"POTENTIAL" CONNECTIVITY IN LOCAL CORTICAL CIRCUITS

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Synaptic connectivity among cortical neurons may vary with time due to the growth and retraction of dendritic spines [1]. This suggests that the invariant description of cortical circuits should be formulated on a level of more stable features of connectivity, i.e. the layout of axonal and dendritic branches. To describe the relative branch layout for two neurons with overlapping axonal and dendritic arbors, we use the number of "potential synapses" [2]. A potential synapse is a location in the neuropil where an axon of one neuron is present within a certain distance from a dendrite of another. Particular values of this distance depend on the type of synapse. For synapses on spines, this distance is on the order of the average spine length (typically 2μm). For shaft synapses, it is roughly equal to the sum of dendritic and axonal radii (about 0.4μm). Defined this way, a potential synapse is a requirement for a physiological synapse.

We developed a method which allows us to determine the expected number of potential synapses between and within classes of excitatory and inhibitory neurons, depending on their laminar positions and relative separation in the direction parallel to the cortical surface. Based on the morphology of a number of 3D reconstructed spiny stellate cells, pyramidal cells, and basket cells from different cortical layers of the cat visual cortex, we are able to produce cortical connectivity maps for the expected number of potential synapses. In addition, we calculated the maps of probability of potential connectivity, i.e. probability of having at least one potential synapse as a function of relative somata positions.

Neurons from the cat visual cortex were filled *in vivo* with biocytin and reconstructed in 3D with the help of Neurolucida [3, 4]. Because neurons were filled *in vivo*, their axonal branches are preserved in their entirety. Reconstructions have been corrected for tissue shrinkage in depth due to drying of slides. A typical reconstructed neuron is shown in Figure 1. Since this project addresses local connectivity only, we limit our analysis to a 1mm cylinder around the neuron.

Calculation of the average number of potential synapses. We use the reconstructed axonal and dendritic arbors of pyramidal, spiny stellate, and basket cells from different cortical layers of different animals to calculate density templates. In turn, density templates are used to calculate the number of potential synapses as a function of distance between neurons. Similar methods have been used in [2, 5-10].

To generate the density templates we calculate line (skeleton) density of branches $\rho_0(\vec{r},\hat{n})$, measured in μm/μm^3 (Figure 2 explains the notation used):

$$\rho_0(\vec{r},\hat{n}) = \sum_i l_i \delta\left(\vec{r}-\vec{r}_i\right)\delta\left(\hat{n}-\hat{n}_i\right). \quad (1)$$

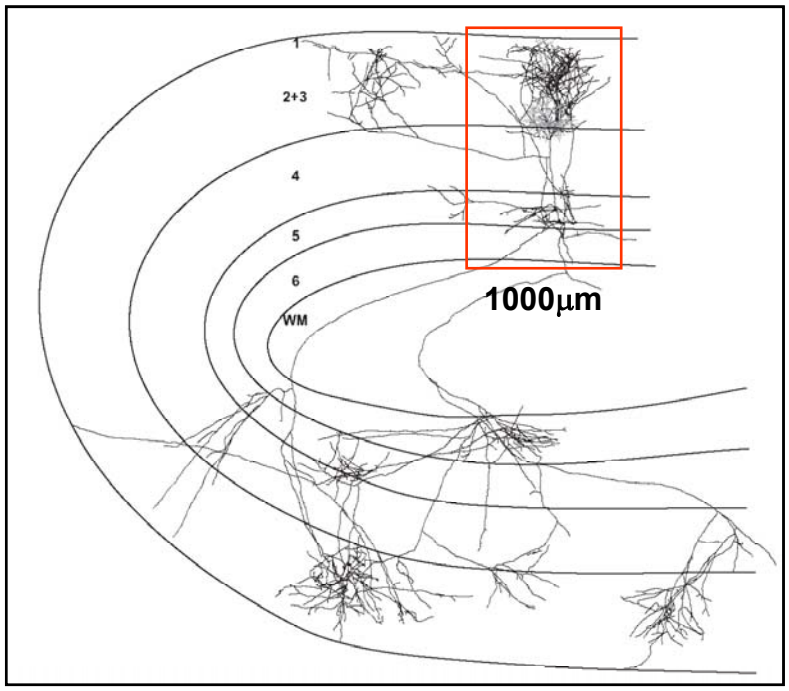

Figure 1. Reconstruction of layer 2/3 pyramidal neuron from cat visual cortex. Axon is shown in black, dendrite is in gray. We restrict our analysis of potential connectivity to local circuits and, therefore, limit axonal processes to the 1000μm cylinder surrounding the soma (red line).

We next pass this density through the gaussian filter G and average it by rotating the arbor around the z-axis perpendicular to the cortical layers and passing through the soma center:

$$\rho_{a,d}(\vec{r},\hat{n}) = \int \rho^0_{a,d}(\vec{r}',\hat{n})\, G(\vec{r}-\vec{r}')d^3r' \frac{d\varphi}{2\pi}$$
$$G(\vec{r}) = \exp\left[-\vec{r}^{\,2}/2\sigma^2\right]/\left(2\pi\sigma^2\right)^{3/2} \qquad (2)$$

Such averaging is justified because dendritic shapes are approximately isotropic [11], and so are local axons. Although such averaging is not valid for long-range horizontal connections, this is not a problem since they were excluded from consideration due to our focus on local circuits. An example of a resulting density template for the pyramidal dendrite from Figure 1 is shown in Figure 2. Finally, we average the density distribution among available neurons (from different animals) of the same class and laminar position.

Having density templates allows us to evaluate the average number of potential synapses for pairs of neurons as a function of the distance between them, $N_p\left(\vec{R}\right)$. This calculation is done by using the following expression:

$$N_p\left(\vec{R}\right) = 2s\int \rho_a(\vec{r}_a,\hat{n}_a)\rho_d(\vec{r}_d,\hat{n}_d)\left|\sin\left(\widehat{\hat{n}_a,\hat{n}_d}\right)\right|\delta\left(\vec{r}_a-\vec{r}_d-\vec{R}\right)d^3r_a d^3r_d d^3\Omega_a d^3\Omega_d \qquad (3)$$

where $\rho_{a,d}$ are the axonal and dendritic template densities, s is the parameter entering the definition of potential synapse (for pyramidal neurons with spiny dendrites it is equal to the average spine length, for GABAergic interneurons with smooth dendrites it is the average dendritic caliber).

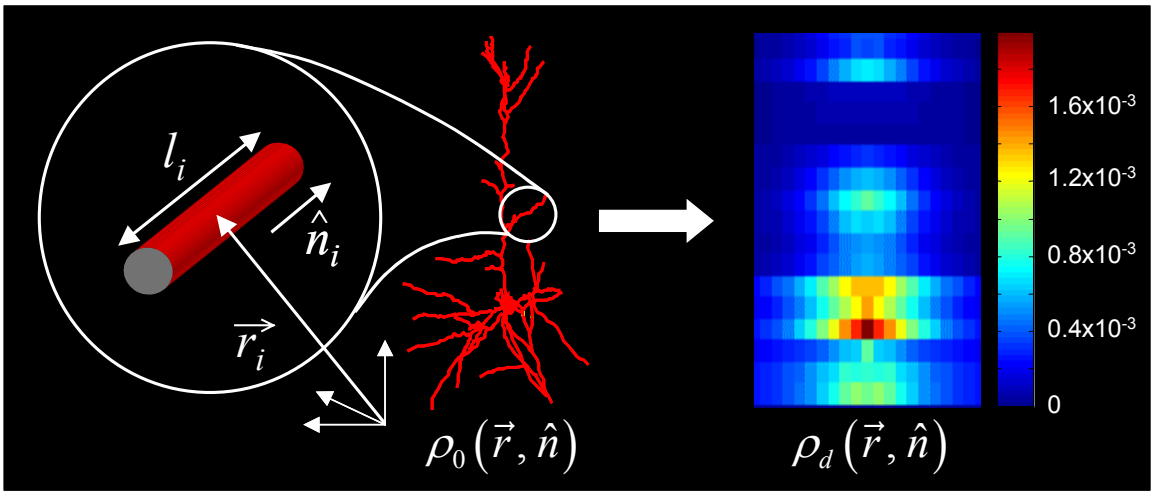

Figure 2. Generation of neuronal template. Skeleton density of dendritic arbor of layer 2/3 pyramidal neuron is denoted by ρ_0. The dendritic template density ρ_d is obtained by filtering and averaging the skeleton density as described in the text. Template density (in μm/μm^3) is represented by the pixel color according to the colorbar. Pixel size is 25μm.

As a result of the described procedure we obtained the number of potential synapses as a function of the relative positions of two neurons. One result of such calculation is presented in Figure 3A, which shows layer 4 spiny stellate neuron (white triangle) and the domain of other neurons receiving potential connections from it. The color of each pixel reflects the number of potential synapse received by a spiny neuron in that pixel. Results of another calculation shows a similar map for layer 2/3 pyramidal cell, Figure 3B. White contours in these maps represent the domains of potential connectivity threshold at one potential synapse. Such maps could be obtained for neurons at any laminar positions. We are in the process of recalculating these maps on a larger set of reconstructed neurons in order to extend them to layers 5 and 6, as well as to include potential connectivity with cortical basket cells.

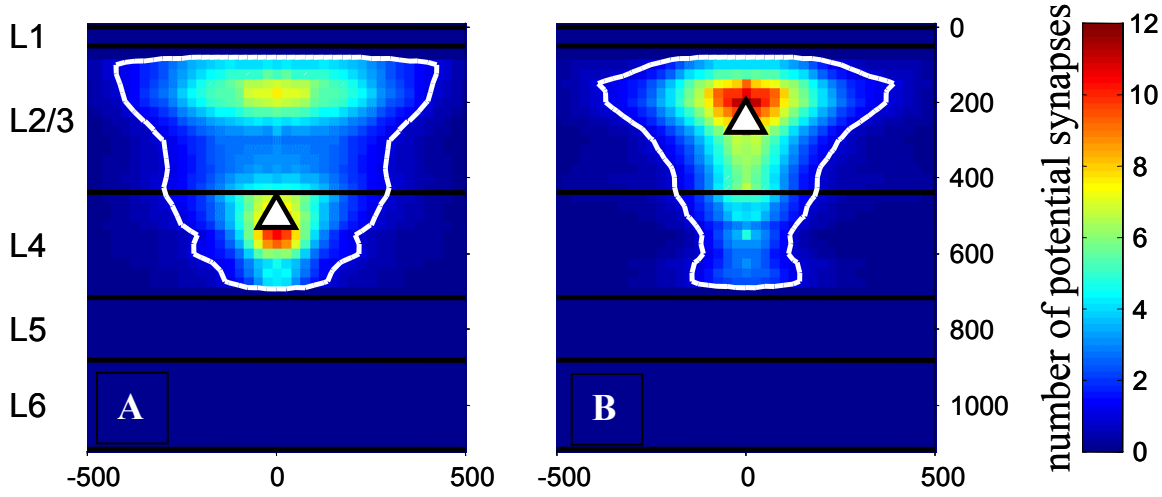

Figure 3. Maps of potential connectivity. **A**. Number of potential synapses made by layer 4 spiny stellate neuron (triangle) onto another spiny neuron located at a given pixel is given by the colorcode. **B**. Similar map for layer 2/3 pyramidal neuron. Contours corresponding to one potential synapse are shown in white. The bottom and right axis in A and B show the scale in micrometers. Left axis shows cortical layers.

Calculation of the probability of potential connection. In addition to the expected number of potential synapses, we calculate the probability of having at least one potential synapse, or the probability of potential connection. Because potential synapse is a necessary condition for an actual synapse, this quantity provides an upper limit on the probability of an actual synaptic connection. To calculate the potential connection probability we use the following Monte-Carlo algorithm. We place a pair of neurons at distance $\vec{R}$ from each other. Then, we choose a random orientation of the dendritic arbor (around the z-axis passing through its soma) and a random orientation of the axonal arbor (around its z-axis). We detect potential synapses by searching for axonal branches approaching dendritic branches closer than the scale *s*. Once a single potential synapse is found, or if none are found, we choose another random orientation pair. The probability of potential connectivity is estimated by the fraction of orientation pairs that have at least one potential synapse. Example of such probability distribution maps for neurons from Figures 3A and 3B are shown in Figures 4A and 4B respectively. The color of a given pixel indicates the probability of a potential connection from the original neuron (white triangle) onto another neuron located at that pixel. White lines correspond to 0.75 probability contours.

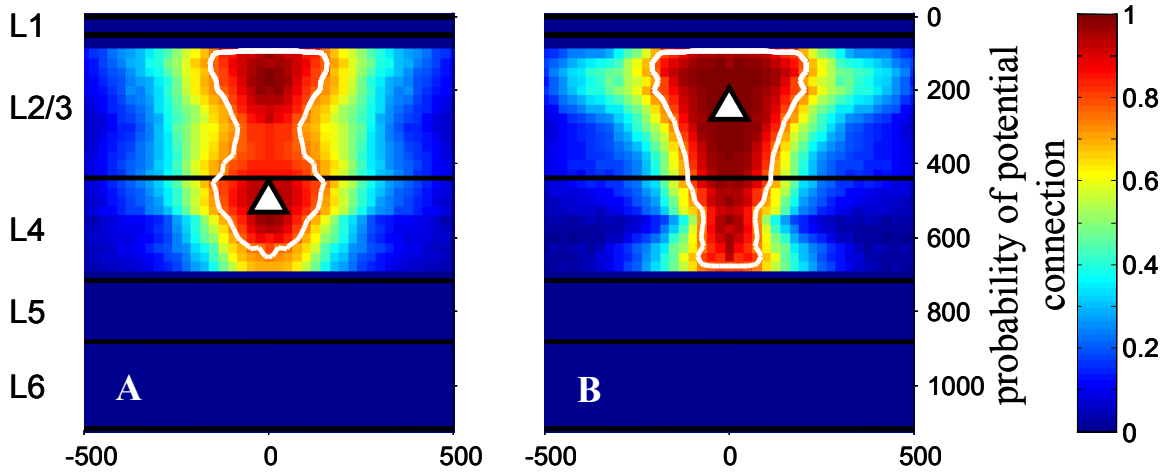

Figure 4. Maps of probability of potential connection (i.e. probability of having at least one potential synapse). **A**. Probability of potential synapses made by layer 4 spiny stellate neuron (triangle) onto another spiny neuron located at a given pixel is given by the colorcode. **B**. Similar map for layer 2/3 pyramidal neuron. White contours correspond to 0.75 probability. The bottom and right axis in A and B show the scale in micrometers. Left axis shows cortical layers.

Calculations of potential connectivity are subject to variability, which must be estimated for correct interpretation of the results. One source of variability is in the averaging procedure applied to a particular arbor. Because arbor density distributions may deviate from isotropy, this variability will be verified in typical cases by re-calculating potential connectivity without that averaging. This will provide an estimate of variability in our results. Because such calculation is computationally expensive, it cannot be applied to all reconstructed arbors. Another source of variability is the difference in arbor shapes between different neurons. We will be able to estimate the impact of such difference on our findings, both the average number of potential synapses and the probability of potential connection, by analyzing several neurons of the same class and layer. Naturally, the precision of the calculation will increase with the number of reconstructed neurons because they can be divided into finer and finer classes.

By combining 3D reconstructions of neurons from different layers, we will start assembling a comprehensive potential wiring diagram of the neocortex. This potential connectivity diagram will combine inter- and intra-layer connections and describe the spread of connectivity in the cortical plane. Such information may be used for several purposes. First, potential connectivity

provides an upper limit of actual connectivity. The average number of actual synapses can be calculated by multiplying the number of potential synapses by the filling fraction (fraction of potential synapses that correspond to actual synapses) [2]. The resulting wiring diagram gives a foundation for realistic modeling of neuronal dynamics, beyond random networks. Second, by comparing the maps of potential connectivity with actual connectivity data, we will be able to determine potential for plasticity in cortical connectivity due to the formation and elimination of synapses. Finally, potential connectivity may be an appropriate description of cortical circuits in the face of ongoing remodeling of connectivity. In particular, potential connectivity domain may provide a working definition of a cortical column, the size of which is between the mini-columns [12] and the hypercolumns [13].

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