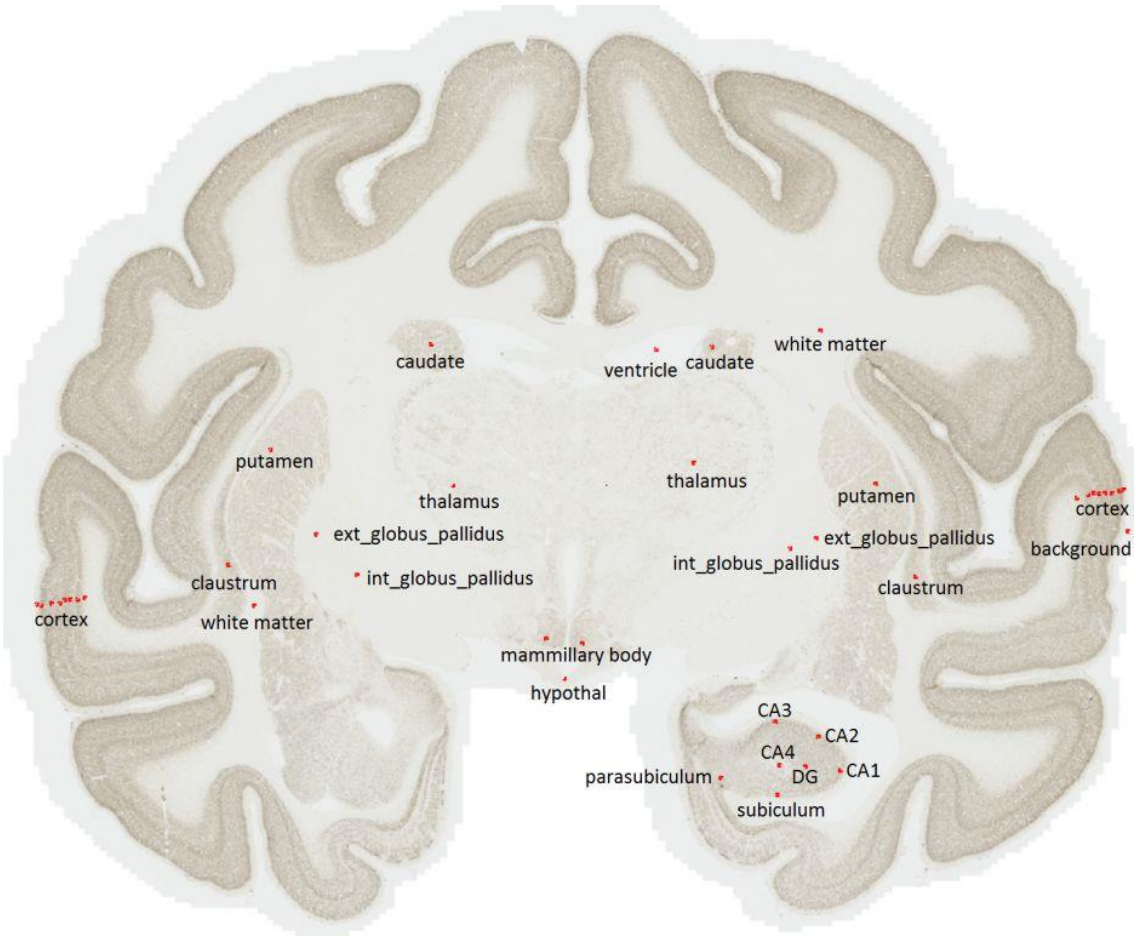
# *Supplementary Material*

## 1 Major anatomical regions studied in this work

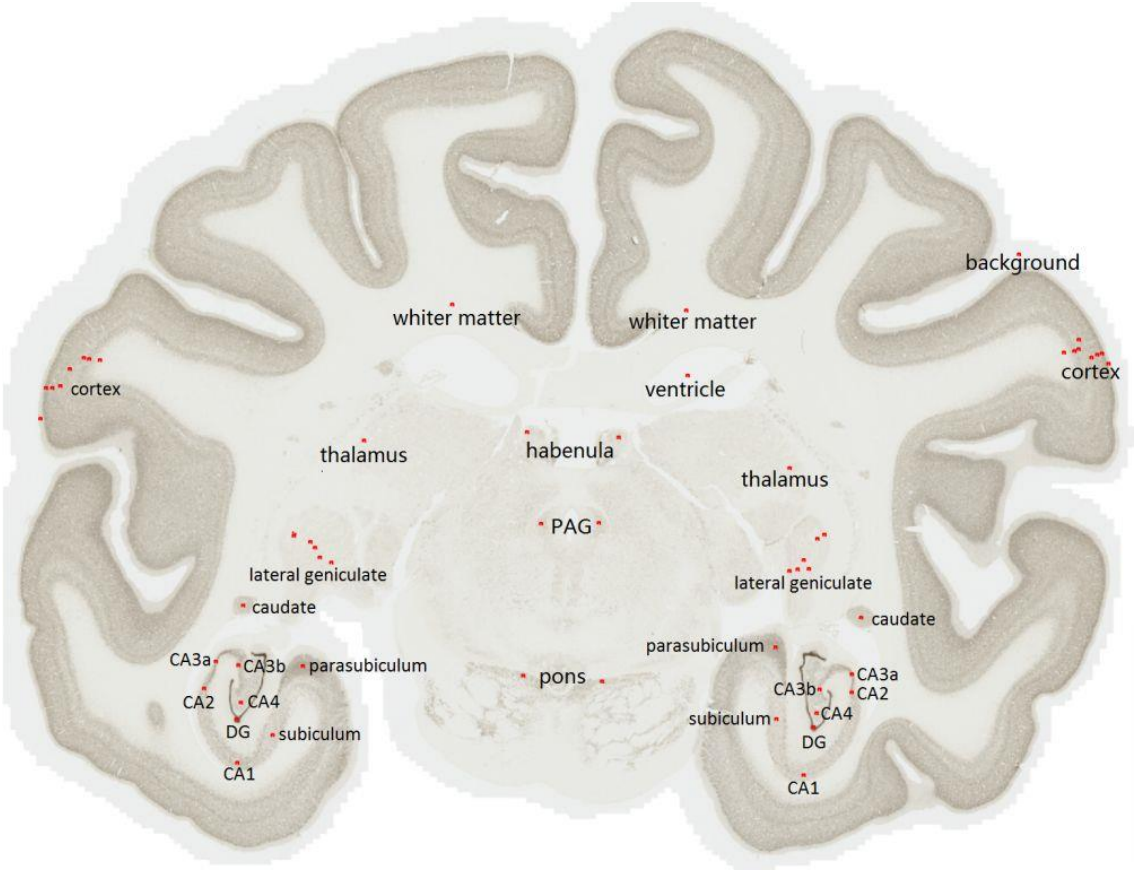
**Supplementary Table 1.** List of the major anatomical regions used in three different datasets. Se: *segmentation dataset*, I: *individualization dataset*, St: *stereology dataset*.

| N° | Anatomical regions | | Dataset |
|---|---|---|---|
| | Major regions | Sub-regions | |
| 1 | Cortex | Cortex layer 1-6 | Se / I / St |
| 2 | Caudate | / | Se / I / St |
| 3 | Thalamus | / | Se / I / St |
| 4 | Claustrum | / | Se / I / St |
| 5 | Putamen | / | Se / I |
| 6 | Hippocampus | CA1-4 (Cornu Ammonis 1-4) | Se / I |
| | | Dentate gyrus | Se / I / St |
| 7 | Subiculum | Subiculum | Se / I / St |
| | | Para-subiculum | |
| 8 | Globus pallidus | Globus pallidus internal | Se |
| | | Globus pallidus external | |
| 9 | Hypothal (Hypothalamus) | / | Se |
| 10 | Habenula | / | Se |
| 11 | Mammillary body | / | Se |
| 12 | LG (lateral geniculate) | / | Se |
| 13 | PAG (periaqueductal gray) | / | Se |
| 14 | Pons | / | Se |
| 15 | White matter | / | Se / I |
| 16 | Ventricle | / | Se / I |
| 17 | Background | / | Se |

## 2 Dataset for the segmentation of neuronal staining – *segmentation dataset*




**Supplementary Figure 1.** Image of brain section N° 81 of 216,000x178,000 pixels (47.57 mm x 39.16 mm), ~ 130 GB. The red squares represent the spatial location of images of 512x512 pixels in the *segmentation dataset*. The legends correspond to anatomical structures to which they belong.




**Supplementary Figure 2**. Image of brain section N° 101 of 236,000x181,000 pixels (52 mm x 39.9 mm), ~ 148 GB. The red squares represent the spatial location of images of 512x512 pixels in the *segmentation dataset*. The legends correspond to anatomical structures to which they belong.

**Supplementary Table 2.** List of *segmentation dataset* images for the segmentation of neuronal staining. Side: L – Left, R – Right, C – central. L/V: Learning set / Validation set. The size of images is 512x512 pixels.

| N° | Image name | Side | Region | Position X | Position Y | L/V |
|---|---|---|---|---|---|---|
| 1 | CJ1301_slide81unsharp_BG_R | R | background | 215024 | 100457 | L |
| 2 | CJ1301_slide81unsharp_CA1_R | R | CA1 | 160066 | 146153 | L |
| 3 | CJ1301_slide81unsharp_CA2_R | R | CA2 | 155891 | 139728 | L |
| 4 | CJ1301_slide81unsharp_CA3_R | R | CA3 | 147641 | 136853 | L |
| 5 | CJ1301_slide81unsharp_CA4_R | R | CA4 | 148456 | 145163 | L |
| 6 | CJ1301_slide81unsharp_caudate_L | L | caudate | 81930 | 64713 | V |
| 7 | CJ1301_slide81unsharp_caudate_R | R | caudate | 135517 | 65280 | L |
| 8 | CJ1301_slide81unsharp_claustrum_L | L | claustrum | 43161 | 106864 | V |

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|---|---|---|---|---|---|---|
| 9 | CJ1301_slide81unsharp_claustrum_R | R | claustrum | 174451 | 109137 | L |
| 10 | CJ1301_slide81unsharp_ctx_L_layer1 | L | cortex layer1 | 6538 | 114411 | V |
| 11 | CJ1301_slide81unsharp_ctx_L_layer2 | L | cortex layer2 | 7375 | 114628 | L |
| 12 | CJ1301_slide81unsharp_ctx_L_layer3 | L | cortex layer3 | 9386 | 114274 | V |
| 13 | CJ1301_slide81unsharp_ctx_L_layer4 | L | cortex layer4 | 10962 | 114238 | V |
| 14 | CJ1301_slide81unsharp_ctx_L_layer5a | L | cortex layer5a | 11864 | 113558 | L |
| 15 | CJ1301_slide81unsharp_ctx_L_layer5b | L | cortex layer5b | 12980 | 113494 | L |
| 16 | CJ1301_slide81unsharp_ctx_L_layer6a | L | cortex layer6a | 14313 | 113494 | L |
| 17 | CJ1301_slide81unsharp_ctx_L_layer6b | L | cortex layer6b | 15736 | 113172 | V |
| 18 | CJ1301_slide81unsharp_ctx_R_layer1 | R | cortex layer1 | 214234 | 92503 | L |
| 19 | CJ1301_slide81unsharp_ctx_R_layer2 | R | cortex layer2 | 213394 | 92727 | V |
| 20 | CJ1301_slide81unsharp_ctx_R_layer3 | R | cortex layer3 | 211980 | 93207 | L |
| 21 | CJ1301_slide81unsharp_ctx_R_layer4 | R | cortex layer4 | 210620 | 93159 | L |
| 22 | CJ1301_slide81unsharp_ctx_R_layer5a | R | cortex layer5a | 209532 | 93303 | V |
| 23 | CJ1301_slide81unsharp_ctx_R_layer5b | R | cortex layer5b | 208508 | 93127 | V |
| 24 | CJ1301_slide81unsharp_ctx_R_layer6a | R | cortex layer6a | 207388 | 93463 | V |
| 25 | CJ1301_slide81unsharp_ctx_R_layer6b | R | cortex layer6b | 205276 | 94103 | L |
| 26 | CJ1301_slide81unsharp_DG_R | R | dentate gyrus | 153366 | 145403 | L |
| 27 | CJ1301_slide81unsharp_globus_pallidus_ext_L | L | globus pallidus | 59954 | 101070 | V |
| 28 | CJ1301_slide81unsharp_globus_pallidus_ext_R | R | globus pallidus | 155441 | 101799 | L |
| 29 | CJ1301_slide81unsharp_globus_pallidus_int_L | L | globus pallidus | 67746 | 108801 | L |
| 30 | CJ1301_slide81unsharp_globus_pallidus_int_R | R | globus pallidus | 150434 | 103746 | V |
| 31 | CJ1301_slide81unsharp_hypothal | C | hypothal | 107307 | 128570 | L |
| 32 | CJ1301_slide81unsharp_mam_body_L | L | mammillary_body | 103854 | 120974 | L |
| 33 | CJ1301_slide81unsharp_mam_body_R | R | mammillary_body | 110739 | 121899 | V |
| 34 | CJ1301_slide81unsharp_putamen_L | L | putamen | 51147 | 84883 | L |
| 35 | CJ1301_slide81unsharp_putamen_R | R | putamen | 166812 | 91399 | V |
| 36 | CJ1301_slide81unsharp_subiculum_para_R | R | para-subiculum | 137257 | 147603 | L |
| 37 | CJ1301_slide81unsharp_subiculum_R | R | subiculum | 148216 | 150803 | L |
| 38 | CJ1301_slide81unsharp_thalamus_L | L | thalamus | 86018 | 91673 | L |
| 39 | CJ1301_slide81unsharp_thalamus_R | R | thalamus | 132095 | 87392 | V |
| 40 | CJ1301_slide81unsharp_ventricle_R | R | ventricle | 124922 | 65719 | V |

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|---|---|---|---|---|---|---|
| 41 | CJ1301_slide81unsharp_WM_L | L | white matter | 48104 | 114674 | V |
| 42 | CJ1301_slide81unsharp_WM_R | R | white matter | 156246 | 62111 | L |
| 43 | CJ1301_slide101unsharp_BG_R | R | background | 212245 | 52644 | V |
| 44 | CJ1301_slide101unsharp_CA1_L | L | CA1 | 49025 | 158817 | L |
| 45 | CJ1301_slide101unsharp_CA1_R | R | CA1 | 167285 | 161394 | V |
| 46 | CJ1301_slide101unsharp_CA2_L | L | CA2 | 42084 | 143295 | L |
| 47 | CJ1301_slide101unsharp_CA2_R | R | CA2 | 177325 | 144103 | V |
| 48 | CJ1301_slide101unsharp_CA3a_L | L | CA3a | 44549 | 137657 | L |
| 49 | CJ1301_slide101unsharp_CA3a_R | R | CA3a | 177356 | 140325 | V |
| 50 | CJ1301_slide101unsharp_CA3b_L | L | CA3b | 49255 | 138429 | V |
| 51 | CJ1301_slide101unsharp_CA3b_R | R | CA3b | 170600 | 143485 | L |
| 52 | CJ1301_slide101unsharp_CA4_L | L | CA4 | 49763 | 146237 | L |
| 53 | CJ1301_slide101unsharp_CA4_R | R | CA4 | 169976 | 148447 | V |
| 54 | CJ1301_slide101unsharp_caudate_L | L | caudate | 50258 | 125958 | L |
| 55 | CJ1301_slide101unsharp_caudate_R | R | caudate | 179257 | 128488 | V |
| 56 | CJ1301_slide101unsharp_ctx_L_layer1 | L | cortex layer1 | 7920 | 86958 | L |
| 57 | CJ1301_slide101unsharp_ctx_L_layer2 | L | cortex layer2 | 9075 | 80531 | V |
| 58 | CJ1301_slide101unsharp_ctx_L_layer3 | L | cortex layer3 | 10405 | 80533 | L |
| 59 | CJ1301_slide101unsharp_ctx_L_layer4 | L | cortex layer4 | 12079 | 80133 | L |
| 60 | CJ1301_slide101unsharp_ctx_L_layer5a | L | cortex layer5a | 14044 | 76592 | V |
| 61 | CJ1301_slide101unsharp_ctx_L_layer5b | L | cortex layer5b | 16993 | 74252 | V |
| 62 | CJ1301_slide101unsharp_ctx_L_layer6a | L | cortex layer6a | 18092 | 74491 | V |
| 63 | CJ1301_slide101unsharp_ctx_L_layer6b | L | cortex layer6b | 20348 | 74728 | L |
| 64 | CJ1301_slide101unsharp_ctx_R_layer1 | R | cortex layer1 | 230933 | 75485 | V |
| 65 | CJ1301_slide101unsharp_ctx_R_layer2 | R | cortex layer2 | 229580 | 73350 | L |
| 66 | CJ1301_slide101unsharp_ctx_R_layer3 | R | cortex layer3 | 228587 | 73598 | V |
| 67 | CJ1301_slide101unsharp_ctx_R_layer4 | R | cortex layer4 | 227383 | 74206 | V |
| 68 | CJ1301_slide101unsharp_ctx_R_layer5a | R | cortex layer5a | 224744 | 70457 | L |
| 69 | CJ1301_slide101unsharp_ctx_R_layer5b | R | cortex layer5b | 224692 | 72436 | L |
| 70 | CJ1301_slide101unsharp_ctx_R_layer6a | R | cortex layer6a | 223797 | 72825 | L |
| 71 | CJ1301_slide101unsharp_ctx_R_layer6b | R | cortex layer6b | 221626 | 73142 | V |
| 72 | CJ1301_slide101unsharp_DG_L | L | dentate gyrus | 48819 | 149736 | L |

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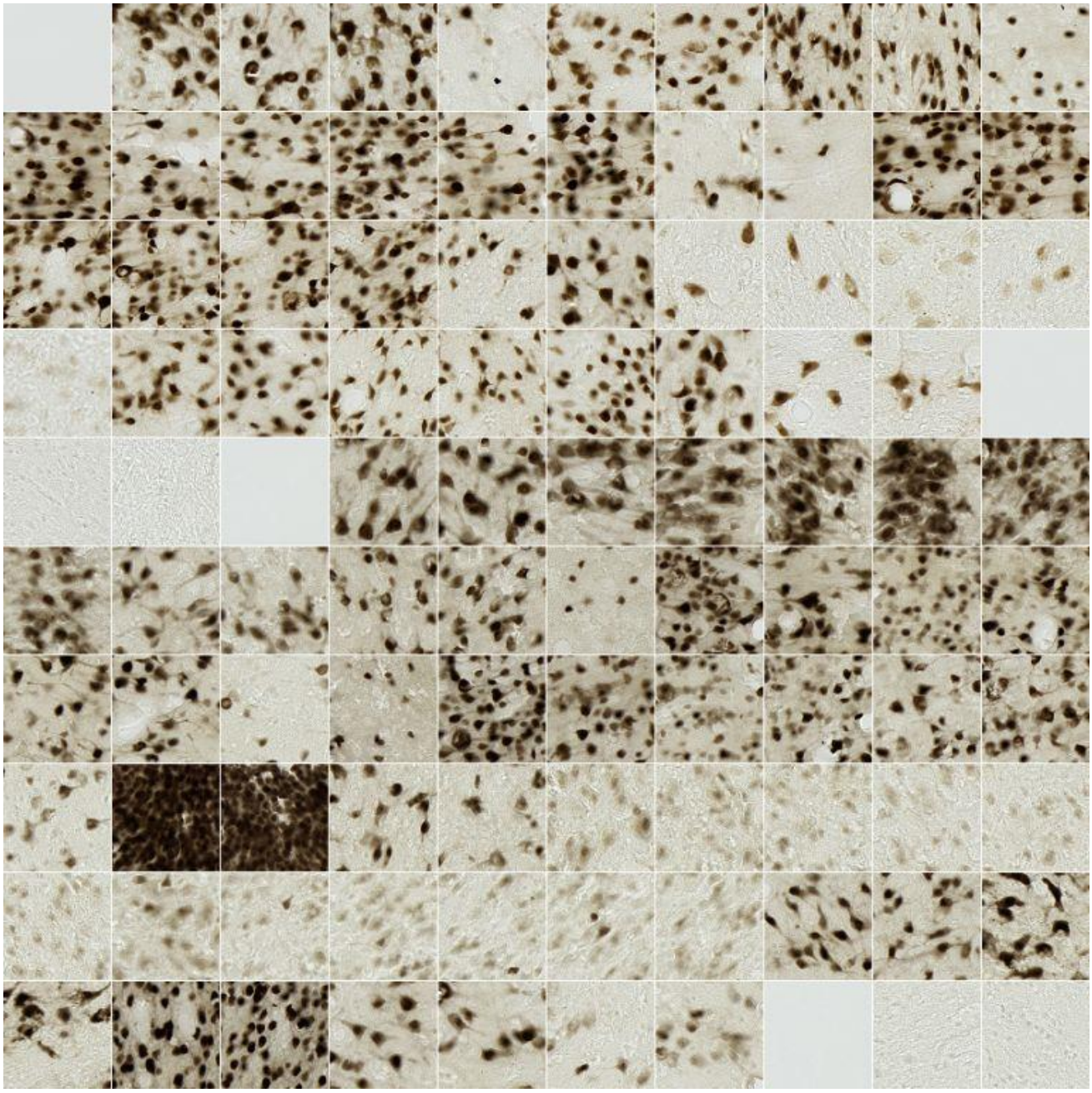
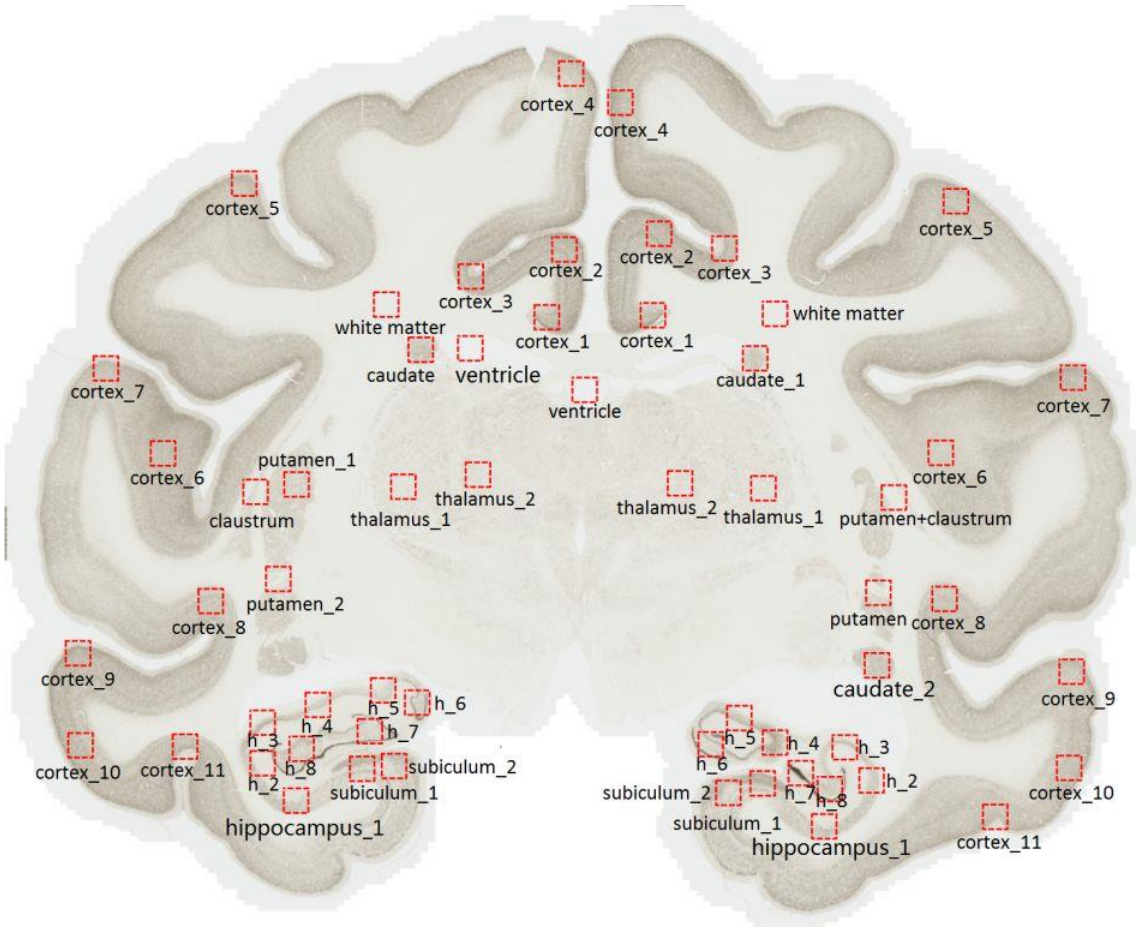
|---|---|---|---|---|---|---|
| 73 | CJ1301_slide101unsharp_DG_R | R | dentate gyrus | 169343 | 151390 | V |
| 74 | CJ1301_slide101unsharp_habenula_L | L | habenula | 109521 | 89758 | V |
| 75 | CJ1301_slide101unsharp_habenula_R | R | habenula | 128654 | 90860 | L |
| 76 | CJ1301_slide101unsharp_LG_L_1 | L | LG | 68660 | 116983 | L |
| 77 | CJ1301_slide101unsharp_LG_L_2 | L | LG | 66256 | 115925 | V |
| 78 | CJ1301_slide101unsharp_LG_L_3 | L | LG | 65241 | 113883 | L |
| 79 | CJ1301_slide101unsharp_LG_L_4 | L | LG | 64272 | 112680 | V |
| 80 | CJ1301_slide101unsharp_LG_L_5 | L | LG | 60837 | 111409 | L |
| 81 | CJ1301_slide101unsharp_LG_L_6 | L | LG | 60914 | 110889 | V |
| 82 | CJ1301_slide101unsharp_LG_R_1 | R | LG | 164301 | 118741 | V |
| 83 | CJ1301_slide101unsharp_LG_R_2 | R | LG | 166026 | 118416 | L |
| 84 | CJ1301_slide101unsharp_LG_R_3 | R | LG | 167298 | 116469 | V |
| 85 | CJ1301_slide101unsharp_LG_R_4 | R | LG | 168376 | 118300 | L |
| 86 | CJ1301_slide101unsharp_LG_R_5 | R | LG | 170150 | 112041 | L |
| 87 | CJ1301_slide101unsharp_LG_R_6 | R | LG | 171612 | 111158 | V |
| 88 | CJ1301_slide101unsharp_PAG_L | L | PAG | 112445 | 108877 | V |
| 89 | CJ1301_slide101unsharp_PAG_R | R | PAG | 124524 | 108709 | L |
| 90 | CJ1301_slide101unsharp_pons_L | L | pons | 108715 | 140626 | L |
| 91 | CJ1301_slide101unsharp_pons_R | R | pons | 125184 | 141824 | V |
| 92 | CJ1301_slide101unsharp_subiculum_para_L | L | para-subiculum | 62704 | 138606 | L |
| 93 | CJ1301_slide101unsharp_subiculum_para_R | R | para-subiculum | 161407 | 134685 | V |
| 94 | CJ1301_slide101unsharp_subiculum_L | L | subiculum | 56393 | 153016 | L |
| 95 | CJ1301_slide101unsharp_subiculum_R | R | subiculum | 161588 | 149664 | V |
| 96 | CJ1301_slide101unsharp_thalamus_L | L | thalamus | 75488 | 91524 | V |
| 97 | CJ1301_slide101unsharp_thalamus_R | R | thalamus | 164357 | 97270 | L |
| 98 | CJ1301_slide101unsharp_ventricle_R | R | ventricle | 143068 | 77944 | L |
| 99 | CJ1301_slide101unsharp_WM_L | L | white matter | 93897 | 63118 | L |
| 100 | CJ1301_slide101unsharp_WM_R | R | white matter | 142766 | 64349 | V |



**Supplementary Figure 3.** *Segmentation dataset* for the segmentation of neuronal staining. From left to right, from top to bottom: image number is from N° 1 to N° 100.

## 3 Dataset for the individualization of neurons – *individualization dataset*




**Supplementary Figure 4.** Image of brain section N° 91 of 230,000x188,000 pixels (50.74 mm x 41.33 mm), ~ 145 GB. The red squares represent the spatial location of images of 5000x5000 pixels in the *individualization dataset*. "h" is an abbreviation for the name of the region of the hippocampus. The numbers at the end of each region corresponds to the number of the images in these regions.

**Supplementary Table 3.** List of *individualization dataset* images for the individualization of neurons. Side: L – Left, R – Right, C – Central. The size of images is 5000x5000 pixels. Thereinto, the images of N° 3, 24, 25 and 46 are so fuzzy that expert cannot distinguish neurons.

| N° | Image name | Side | Region | Position X | Position Y |
|---|---|---|---|---|---|
| 1 | CJ1301_slide91unsharp_caudate_L | L | caudate | 82522 | 67838 |
| 2 | CJ1301_slide91unsharp_caudate_R_1 | R | caudate | 150612 | 69601 |
| 3 | CJ1301_slide91unsharp_caudate_R_2 | R | caudate | 175410 | 132107 |
| 4 | CJ1301_slide91unsharp_claustrum_L | L | claustrum | 48891 | 96789 |

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|---|---|---|---|---|---|
| 5 | CJ1301_slide91unsharp_claustrum_R | R | claustrum | 178852 | 97930 |
| 6 | CJ1301_slide91unsharp_ctx_L_1 | L | cortex | 108174 | 61247 |
| 7 | CJ1301_slide91unsharp_ctx_L_2 | L | cortex | 111775 | 47451 |
| 8 | CJ1301_slide91unsharp_ctx_L_3 | L | cortex | 92715 | 52930 |
| 9 | CJ1301_slide91unsharp_ctx_L_4 | L | cortex | 113133 | 11625 |
| 10 | CJ1301_slide91unsharp_ctx_L_5 | L | cortex | 46576 | 34018 |
| 11 | CJ1301_slide91unsharp_ctx_L_6 | L | cortex | 30036 | 88960 |
| 12 | CJ1301_slide91unsharp_ctx_L_7 | L | cortex | 18414 | 71644 |
| 13 | CJ1301_slide91unsharp_ctx_L_8 | L | cortex | 39760 | 119015 |
| 14 | CJ1301_slide91unsharp_ctx_L_9 | L | cortex | 12737 | 129645 |
| 15 | CJ1301_slide91unsharp_ctx_L_10 | L | cortex | 13244 | 148385 |
| 16 | CJ1301_slide91unsharp_ctx_L_11 | L | cortex | 34526 | 148654 |
| 17 | CJ1301_slide91unsharp_ctx_R_1 | R | cortex | 129792 | 60821 |
| 18 | CJ1301_slide91unsharp_ctx_R_2 | R | cortex | 131056 | 44222 |
| 19 | CJ1301_slide91unsharp_ctx_R_3 | R | cortex | 144342 | 47234 |
| 20 | CJ1301_slide91unsharp_ctx_R_4 | R | cortex | 123157 | 17547 |
| 21 | CJ1301_slide91unsharp_ctx_R_5 | R | cortex | 191482 | 37605 |
| 22 | CJ1301_slide91unsharp_ctx_R_6 | R | cortex | 188387 | 88838 |
| 23 | CJ1301_slide91unsharp_ctx_R_7 | R | cortex | 215311 | 73538 |
| 24 | CJ1301_slide91unsharp_ctx_R_8 | R | cortex | 189168 | 118593 |
| 25 | CJ1301_slide91unsharp_ctx_R_9 | R | cortex | 215041 | 133395 |
| 26 | CJ1301_slide91unsharp_ctx_R_10 | R | cortex | 214521 | 153002 |
| 27 | CJ1301_slide91unsharp_ctx_R_11 | R | cortex | 199475 | 162973 |
| 28 | CJ1301_slide91unsharp_hip_L_1 | L | hippocampus | 57130 | 159664 |
| 29 | CJ1301_slide91unsharp_hip_L_2 | L | hippocampus | 50320 | 152057 |
| 30 | CJ1301_slide91unsharp_hip_L_3 | L | hippocampus | 50324 | 143779 |
| 31 | CJ1301_slide91unsharp_hip_L_4 | L | hippocampus | 61567 | 140170 |
| 32 | CJ1301_slide91unsharp_hip_L_5 | L | hippocampus | 74853 | 137205 |
| 33 | CJ1301_slide91unsharp_hip_L_6 | L | hippocampus | 81808 | 139695 |
| 34 | CJ1301_slide91unsharp_hip_L_7 | L | hippocampus | 72156 | 145516 |
| 35 | CJ1301_slide91unsharp_hip_L_8 | L | hippocampus | 58265 | 149115 |
| 36 | CJ1301_slide91unsharp_hip_R_1 | R | hippocampus | 164647 | 164902 |

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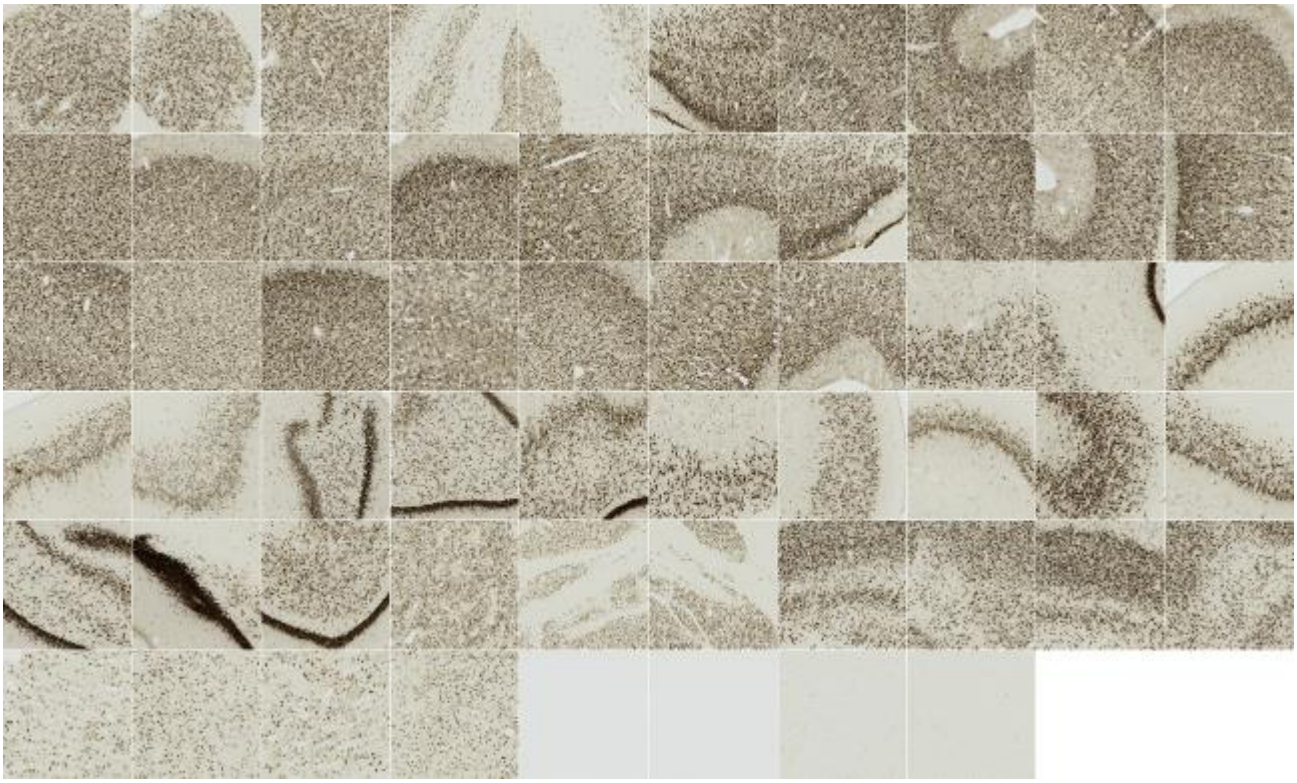
|---|---|---|---|---|---|
| 37 | CJ1301_slide91unsharp_hip_R_2 | R | hippocampus | 174314 | 155545 |
| 38 | CJ1301_slide91unsharp_hip_R_3 | R | hippocampus | 168837 | 148803 |
| 39 | CJ1301_slide91unsharp_hip_R_4 | R | hippocampus | 154655 | 147824 |
| 40 | CJ1301_slide91unsharp_hip_R_5 | R | hippocampus | 147370 | 142851 |
| 41 | CJ1301_slide91unsharp_hip_R_6 | R | hippocampus | 141545 | 148185 |
| 42 | CJ1301_slide91unsharp_hip_R_7 | R | hippocampus | 159898 | 154049 |
| 43 | CJ1301_slide91unsharp_hip_R_8 | R | hippocampus | 165822 | 157309 |
| 44 | CJ1301_slide91unsharp_putamen_L_1 | L | putamen | 57213 | 95283 |
| 45 | CJ1301_slide91unsharp_putamen_L_2 | L | putamen | 53517 | 114512 |
| 46 | CJ1301_slide91unsharp_putamen_R | R | putamen | 175690 | 117341 |
| 47 | CJ1301_slide91unsharp_subiculum_L_1 | L | subiculum | 70588 | 153176 |
| 48 | CJ1301_slide91unsharp_subiculum_L_2 | L | subiculum | 77069 | 152620 |
| 49 | CJ1301_slide91unsharp_subiculum_R_1 | R | subiculum | 152133 | 156150 |
| 50 | CJ1301_slide91unsharp_subiculum_R_2 | R | subiculum | 145144 | 158084 |
| 51 | CJ1301_slide91unsharp_thalamus_L_1 | L | thalamus | 78962 | 95737 |
| 52 | CJ1301_slide91unsharp_thalamus_L_2 | L | thalamus | 94158 | 93285 |
| 53 | CJ1301_slide91unsharp_thalamus_R_1 | R | thalamus | 152284 | 96060 |
| 54 | CJ1301_slide91unsharp_thalamus_R_2 | R | thalamus | 135308 | 95043 |
| 55 | CJ1301_slide91unsharp_vtcl_L | L | ventricle | 92600 | 67573 |
| 56 | CJ1301_slide91unsharp_vtcl_med | C | ventricle | 1158889 | 76022 |
| 57 | CJ1301_slide91unsharp_WM_L | L | white matter | 75488 | 58650 |
| 58 | CJ1301_slide91unsharp_WM_R | R | white matter | 154657 | 60546 |

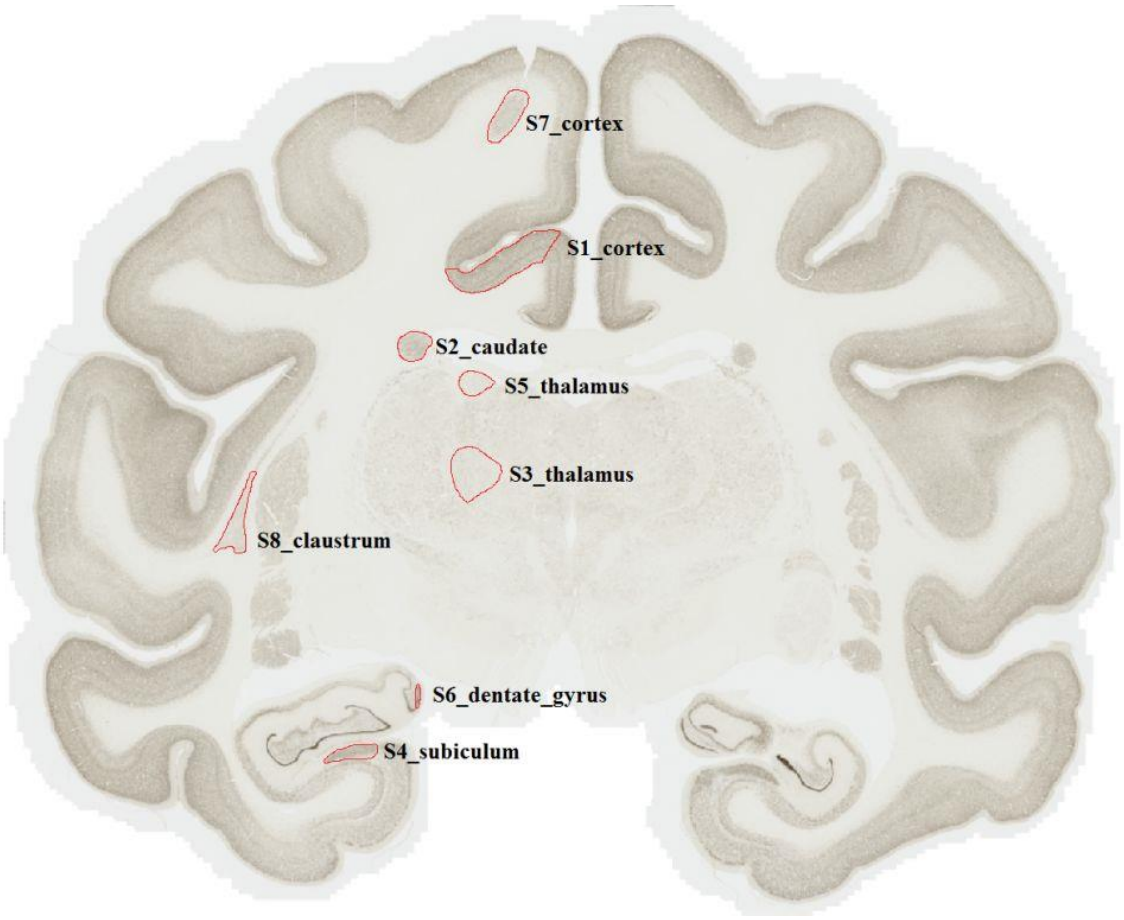


**Supplementary Figure 5.** *Individualization dataset* images for the individualization of neurons. From left to right, from top to bottom: images are numbered from N° 1 to N° 58. Thereinto, the images of N° 3, 24, 25 and 46 were so fuzzy that expert couldn't distinguish neurons.

Note:

- The images of N° 1-2, 4-23, 26-45, 47-54 were used to compare the number of neurons estimated automatically and manually.
- The images of N° 1, 4, 7, 12, 33-34, 44, 47, 51 were used to study the location of the centroids and the contours of the neurons.

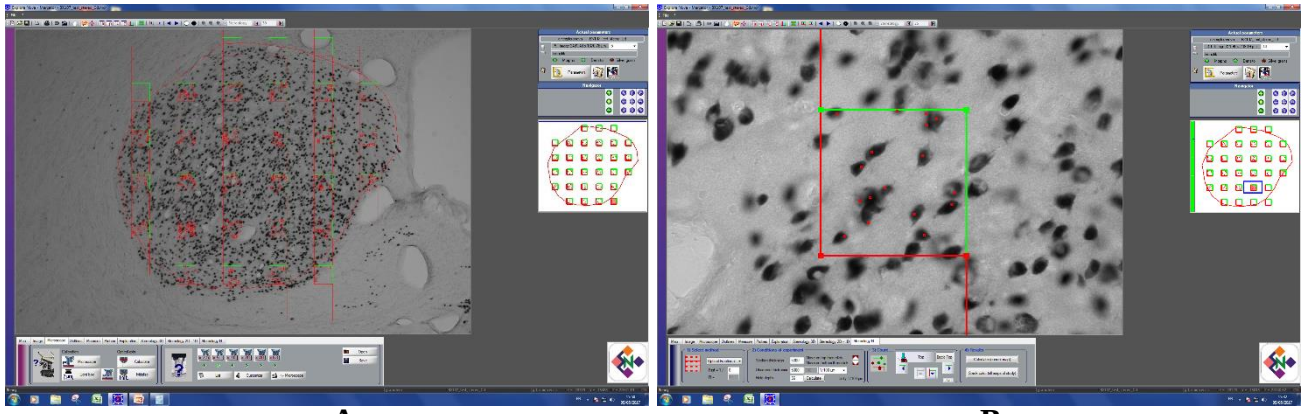
## 4 Dataset for the quantitative stereological counting – *stereology dataset*




**Supplementary Figure 6.** Image of brain section N° 91. The regions delineated in red are the regions of interest selected to carry out the quantitative stereological counting which was used as the ground truth to validate the proposed method of individualization. S1 - 8 correspond to the number of the ROIs.

## 5 Stereological counting

Two experts (biologists b1 and b2) who master stereology techniques estimated the number of neurons on the *stereology dataset* (Supplementary Figure 6) dedicated to stereological counting. Each biologist counted the neurons twice independently on the same anatomical region directly under a microscope using Mercator (Explora Nova, Supplementary Figure 7). In each anatomical region, the biologists first traced the contours of the region to be studied (both expert used the same delineated contours), and then performed random systematic sampling using a number of optical dissectors with the optimal parameters determined in order to obtain an unbiased estimation (the optical dissector size, the distance between two optical dissectors are displayed in "Sampling" column in Supplementary Table 4). The sampling fraction ranged from 1/100 to 1/4 in this study. In each optical dissector (Supplementary Figure 7B), there was a green border (the acceptance line) and a red border (the rejection line) that gave the rules for taking or not into account the neurons that intersect them. The biologists then pointed out the centers of neurons that were located inside the optical dissectors or touching the green borders. Neurons touching the red borders were not counted. Working directly under microscope, it is possible for biologists to modify the focus according to the section depth which facilitates the identification of overlapping or cut neurons. In order to be able to compare with image processing approach which deal with flatten images (single focus setting), we adapted this technique and we did not take into account dead zones.



**A** **B**

**Supplementary Figure 7.** (**A**) Mercator software. The biologist first delineated the region of interest – the red outline corresponding to the caudate region. With the optimal parameters, a sampling of optical dissectors was performed and the neurons were counted by the biologists. (**B**) Illustration of the counting of neurons in an optical dissector. Neurons are counted if they are entirely inside the optical dissector or if they intersect the acceptance lines (green) without touching the rejection lines (red). The small red square on neuron are centers pointed out by biologists.

Once the neuron counting achieved on this sampling, the software Mercator have estimated the total number of neurons in this region according to the equation calculated as:

$$N_{total} = \frac{1}{ssf} \times \frac{1}{asf} \times \frac{1}{hsf} \times \sum Q^{-} = \frac{1}{asf} \times \sum Q^{-}$$

where *ssf* is the section sampling fraction, *asf* is the area sampling fraction, *hsf* is the height of the dissector sample relative to the section thickness, $N_{total}$ is the total number of neurons counted in the optical dissectors. In this study, *ssf*=1, *hsf*=1.

**Supplementary Table 4.** Information of the second sampling about stereological counting, concerning sampling fraction for each anatomical region (the optical dissector (OD) size (μm × μm) / the square of the distance between two optical dissectors (μm)), the number (Nb) of counted optical dissector, the number of counted neurons in all optical dissectors, the total area of all optical dissectors, the region area and the number of neurons evaluated in each anatomical region. In this study, *asf*=Counted area / Region area. Nb of evaluated neurons = Nb of counted neurons / (Counted area / Region area).

| Regions | Expert | Sampling | Nb of counted OD | Nb of counted neurons | Counted area ($\mu m^2$) | Region area ($\mu m^2$) | Nb of evaluated neurons |
|---|---|---|---|---|---|---|---|
| S1_cortex | b1 | 50×50/400×400 | 28 | 226 | 70000 | 5892227 | 19023 |
| | b2 | 50×50/400×400 | 28 | 211 | 70000 | | 17761 |
| S2_caudate | b1 | 50×50/100×100 | 63 | 263 | 157500 | 1558283 | 2602 |
| | b2 | 50×50/100×100 | 68 | 266 | 170000 | | 2438 |
| S3_thalamus | b1 | 50×50/200×200 | 62 | 88 | 155000 | 4239108 | 2407 |
| | b2 | 50×50/200×200 | 69 | 90 | 172500 | | 2212 |
| S4_subiculum | b1 | 50×50/200×200 | 15 | 107 | 37500 | 1235170 | 3524 |
| | b2 | 50×50/200×200 | 18 | 130 | 45000 | | 3568 |
| S5_thalamus | b1 | 50×50/100×100 | 55 | 64 | 137500 | 1327570 | 618 |
| | b2 | 50×50/100×100 | 57 | 49 | 142500 | | 456 |
| S6_dentate_gyrus | b1 | 20×20/40×40 | 29 | 88 | 11600 | 126718 | 961 |
| | b2 | 20×20/40×40 | 30 | 98 | 12000 | | 1035 |
| S7_corte× | b1 | 50×50/400×400 | 11 | 45 | 27500 | 2332417 | 3817 |
| | b2 | 50×50/400×400 | 11 | 57 | 27500 | | 4834 |
| S8_claustrum | b1 | 50×50/200×200 | 39 | 172 | 97500 | 2309251 | 4074 |
| | b2 | 50×50/200×200 | 34 | 127 | 85000 | | 3450 |

## 6 Statistics calculated for average neuron radius in different anatomical regions

**Supplementary Table 5.** Six summary statistics calculated for average neuron radius in different anatomical region. The unit is micrometer.

| Anatomical regions | min | 1st Qu. | median | mean | 3rd Qu. | max |
|---|---|---|---|---|---|---|
| Caudate | 1.26 | 4.02 | 5.05 | 4.91 | 5.79 | 10.36 |
| Claustrum | 1.28 | 2.99 | 3.69 | 3.80 | 4.52 | 9.30 |
| Cortex | 1.34 | 3.74 | 4.64 | 4.77 | 5.69 | 17.80 |
| Hippocampus | 1.02 | 3.96 | 4.91 | 5.08 | 6.02 | 20.38 |
| Putamen | 1.44 | 3.31 | 3.98 | 4.033 | 4.73 | 9.41 |
| Subiculum | 1.37 | 4.24 | 5.16 | 5.08 | 5.94 | 11.71 |
| Thalamus | 1.29 | 3.34 | 4.21 | 4.49 | 5.48 | 11.64 |