difference in the scale). Excitatory projections within the cortical column have clear directionality toward the pial surface due to the flame shape morphology of excitatory axons (Fig. 12A), whereas projections among inhibitory basket cells are much more balanced and slightly stronger in the direction of the white matter (Fig. 12B).

**Discussion**

**Significance of the Neurogeometric Description of Synaptic Connectivity**

Although potential synaptic connectivity estimated from neurogeometry is not equivalent to functional connectivity, it is useful in several respects. First, potential connectivity provides an estimate of actual connectivity. The average number of actual synapses can be calculated by multiplying the number of potential synapses by the filling fraction, which is an independently measurable fraction of potential synapses that correspond to actual synapses (Stepanyants et al. 2002). Moreover, functional strengths of interlaminar projections are typically well correlated with the expected numbers of potential connections.
synapses for these projections, although there are important exceptions (Shepherd et al. 2005). The resulting wiring diagrams give a foundation for realistic modeling of neuronal dynamics beyond random networks.

Second, whereas actual synaptic connectivity can change over time in the developing and adult cortices (Lendvai et al. 2000; Trachtenberg et al. 2002; Holtmaat et al. 2005) due to the formation and elimination of dendritic spines and sprouting axon terminals, potential connectivity is more stable (Trachtenberg et al. 2002; Holtmaat et al. 2005), as it is determined by the layouts of axonal and dendritic branches. The number of potential synapses provides a limit for connectivity that can be achieved by structural synaptic plasticity, that is, through local synaptogenesis, without remodeling of axons and dendrites.

Third, our analysis suggests that local potential connectivity in different animals (same species, age, brain area, neuron classes, etc.) could be less variable than actual connectivity. For example, the coefficient of variation in the numbers of actual synaptic contacts in the rat somatosensory cortex is in the 1.9–3.2 range if both synaptically connected and not connected neuron pairs directly on top of each other are considered (Feldmeyer et al. 1999, 2002, 2006; Markram et al. 1997). This range is much higher than the range of corresponding coefficients of variation in potential connectivity, which is 0.1–0.3 in this study (Fig. 10.4). The coefficient of variation in the number of actual synapses reduces to 0.1–0.3 level only if synaptically not connected pairs of neurons are ignored. The difference in the coefficients of variation in the numbers of potential and actual synapses can be attributed in part to the fact that a potential synapse is a requirement for an actual synapse. The number of potential synapses between 2 cortical neurons with overlapping local axonal and dendritic arbors depends mainly on the class of neurons, their laminar positions, and relative lateral separation. The number of actual synapses in addition to neuron positions will depend on their properties, that is, orientation preference. Therefore, potential connectivity may be an appropriate description of cortical circuits in the face of ongoing remodeling of dendritic spines and variability of synaptic connectivity among different animals.

Maps of potential connectivity provide a basis for definition of structural columns, which may be the source of functional columnarity of the cortex (Hubel and Wiesel 1963; Ohki et al. 2006). In particular, structural columns in the cortex can be defined based on the domains of potential divergence/convergence among excitatory neurons (Figs 5 and 8A). In these domains, the reference neurons are potentially pre/postsynaptic to all other neurons, and any divergence/convergence pattern can be achieved by local synaptogenesis. Because domains for projections involving inhibitory basket cells (Fig. 6) are much smaller in size (if they exist at all), inhibitory basket cell circuits within the structural column have a more limited

Figure 10. Expected number of potential synapses for neurons directly on top of each other (neurons with zero lateral separation). (A) Potential connectivity between excitatory neurons directly on top of each other, $e \rightarrow e (s = 2.0 \mu m)$. Left and top axes correspond to laminar positions of potentially pre- and postsynaptic neurons within the common cortical template. (B) $e \rightarrow i$ Potential connectivity ($s = 0.5 \mu m$). (C) $i \rightarrow e$ Potential connectivity ($s = 0.5 \mu m$). Note the differences in scales. Thin white contours are isolines for the coefficient of variation of the expected maximum number of potential synapses. Gray pixels indicate positions where confidence in the estimate was low due to small number of reconstructed neurons (see Materials and Methods). Left and top axes show cortical layers. Bottom axis provides the scale in micrometers.
potential for remodeling. The radius of structural columns (100–300 \(\mu\)m) depends strongly on the laminar positions of potentially pre- and postsynaptic neurons (Fig. 8A) and falls between the sizes of the minicolumn (Peters and Payne 1993; Mountcastle 1997; Rockland and Ichinohe 2004) and the hypercolumn (Hubel and Wiesel 1977). The dependence of structural column size on depth in the cortex hinders the description of connectivity, yet it is not unexpected because neural response properties depend on laminar position as well (Bauer et al. 1980, 1983; Hirsch et al. 2002; Martinez et al. 2002, 2005; Amirikian and Georgopoulos 2003).

**Possible Sources of Errors**

Correct interpretation of potential connectivity maps requires an understanding of possible sources of errors. As with any experimental measurements, our results are prone to measurement, estimation, and sampling bias and corresponding errors (Kotz et al. 1982).

Measurement bias in our data set arises from imperfect neuronal reconstructions and must be carefully controlled (Ascoli et al. 2001; Scorcioni et al. 2004). Incompletely labeled arbors and branches truncated due to tissue slicing result in systematic underestimation of potential connectivity. This is a major concern for the reconstruction of axonal arbors in particular. This bias is independent of the sample size. To minimize this measurement bias, all the reconstructed neurons in this study were labeled in vivo and were judged to be well filled, that is, axonal and dendritic processes appeared to end abruptly rather than fading away. It should be noted that for each retrogradely labeled cell, the termination field of the axon collateral, which entered the injection site, could not be reconstructed due to the dense tracer deposit. We estimate that such axon collaterals represent only a minor fraction of the total axonal field of the cell.

Estimation bias in our analysis arises from the Gaussian smoothing procedure where sharp variations in the maps are smoothed out or averaged (see Supplementary Fig. S3). These large variations could turn out to be real if a larger sample of reconstructed neurons is analyzed. We evaluate the estimation bias to be less than 15%. This was done by comparing our results with the results obtained when using smaller values of the smoothing parameter \(\sigma\).

In general, there is no good way to estimate the sampling bias, which in this study could result from having an unrepresentative population of reconstructed neurons. Errors due to this bias do not necessarily decrease with the increasing sample size. In an attempt to understand possible sampling bias in our data, we compare our reconstructed excitatory neurons with the classification of excitatory neurons from the cat visual cortex used in Binzegger et al. (2004). Two classes of neurons are not represented in our sample, that is, pyramidal cells with cell bodies in L5 and L6 and axons in layers 5/6. This bias will result

![Figure 11. Structural projection strength. (A) Structural strength of projections (see the text for definition) for e \(\rightarrow\) e potential connectivity (\(s = 2.0 \mu\)m). Left and top axes correspond to laminar positions of potentially pre- and postsynaptic neurons within the common cortical template. (B) Same for e \(\rightarrow\) i potential connectivity (\(s = 0.5 \mu\)m). (C) i \(\rightarrow\) e (\(s = 0.5 \mu\)m). (D) i \(\rightarrow\) i (\(s = 0.5 \mu\)m). Note the 100-fold difference in scales. Thin white contours are isolines for the coefficient of variation of the structural projection strength. Gray pixels indicate positions where confidence in the estimate was low due to small number of reconstructed neurons (see Materials and Methods). Left and top axes show cortical layers. Bottom axis provides the scale in micrometers.](image-url)
in the underestimate of potential connectivity in L5/6. However, this effect is reduced by the fact that the above neuron classes comprise only 21% and 25% of the overall excitatory neuron populations in L5 and L6 (Binzegger et al. 2004). In addition, 2 out of 3 L6 cells in our sample have complex receptive fields (Hirsch, Gallagher, et al. 1998) and only sparsely project to L4. Because 60% of L6 pyramids are L4 projecting (McGuire et al. 1984; Katz 1987; Ahmed et al. 1994), potential connectivity from L6 to L4 is likely to be underestimated.

Another source of errors is in the variability in arbor shapes of individual neurons belonging to the same class. This variability is especially striking for axonal arbors. We estimate the impact of variability in arbor shapes on our findings, both the average number of potential synapses and the probability of potential connection, by bootstrap resampling with replacement procedure. Naturally, the precision of the calculations will increase with the number of reconstructed neurons. Yet, as the number of reconstructions grows, one may want to divide them into finer and finer classes. Such classification will become crucial for a more precise description of synaptic connectivity.

**Limitations of the Neurogeometric Description of Synaptic Connectivity**

It is essential to underline the limitations of our method in predicting the actual synaptic connectivity. First, as was already mentioned, we do not have precise knowledge of the value of parameter $s$ for every connection type considered. Instead, in generating the maps of potential connectivity, we used generic values of 2 μm for synapses on spines and 0.5 μm for shaft synapses. Because the number of potential synapses scales linearly with $s$ (Stepanyants et al. 2002, 2004), our results, at minimum, provide correct spatial distributions and relative values of potential synapses. These results can be easily rescaled when more precise estimates of average values of $s$ become available in the future. Second, the expected number of actual synapses for projections between groups of neurons is related to the number of potential synaptic connections through the ratio called the filling fraction (Stepanyants et al. 2002; Stepanyants and Chklovskii 2005).

The filling fraction, which can be measured independently from electron microscopy or estimated from average morphometric data, provides the link from potential to actual connectivity. For $e \rightarrow e$ projections, the filling fraction is estimated to be in the 0.1–0.3 range for many species and cortical areas (Stepanyants et al. 2002). However, the precise values of the filling fractions for different projections in the cat visual cortex are unknown. So, the calculated numbers of potential synapses are at best proportional to the numbers of actual synapses. Indeed, a good correlation between potential connectivity and functional projection strengths was observed for several projections in the rat barrel cortex (Shepherd et al. 2005).

For some projections in Shepherd et al. (2005), however, correlation was poor. Low correlation was also reported in Kalisman et al. (2005) between excitatory postsynaptic potential amplitude and number of axon-dendritic touch sites. This points to specificity beyond that provided by potential connectivity calculation. Specificity in synaptic connectivity is the third limitation that needs to be understood for a correct interpretation of the results. In this study, we are combining neurons from different brains, and as a result, the positions of axonal and dendritic branches of these neurons are uncorrelated on a micrometer scale. In contrast, the analysis of neuron pairs reconstructed from the same tissue showed that positions of axonal and dendritic branches can be positively correlated leading to larger numbers of potential synapses than what would be predicted by our method (Stepanyants et al. 2004). Positive correlation had been observed in the relative layout of many classes of GABAergic interneuron axons and dendrites of neurons postsynaptic to these axons. This positive correlation resulted in a 1.3- to 2.7-fold increase in the number of potential synapses. No significant correlation in the relative layout of branches was detected for neurons that are synaptically not connected. In addition, no correlation was observed in the layout of excitatory neuron axons. Though such analysis was not performed for the cat inhibitory basket cell axons, there are numerous examples of basket cell specificity in the literature (e.g., for L3 large basket cell, see Somogyi et al. 1983; for L5 basket cell, see Kisvárday et al. 1987) indicating that we
underestimate the numbers of potential synapses established by these axons and their postsynaptic dendrites.

Comparison with Other Studies of Connectivity in the Cat V1

Direct comparison of our findings with the results from other studies of connectivity in the cat visual cortex is hindered by the above-mentioned lack of knowledge of the filling fraction, uncertainty in the parameter $s$, and the possibility of structural specificity. Another complication arises from the fact that in this study, axonal arbors of excitatory neurons are significantly truncated due to the emphases on local potential connectivity. This results in the reduction of the overall number of potential synapse mediated by excitatory axons. Because the relative length of longer range projections and the numbers of synapses they provide is unknown, we are unable to compare our results to the counts of asymmetric synapses on dendrites of excitatory neurons and inhibitory basket cells available in the literature. In the following, we make comparisons of potential connectivity to average numbers of synapses between pairs of neurons and counts of total numbers of synapses on axons and dendrites of individual neurons.

$e \rightarrow i$ Pairs

In the cat visual cortex, in $3$ synaptically connected L2/3 pyramidal cell to basket cell pairs, the numbers of electron microscopically determined synaptic junctions were $1$, $2$, and $2$ (Buhl et al. 1997) or $1.7$ on average. In comparing this result with our estimate, where a L2/3 excitatory neuron establishes on average $0.6–0.8$ potential synapses with a L2/3 inhibitory basket cell (Fig. 10B), it is important to keep in mind that the averaging is done differently. Whereas in our estimate, the number of potential synapses is averaged over both potentially connected and not connected neuron pairs, only connected pairs are considered in Buhl et al. (1997). Therefore, we recalculate the expected number of potential synapses excluding pairs with no potential synapses from averaging. The result is $1.6 \pm 0.3$ potential synapses, which is consistent with the $1.7$ actual synapses estimate under the assumption that synaptically connected $e \rightarrow i$ pairs convert nearly all potential synapses into actual.

$i \rightarrow e$ Pairs

Basket cells on the border of L3/4 establish 6–8 synapses per connection with excitatory neurons (Somogyi et al. 1983). Of these synapses, $43\%$ are made on perikarya, $26\%$ on spines, and $31\%$ on dendritic shafts (Somogyi et al. 1983). Hence, on average $1.9–2.5$ synapses are made on dendritic shafts of postsynaptic excitatory neurons (per connection). Our estimate of expected $i \rightarrow e$ potential connectivity on dendritic shafts near the L3/4 border is $1.9 \pm 0.4$ (all pairs), Figure 10C, or $2.7 \pm 0.5$ (potentially connected pairs only). As expected, this number is no less than the number of actual synapses.

$i \rightarrow i$ Pairs

A synaptic connection between a pair of L2/3 basket cells is mediated on average by $7.3 \pm 3.2$ dendritic shaft synapses (Tamás et al. 1998). Our estimate of the corresponding number of potential dendritic shaft synapses (for potentially connected and not connected pairs) is $1.5–2$, Figure 10D. If only potentially connected pairs of neurons are considered, this estimate increases to $2.7 \pm 0.5$ potential synapses, which is still less than the average number of actual synapses. This discrepancy disappears if we take into account the structural specificity (correlation) of $i \rightarrow i$ connections, which can result in a 2.7-fold increase (Stepanyants et al. 2004) in the number of potential synapses.

$e \rightarrow e$ versus $e \rightarrow i$ Connectivity

In Kisvárday et al. (1986), it was estimated that only less than $5\%$ of L3 pyramidal cell targets are putative inhibitory cells. Because only $42\%$ of L3 inhibitory neurons are basket cells (Binzegger et al. 2004), it is reasonable to assume that only about $2\%$ of L3 pyramidal cell targets in L3 are dendrites of inhibitory basket cells. A comparison of Figure 11A,B shows that the average fraction of $e \rightarrow i$ potential synapses in L3 is also about $2\%$. Hence, the number of actual $e \rightarrow i$ synapses is made in the same proportion as the number of potential $e \rightarrow i$ synapses. This result is consistent with the Peter’s rule (Peters and Feldman 1976; Peters 1979; Binzegger et al. 2004) and the observation that axons of excitatory neurons show no structural specificity (correlation) toward their targets (Stepanyants et al. 2004).

$i \rightarrow e$ Connectivity

In L4, inhibitory basket cell estimates are accounted for 145–195 inhibitory dendritic shaft synapses on spiny stellate cells (Budd 2000). This estimate was based on the assumption that basket cells comprise 26–35\% of all GABAergic neurons in L4. Because we assume that this fraction is 78\% in L4, the number of basket cell synapses has to be increased to 440. Consistent with this number, our estimate derived from Figure 11C results in the average of $870 \pm 210$ potential synapses for this projection.

$i \rightarrow i$ Connectivity

In Ahmed et al. (1997), it was estimated that a L4 inhibitory basket cell receives about 180 dendritic shaft synapses from other basket cells. In Budd (2000), this number is estimated to be in the 71–107 range and if rescaled to 78% fraction of L3 inhibitory neurons is 220 synapses. Our estimate derived from Figure 11D is 860 ± 190, which is consistent.

Basket Cell Divergence

The average number of boutons on the inhibitory basket cell axons in this study is $5060 \pm 580$ ($n = 8$) for L2/3 cells and $4100 \pm 420$ ($n = 3$) for L4 cells. In Anderson et al. (2002) these numbers are estimated to be $4900$ for L2/3 cells and $2700$ for L4 cells, where the latter number is smaller possibly due to the presence of clump basket cells in the sample (clump cells are excluded from our analysis). As expected, potential divergence of L2/3 and L4 inhibitory basket cells is higher, 11 000 ± 1800 and 7500 ± 1200, and is made roughly in the same proportion.

Conclusion

In this paper, we attempt a statistical reconstruction of potential synaptic connectivity in local cortical circuits of the cat V1. By using 3D reconstructions of single neurons, we calculate the principal parameters in the distribution of the number of potential synapses, providing a quantitative description of structural organization of local cortical circuits, that is, for neurons separated laterally by less than 500 μm. Potential connectivity between excitatory neurons is organized in
columnar domains (Figs 5 and 7). These structural columns extending from the white matter to the pial surface have laminar specific radii in the range of 100–300 μm (Fig. 8). The expected numbers of potential synapses are maximal for neurons directly on top of each other (Fig. 10) and decay rapidly with increased lateral displacement. Maps of probability of potential connectivity, that is, probability of finding potentially connected pairs of neurons (Fig. 7) exhibit similar columnar architecture. In contrast to e → e connectivity, potential connectivity involving inhibitory basket cells is substantially weaker and is typically organized in compact rather than vertically elongated columnar domains (Fig. 6). The analysis of the overall local potential e ↔ e divergence and convergence summarized in Figure 9 reveals important differences in the architecture of supragranular versus infragranular layers. Layers 2/3 receive on average 3-fold higher potential input than L5 and L6. An interesting next step is to see whether this difference is offset by potential connectivity of the long-range projections, which were excluded from this analysis. In contrast, i ↔ i divergence is not significantly different from convergence and the divergence/convergence ratio in L3 and L4 is one, Figure 9B.

Supplementary Material
Supplementary figures can be found at http://www.cercor.oxfordjournals.org/.

Notes
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References
Ramón y Cajal S. 1891. Sur la structure de l’ecorce cérébrale de quelques mammifères. La Cellule. 7:125-176.