Supplementary File 1: Supplementary Tables

Cell-type specific innervation of cortical pyramidal cells at their apical dendrites

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**Supplementary table 1**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | S1 | V2\* | PPC\* | ACC | LPtA | PPC-2 |
| Mouse age (postnatal days) | 28 | 56 | 56 | 56 | 4­­­­­6 | 57 |
| Sample location relative to bregma in mm (AP,ML) | (-1.2, -3.9) | (-2.6,1.7) | (-2,-1.7) | (0.8,-0.15) | (-2.1, -1.3) | (-2, -1.5) |
| Sample section thickness (µm) | 1000 | 600 | 600 | 600 | 1000 | 600 |
| SEM type (FEI, USA) | Magellan | Quanta | Quanta | Quanta | Verios | Magellan |
| Electron landing energy (KeV) | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 |
| Beam current (nA) | 3.2 | 0.2 | 0.2 | 0.11-0.2 | 0.8 | 1.6 |
| Beam dwell time (µs) | 0.1 | 2.1 - 2.8 | 2.3 | 2.1 - 2.8 | 0.5 | 0.2 |
| Tile configuration in plane (x, y) | 3 x 3 | 1 x 2 | 1 x 2 | 1 x 3 | 4 x 5 | 5x5, 5x10 |
| Overlap between tiles (x, y) | (21%, 11%) | (N/A, 8%) | (N/A, 6%) | (N/A, 4.5%) | (8%, 12%) | (6%, 7%) |
| Single tile resolution (pixels) | 3072x2048 | 6144x4096 | 6144x4096 | 6144x4096 | 3072x2048 | 4096x3536 |
| voxel size (nm3) | 11.24x11.24x28 | 12x12x30 | 12x12x30 | 12x12x30 | 11.24x11.24x30 | 11.24x11.24x30 |
| Final high-resolution volume (µm3) | 66x89x202 | 72x91x153 | 72x93x141 | 70x141x98 | 130x110x85 | 200x(185-370)x200 |
| Dataset distance from pia (µm) | 125 | 215 | 170 | 110 | 20 | 10 |
| Low-resolution EM volume existence\*\* |  |  |  |  | X | X |
| Staining approach | Manual | AMW assistance | AMW assistance | AMW assistance | AMW assistance | Manual |
| Staining protocol | Conventio-nal | Modified Hua | Modified Hua | Modified Hua | Modified Hua | Modified Hua |

\* From opposing hemispheres of same animal

\*\* Used for finding the apical dendrite’s cell body of origin

**Experimental parameters.**

This table summarizes experimental parameters used for sample preparation and volumetric electron microscopy in 6 datasets from 5 cortical regions.

**Supplementary table 2**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | V2 | PPC | ACC | PPC-2 |
| 50% Ethanol | 30 min @ 4°C | 30 min @ 4°C | 30 min @ 4°C | 30 min @ RT (cooled) |
| 75% Ethanol | 45 min @ 4°C | 45 min @ 4°C | 45 min @ 4°C | 30 min @ RT (cooled) |
| 100% Ethanol | 45 min @ RT | 45 min @ RT | 45 min @ RT | 2 times, 30 min @ RT |
| Pure acetone | 3 times , 45 min each @ RT | 3 times , 45 min each @ RT | 3 times , 45 min each @ RT | 4 times, 20 min each @ RT |
| 50% Spurr’s resin in acetone | 3 hr @ RT (no rotation, closed tube). Next, open tube for 90 min initial rotation + overnight @ RT | 3 hr @ RT (no rotation, closed tube). Next, open tube for 90 min initial rotation + overnight @ RT | 3 hr @ RT (no rotation, closed tube). Next, open tube for 90 min initial rotation + overnight @ RT | 4 hr @ RT, closed tube cap, slow rotation |
| 75% Spurr’s resin | N/A | N/A | N/A | Overnight @ RT. Slow rotation, closed caps. |
| 100% Spurr’s resin | 6 hr @ RT | 6 hr @ RT | 6 hr @ RT | 2 times, 3 hr @ RT each. No rotation |

**Dehydration and embedding times and temperatures**.

Time and duration of each dehydration and embedding step for samples from 3 cortical region (n=4).

**Supplementary table 3**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | S1 | V2 | PPC | ACC | LPtA | PPC-2 |
| Main bifurcation input mapping | X | X | X | X |  | X |
| Reconstruction of all apical dendrites |  |  |  | X |  |  |
| Synapse size estimation | X | X | X | X |  |  |
| Spine innervation fraction |  | X | X | X | X |  |
| Double spine fraction | X | X | X | X |  |  |
| Fractional innervation of inhibitory axons | X | X | X | X |  |  |
| Cell type comparisons of inhibitory fraction | X (only L2) | X(only L2) | X(only L2) | X(only L2) | X | X |
| Path distance to soma dependency | X (only L2) | X(only L2) | X(only L2) | X(only L2) | X | X |
| Profile of inhibitory fraction along upper cortex | X | X | X | X | X | X |

**Overview of analyses carried out in the 6 datasets.**

**Supplementary Table 4**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Dataset layer origin | L1 | | L2 | |
| Seed structure | Shaft | Spine | Shaft | Spine |
| Layer 2 | 14,29% (n = 14) | 100% (n = 5) | 0% (n = 92) | 97,1% (n = 35) |
| Layer 3 | 3,7% (n = 27) | 100% (n = 16) | N/A | N/A |
| Layer 5tt | 22,5% (n = 40) | 93,3% (n = 30) | N/A | N/A |
| Deep (Layer 3/5) | N/A | N/A | 1,1% (n = 91) | 98,4% (n = 61) |
| Layer 5st | 60% (n=10) | N/A | 44,44% (n = 9) | N/A |

**Fraction of spine-preferring input on spine and shaft of ADs.**

The fraction of spine-preferring (likely excitatory) synapse onto shaft and spine of apical dendrites for L2-5 pyramidal cell types in layers 1 and 2. N refers to number of axons.

**Supplementary Table 5**

|  |  |  |
| --- | --- | --- |
|  | Somatic depth relative to pia (µm) | |
| Dataset name | PPC-2 | LPtA |
| Layer 2 | 208-250 | 268-295 |
| Layer 2MN | 166, 172 | N/A |
| Layer 3 | 304-376 | 327-442 |
| Layer 5tt | 524-582 | 621-657 |
| Layer 5st | 507-620 | N/A |

**Somatic depth of pyramidal neurons residing in layers 2-5 (n = 40, 11 for PPC-2 and LPtA, respectively).**