Supplementary Material

Three-Dimensional Spatial Distribution of Synapses in the Neocortex: a Dual-Beam Electron Microscopy Study
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Supplementary tables

| Sample no. and animal id. | Counting frame volume (µm³) | Absolute no. of AS | Absolute no. of SS | Absolute no. of ALL | Number of AS per µm³ | Number of SS per µm³ | Number of ALL per µm³ |
|--------------------------|-----------------------------|-------------------|-------------------|---------------------|----------------------|---------------------|----------------------|
| 1 (W31)                  | 149.1262                    | 135               | 12                | 147                 | 0.9053               | 0.0805              | 0.9857               |
| 2 (W31)                  | 157.1471                    | 101               | 8                 | 109                 | 0.6427               | 0.0509              | 0.6936               |
| 3 (W33)                  | 186.4505                    | 158               | 15                | 173                 | 0.8474               | 0.0805              | 0.9279               |
| 4 (W33)                  | 176.4412                    | 157               | 21                | 178                 | 0.8898               | 0.1190              | 1.0088               |
| 5 (W33)                  | 176.2756                    | 148               | 19                | 167                 | 0.8396               | 0.1078              | 0.9474               |
| 6 (W33)                  | 175.5540                    | 156               | 9                 | 165                 | 0.8886               | 0.0513              | 0.9399               |
| 7 (W33)                  | 191.2839                    | 171               | 18                | 189                 | 0.8940               | 0.0941              | 0.9881               |
| 8 (W35)                  | 247.5782                    | 186               | 12                | 198                 | 0.7513               | 0.0485              | 0.7997               |
| 9 (W35)                  | 178.4011                    | 191               | 10                | 201                 | 1.0706               | 0.0561              | 1.1267               |
| 10 (W35)                 | 165.0586                    | 152               | 16                | 168                 | 0.9209               | 0.0969              | 1.0178               |
| Average                  | 180.3316                    | 155.5             | 14                | 169.5               | 0.8623               | 0.0776              | 0.9399               |
| Total                    | 1803.3165                   | 1555              | 140               | 1695                |                      |                     |                      |

Table S1. Volume of tissue samples and actual counts of synaptic junctions per sample in the neuropil of layer III. The counting frame volumes and synaptic densities have been corrected for tissue shrinkage. AS: asymmetric synaptic junctions; SS: symmetric synaptic junctions; ALL: asymmetric and symmetric synaptic junctions.
### Two-sample Kolmogorov-Smirnov tests (p values)

| Sample No. | Experimental samples vs homogeneous Poisson processes | Experimental samples vs simulated realizations of RSA |
|------------|------------------------------------------------------|-----------------------------------------------------|
|            | G         | F         | K         | G         | F         | K         |
| 1          | 0.952     | 0.952     | 0.468     | 0.799     | 0.952     | 0.994     |
| 2          | 0.586     | 0.998     | 0.699     | 0.035     | 0.999     | 0.906     |
| 3          | 0.952     | 1.000     | 0.994     | 0.952     | 0.999     | 0.994     |
| 4          | 0.388     | 0.999     | 0.906     | 0.388     | 1.000     | 0.994     |
| 5          | 0.998     | 0.999     | 0.699     | 0.998     | 1.000     | 0.994     |
| 6          | 0.952     | 0.999     | 0.906     | 0.998     | 1.000     | 0.994     |
| 7          | 0.134     | 0.998     | 0.994     | 0.134     | 0.999     | 0.994     |
| 8          | 0.999     | 0.999     | 0.906     | 0.999     | 1.000     | 0.999     |
| 9          | 0.999     | 0.799     | 0.999     | 0.998     | 0.952     | 0.994     |
| 10         | 0.586     | 0.952     | 0.906     | 0.387     | 0.998     | 0.999     |

**Table S2.** p-values of the Kolmogorov-Smirnov tests performed on the G, F and K functions derived from the actual experimental samples, from homogeneous Poisson processes, and from the mean of 100 simulated realizations of RSA per sample. In this table, only asymmetric synapses have been considered.
Supplementary Methods

Log-normal distribution of Feret’s diameters

We used the Feret’s diameter of synaptic junctions (the diameter of the smaller sphere circumscribing the synaptic junction) as an estimate of their sizes. If \( X \) denotes a random variable measuring the Feret’s diameters of synaptic junctions (in nm), then \( X \) follows a lognormal distribution with parameters \( \mu \) (scale) and \( \sigma > 0 \) (shape) if \( Y = \ln X \) follows a Gaussian distribution \( N(\mu, \sigma) \).

The probability density function is given by:

\[
F(X) = \frac{e^{\frac{-1}{2} \left( \frac{\ln x - \mu}{\sigma} \right)^2}}{x\sigma\sqrt{2\pi}} \quad (1)
\]

where \( \mu = 5.828 \) and \( \sigma = 0.446 \) in our samples.

Since \( Y \) is measured in the scale of log of nm, it is interesting to obtain the expectation and variance of \( X \), given by:

\[
E(X) = \nu = e^{\mu + \frac{\sigma^2}{2}}, \quad Var(X) = \tau^2 = e^{2\mu + \sigma^2}(e^{\sigma^2} - 1) \quad (2)
\]

both estimated by plugging the usual maximum likelihood estimates of \( \mu \) and \( \sigma \) of a Gaussian distribution into Equation (2). The estimates were \( \nu = 375.198 \) nm and \( \tau^2 = 30,981.351 \text{ nm}^2 \).
Supplementary Videos

**Video S1.** [http://cajalbbp.cesvima.upm.es/storage/Video_S1_Merchan_et_al.avi](http://cajalbbp.cesvima.upm.es/storage/Video_S1_Merchan_et_al.avi)

The video shows the use of Espina software for the segmentation of synaptic junctions from a stack of serial sections. In the main window the sections are viewed through the original plane of section, the x-y plane, as they were obtained by the dual-beam electron microscope (FIB/SEM). Other two windows show two alternative planes of section (x-z and y-z). A fourth window shows a 3D representation of the three orthogonal planes. The user can navigate within the image stack using the sliders located at the bottom of the window or by selecting the desired window and using the mouse wheel. In this example, two asymmetric synaptic junctions and one symmetric synaptic junction have been segmented and tagged. Some of their geometrical features, including their spatial position and Feret’s diameter, are determined by the same software.

**Video S2.** [http://cajalbbp.cesvima.upm.es/storage/Video_S2_Merchan_et_al.avi](http://cajalbbp.cesvima.upm.es/storage/Video_S2_Merchan_et_al.avi)

The video shows the formation of a stack of serial sections obtained by FIB/SEM from the neuropil of the rat somatosensory cortex (layer III). Synaptic junctions are then segmented and reconstructed. Asymmetric and symmetric synaptic junctions are shown in green and red respectively. The smallest spheres circumscribing the reconstructed synaptic junctions served to calculate the Feret’s diameters, which were used as an estimation of their size. An unbiased counting frame was also drawn to facilitate the quantification of the number of synapses per unit volume. The geometric centers or centroids of the synaptic junctions were also determined to indicate the spatial position of the synapses. The dimensions of the counting frame in this example were 7.16 x 4.58 x 3.98 µm.

**Video S3.** [http://cajalbbp.cesvima.upm.es/storage/Video_S3_Merchan_et_al.avi](http://cajalbbp.cesvima.upm.es/storage/Video_S3_Merchan_et_al.avi)

The video shows a simulation of a Random Sequential Adsorption (RSA) process in three dimensions. The simulation is performed using a sequential algorithm that first generates a random point that is the center of a sphere with a certain diameter. The diameters used in the simulations were randomly drawn from the probability distribution of the experimentally observed Feret’s diameters (Table 1). This distribution was found to be log-normal (Fig. 2). If the next simulated point with its corresponding Feret’s diameter does not overlap with previously generated points, then it is accepted. Otherwise it is rejected and another random point is generated. The process terminates when the desired number of points has been reached.
Supplementary Figures

Figures S1 to S10. G, F and K functions corresponding to ten samples of the neuropil of layer III in the rat somatosensory cortex. For each figure, in the left column the experimentally observed data (blue traces) have been represented together with those for the corresponding homogeneous Poisson process (red traces) and the mean of 100 simulated realizations of an RSA process (green traces). In the right column the observed functions are depicted in blue and 100 individual RSA simulations in green. Sample number corresponds to figure number.
Figure S7

Figure S8

Figure S9

Figure S10