Are spatial positions of dendritic and axonal branches correlated or independent?

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ABSTRACT

Because direct investigation of synaptic connectivity is difficult, neurobiologists often resort to indirect methods. One method is to infer connectivity from the densities of axonal and dendritic arbors, and the arbor overlap volume. This method is valid only under the assumption that spatial positions of dendritic and axonal branches are uncorrelated. We test this assumption by using 3D reconstructions of neuronal pairs from the rat neocortex. We find that the positions of GABAergic interneuron axons are correlated with their targets, while the positions of pyramidal neuron axons are independent of their targets.

INTRODUCTION

Synaptic connections require physical contacts between axons and dendrites. Therefore, spatial positions of axonal and dendritic branches contain information about potential synaptic connectivity. In particular, 3D light microscopy reconstructions of overlapping axonal and dendritic arbors allow one to infer potential connectivity between the corresponding neurons. Since 3D reconstructions of neuronal pairs are rare one is tempted to use single neuron reconstructions from different animals to estimate arbor overlap and infer potential connectivity (Hellwig, 2000; Jacobs et al., 2002). Such approach is valid only if the spatial positions of dendritic and axonal branches within corresponding arbors are independent. But are they?

To answer this question we developed an algorithm that detects significant correlations between spatial positions of dendritic and axonal branches from 3D reconstructions of overlapping arbors. The algorithm is based on counting how often axonal branches are present in the vicinity (correlation scale) of dendritic branches and comparing these counts with what would be expected if branch positions were independent (null-hypothesis). To generate the null-hypothesis (control) distribution of counts we randomly shift one whole arbor relative to the other and, after each shift, count how often axonal branches are in the vicinity of dendrites. Random shifts destroy short-range correlation (if any) in branch positions between arbors, while preserving the branch density within each arbor. If the actual counts are significantly different from the mean of the control distribution then spatial positions of axonal and dendritic arbors are correlated. We apply this algorithm to the available data from the rat neocortex and find that spatial positions may be both correlated and uncorrelated, depending on the neuronal class. In particular, we find that the positions of GABAergic interneuron (Gi) axons are correlated with their targets, while no correlation is detected between the positions of pyramidal cell (pc) axons and their targets.

Our results have several major implications. First, knowing the magnitudes of correlations in the spatial positions of dendritic and axonal branches allows one to modify the estimates of potential connectivity from the arbor overlap. Second, the existence of correlations indicates specificity in the wiring of cortical circuits on the level of branch positions. Third, detected spatial correlations indicate the existence of developmental mechanisms, such as molecular guidance and selective pruning/sprouting, which need to be investigated experimentally.

METHODS

To quantify the proximity of the axonal and dendritic branches we use two complimentary procedures: potential synapse count and voxel count. Potential synapses (Stepanyants et al.,

2002), Fig. 1, are defined as locations in the neuropil where an axonal branch is present within a certain distance *s* (correlation scale) of a dendritic branch. If *s* is equal to the spine length (typically about 2.5μm) this definition has a simple biological meaning: potential synapse is a necessary (but not sufficient) condition for an actual synapse on spine. For values of *s* of the order of dendritic radius (about 0.4μm) potential synapse is a prerequisite for a shaft synapse.

In a situation when an axon runs alongside a dendrite (a common scenario for axons of some Gi's) we postulate that only one potential synapse can exist between a given pair of axonal and dendritic branches. (Branch is defined as a segment between a bifurcation and an adjacent bifurcation or end point.) Defined this way, the number of potential synapses is only a measure of proximity for axonal and dendritic branches, not sensitive to their relative orientations. In order to capture the higher degree of correlation associated with relative orientation of branches and to demonstrate the robustness of the results to the particular choice of the correlation measure, we introduce the second correlation measure, the voxel count.

To obtain the voxel count, we randomly cover the axon/dendrite overlap volume by spherical voxels of radius *s* (correlation scale). The density of voxels has to be high enough in order to ensure high and uniform coverage, which is defined as voxel density times voxel volume (in our calculations coverage typically equals 100). Count of voxels is the number of voxels containing both axon and dendrite divided by the coverage, Fig. 2. For the sake of clarity we will continue describing the method using the first correlation measure, the number of potential synapses. The same calculations are applied to the voxel counts and lead to similar conclusions.

To determine whether there is a significant correlation between arbors we need to compare the observed number of potential synapses with what would be expected by chance, represented by the control distribution of potential synapse count. It is rather difficult to calculate such control distribution from the first principles because the density of arbor branches is both spatially non-uniform and variable from neuron to neuron. To overcome this difficulty we propose to generate the control distribution by numerically shifting one whole arbor relative to the other and counting potential synapses after each shift, Fig. 3. This shift method destroys short-range correlation (if any) in branch positions between arbors, while preserving the branch density within each arbor. We shift one arbor by a vector randomly chosen from a cube 30μm on the side and centered at the origin, Fig. 3. The choice of the cube size is constrained by two considerations. The shift has to be large enough to establish a different population of potential synapses (i.e. much larger than the correlation scale, *s*) and small enough not to alter arbor overlap significantly (much smaller than the arbor overlap scale of 100-200μm). We made sure that our results are robust to small variations in the cube size.

ANALYSIS OF THE ANATOMICAL DATA

We apply the shift method to neuronal pairs and triplets from the rat neocortex reconstructed in 3D (Tamas et al., 2000). The reconstructed neurons include 5 pc's and 11 Gi's, which are further subdivided into 6 regular spiking non-pyramidal cells (npc's), 2 bitufted cells, 2 axon-axonic cells, and 1 neurogliaform cell. A total of 38 axon-dendrite pairs result from these reconstructions. An example of the reconstructed pc dendrite and npc axon pair is shown in Fig. 1A.

We illustrate in Fig. 4A a typical control distribution for the number of potential synapses at correlation scale s=0.5μm for the npc axon and pc dendrite pair from Fig. 1A obtained after performing 1000 shifts. The observed number of potential synapses, indicated by an arrow in Fig. 4A, is clearly different from the mean of the control distribution. The probability of observing greater or equal number of potential synapses by chance is 0.02 (*p*-value equals 0.02). This indicates the presence of correlation between the npc axon and the pc dendrite. On the other hand, no correlation is found between the pc axon and the npc dendrite, Fig. 4B, where the observed number of potential synapses is well within the range expected by chance.

In order to detect arbor correlations on different length scales, we repeat the calculation for different values of the correlation scale, *s* ($0.2\mu m<s<3\mu m$). The presence of such correlation

would have a transparent biological meaning because the relevant length scales for neuronal connectivity, i.e. the distance between synaptically connected branches are different for different types of synapses. For the shaft synapses in the rat neocortex this distance is about 0.4μm (Braitenberg and Schüz, 1998), while for the spine synapses it is about 2.5μm (Peters and Kaiserman-Abramof, 1970; Spacek and Hartmann, 1983). Figs. 5A and 5B show *p*-values for different *s* calculated for the same arbors as in Figs. 4A and 4B, correspondingly. The possibility of significant correlation exists at the correlation scale of about 0.5μm for the npc axon and the pc dendrite (Fig. 5A), and at the correlation scale of 1.5-2.5μm for the pc axon and the npc dendrite (Fig. 5B). The correlation scale of 0.5μm in Fig. 5A matches the scales of the shaft synapses, appropriate for the npc axon to pc dendrite wiring. However, the correlation scale of 1.5-2.5μm in Fig. 5B is inconsistent with the scale of shaft synapse typical for pc axon to npc dendrite connection. This emphasizes the importance of elimination of chance correlations for the correct interpretation of the shift method results. We eliminate chance correlations by combining ("averaging") the results from different pairs and establishing the correct significance level.

To amplify small but persisting correlations (count of potential synapses or count of voxels, *C*) inherent to a particular connection type we combine the results from a number of different pairs of a particular connection type into a single cumulative *p*-value, $p^{cum}(s)$. The cumulative *p*-value, p^{cum}, is the probability of by chance obtaining the average correlation $\overline{C}$ from the generated control distributions, which is greater or equal to the observed average correlation $\overline{C_{obs}}$ for these pairs:

$$p^{cum} = \Pr\left(\overline{C} \geq \overline{C_{obs}}\right) = \int_{\overline{C} \geq \overline{C_{obs}}} \prod_{i}^{\#\ of\ pairs} \left[\Pr\left(C^i\right) dC^i\right],$$

where C^i is the correlation for the *i*-th pair. $p^{cum}(s)$ is generated by repeating the above procedure for all values of correlation scale *s*. The results of such calculation for different connection types among pc's and Gi's are shown in Fig. 6.

By virtue of looking for correlations between neuronal arbors on different correlation scales simultaneously, we become more susceptible to finding by chance a correlation at one of these scales. To lower the chance of finding false positive correlations we have to find appropriate multiple hypotheses testing corrected significance level (MHTCSL) for the results in Figs. 6B and 6C. We can no longer use the conventional 5% significance level. Because correlations *C* at different correlation scales are not independent, we cannot apply a traditional Bonferroni or Benjamini-Hochberg (Benjamini and Hochberg, 1995) multiple hypotheses test correction. Therefore, we had to use the following bootstrapping procedure.

We calculate MHTCSL from the first principles by performing the following computer experiment. First, we perform a single random shift for each arbor pair of a given connection type. Second, by treating these randomly shifted arbors as the original data we generate new cumulative *p*-values. These two steps are repeated 1000 times. For Fig. 6D, the 5% MHTCSL (red line) is defined as the level, which is crossed by 50 out of 1000 generated cumulative *p*-value curves at any value of correlation scale *s*, ranging from 0.2μm to 3.0μm. For Fig. 6C, we use a different definition. In this case two possible correlations, at around 0.4μm and 1.8-3.0μm, are not significant on their own at 5% MHTCSL (this could be due to the fact that only seven arbor pairs are used to generate Fig. 6C, as opposed to nineteen for Fig. 6D, and the correlation is not amplified enough). 5% MHTCSL for Fig. 6C (red line) is defined as the level, which is crossed by 50 out of 1000 generated cumulative *p*-value curves at least at two values of correlation scale *s*, separated by more than 1μm. The choice of 1μm is inspired by the fact that this is conservative estimate of the typical correlation length for the individual *p*-values as functions of *s*, used to produce Fig. 6C. MHTCSL is not sensitive to small variations of this parameter.

SUMMARY OF DETECTED CORRELATIONS

The results of the analysis for different connection types from Fig. 6 and corresponding MHTCSL are shown in Fig. 7. We find no significant short-range correlation between the axonal and dendritic arbors of the pc, Fig. 6A, or between the pc axons and the Gi dendrites, Fig. 6B. We find significant (at 0.05 MHTCSL) positive correlations between the Gi axons and the pc dendrites, Fig. 7A, at two length scales, s~0.4μm and 1.8-3.0μm. Shorter length scale, s~0.4μm, corresponds to shaft synapses, since this value of s is close to the sum of dendritic and axonal radii (Braitenberg and Schüz, 1998). Longer length scale, 1.8-3.0μm, corresponds to spine synapses, since this is a typical range for a spine length (Peters and Kaiserman-Abramof, 1970; Spacek and Hartmann, 1983). Both kinds of synapses are known to exist between Gi axons and pc dendrites. Finally, we find significant (at 0.05 MHTCSL) correlation between axons and dendrites of Gi's (both from different and same neurons) at the correlation scale s=0.2-0.6μm, Fig. 7B. This corresponds to shaft synapses among Gi's.

A novel feature of our analysis is counting potential rather than actual synapses. One may question the expediency of this analysis, given that neuronal circuitry is determined by actual synapses. To answer this, we point out that unraveling synaptogenesis in cortical circuitry is a very difficult problem. In order to make progress, we break up the problem of synaptogenesis into two more tractable questions: formation of potential synapses and transformation of potential synapses into actual synapses. This paper addresses the first question only, which is justified given that potential synapses are a necessary condition of actual synapses. Moreover, observations of spine plasticity (Trachtenberg et al., 2002) argue for a dynamic cortical circuitry, supporting the idea that potential synapses may be a useful way to characterize the connectivity diagram.

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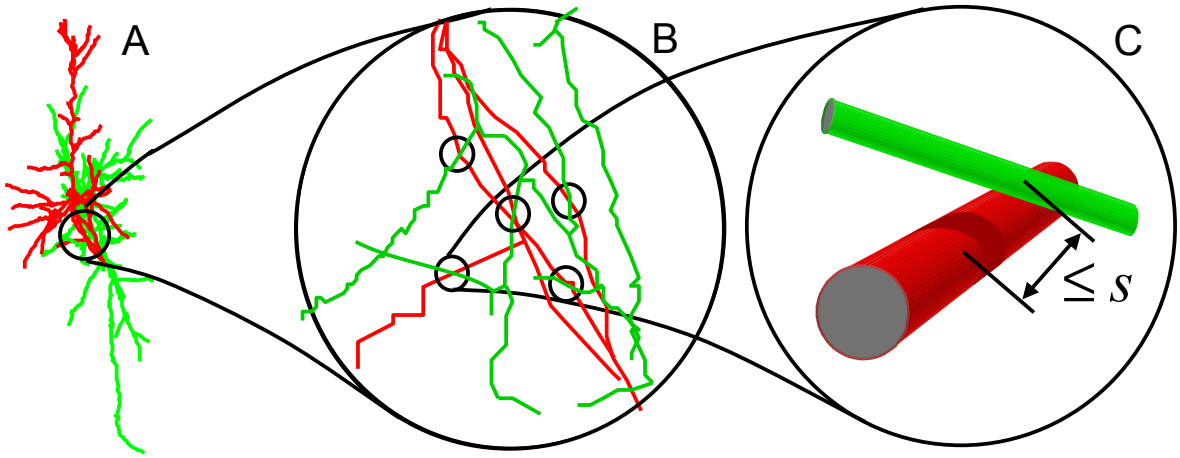

Figure 1. Potential synapses between two arbors. **A:** A 3D reconstruction of pyramidal cell (pc) dendrite (red) and regular spiking non-pyramidal cell (npc) axon (green). **B:** A magnification of the circled region in **A**. Potential synapses between the pc dendrite and the npc axon are shown by black circles. **C**: Further magnification of the circled region in **B**. Potential synapse is a location where an axonal branch is present within the correlation scale *s* of a dendrite.

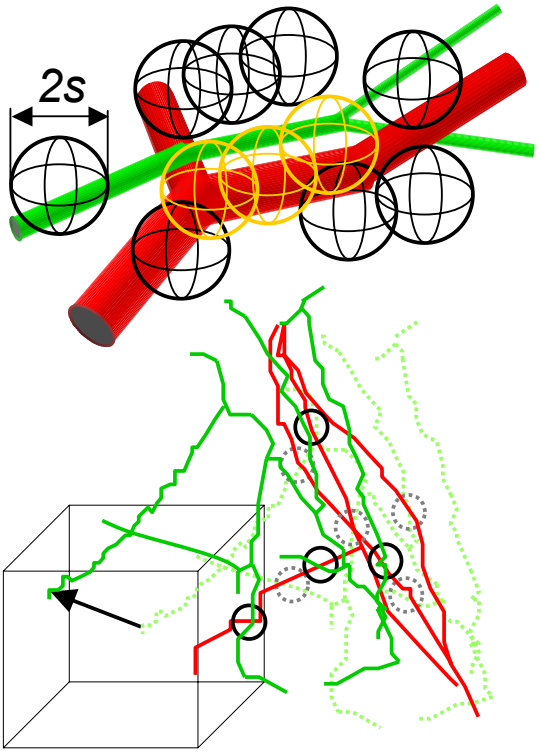

Figure 2. Spherical voxel count. The overlap region between axon (green) and dendrite (red) is randomly covered by spherical voxels (black and yellow spheres) of radius *s*. The density of voxels is chosen high enough to achieve uniform coverage. To obtain the spherical voxel count we calculate the number of voxels containing both axon and dendrite (yellow spheres) and divide it by the coverage.

Figure 3. Illustration of the shift method on a fragment of a 3D reconstruction from Fig. 1B. Potential synapses between the pc dendrite (red) and the npc axon (green dotted line) are shown by black dotted circles. After a small random shift of the npc axon (green) the number of new potential synapses (black circles) is smaller. The arrow, which shows the direction of the shift, is randomly chosen from the cube (see text for details).

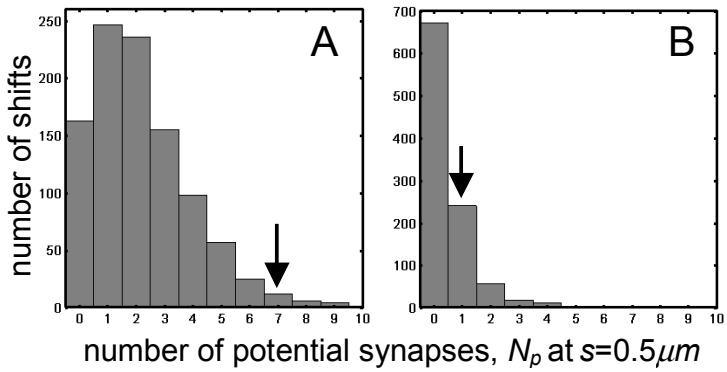

Figure 4. Examples of control histograms for numbers of potential synapses for neurons from Fig. 1A. Correlation scale is s=0.5μm. The arrows show the observed numbers of potential synapses. **A:** A possible correlation between the npc axon and the pc dendrite (p=0.02). **B:** No correlation is detected between the pc axon and the npc dendrite.

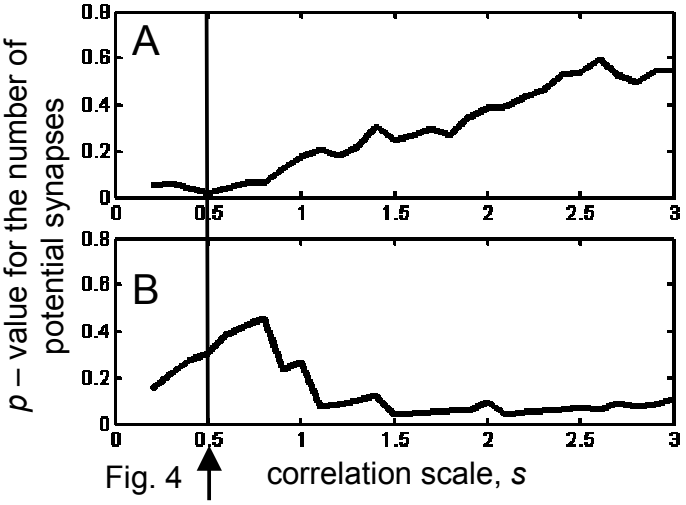

Figure 5. *p*-values as functions of correlation scale, *s*, for the number of potential synapses. Results correspond to the same arbor pairs as in Figs. 4A and 4B. There are possible significant positive correlations at *s*=0.5μm and *s*=1.5-2.5μm in **A** and **B** respectively. The arrow points to the correlation scale *s*=0.5μm, which corresponds to the histograms in Fig. 4.

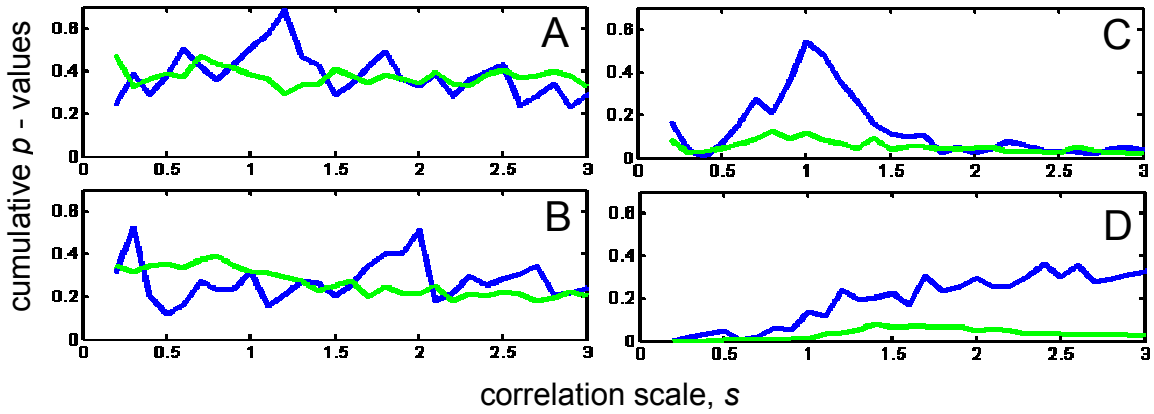

Figure 6. Cumulative *p*-values as functions of correlation scale *s*, for connections among the pc's and the Gi's. **A**: five pairs consisting of the pc axon and dendrite belonging to the same neuron. **B**: seven pairs compose with the pc axon and the Gi dendrite. **C**: seven pairs of the Gi axons and the pc dendrite (same pairs of neurons as in **B**). **D**: nineteen pairs consisting of the Gi axon and dendrite (some axon dendrite pairs belong to the same neuron). Blue and green lines show cumulative *p*-values for potential synapse and spherical voxel counts respectively. No significant correlation is detected in **A** and **B**.

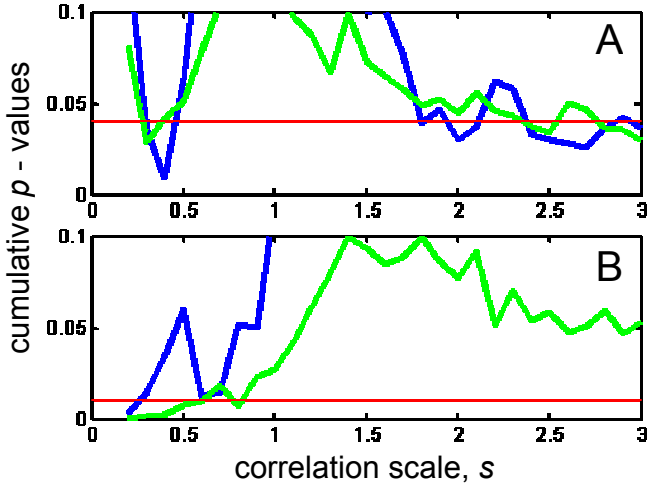

Figure 7. Multiple hypotheses testing corrected significance levels (MHTCSL) for cumulative *p*-values **A**, from Fig. 6C and **B**, from Fig. 6D. MHTCSL (red lines) is set at 5%. There is significant positive correlation in **A,** between the Gi axons and the pc dendrites around *s*=0.4μm and *s*=1.8-3.0μm, and in **B**, between axons and dendrites of the Gi's at *s*=0.2-0.6μm.