Supporting Information

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SI Methods

Animals. We obtained estimates of neuron and other cell numbers across the cortical expanse from four species of primates. All brain tissue for these experiments was obtained from ongoing experiments of other investigators at Vanderbilt University or was purchased, so tissue preparation methods varied across cases but not in any way that would affect our cell counts. The analyzed material came from two flat hemispheres from two different prosimian galagos (*Otolemur garnetti*), one flat hemisphere from a New World owl monkey (*Aotus nancymae*), one hemisphere from an Old World macaque monkey (*Macaca mulatta*), and one flat hemisphere from an Old World baboon (*Papio cynocephalus anubis*). The baboon brain was purchased from the tissue program at the University of Washington National Primate Research Center (http://www.wanprc.org/primate-resources/pathology-tissue-program/).

Dissection of Cortex. All brains were perfused with PBS followed by 2–4% paraformaldehyde fixative. The baboon brain was perfused with PBS only and then was fixed by immersion in 4% paraformaldehyde after flattening and dissection. The cortical hemisphere from each galago, owl monkey, and baboon was separated from subcortical structures, the pia was removed, and the sulci were opened to flatten the cortex, as described elsewhere (1–3). In the owl monkey and in galago #2 (case 08–07), identifiable visual, somatosensory, auditory, and motor areas or regions were dissected after the flat cortex was viewed on a light box, so that myelin-dense sensory areas appeared dark relative to surrounding cortex (Fig. 1*A*). Dissected areas were processed individually. The flattened hemispheres from galago #1 (case 07–104) and the baboon (case 09–27) were dissected into a grid of 5×5 mm^2 tissue pieces (pieces along the margins of the hemisphere were smaller and irregular). Each piece was weighed, and, where possible, pieces were assigned to a cortical area identified by transmitted light. Some pieces were processed individually, and some were combined with small neighboring pieces.

Tissue Processing and Cell Counting. The isotropic fractionator method (4) was used to determine the number of neurons and nonneuron cells in each sample. Details of processing steps are detailed in previous publications (e.g., ref. 5). Briefly, tissue samples from each case were homogenized in a dissociation solution of Triton X-100 and sodium citrate in distilled water, using glass Pyrex Tenbroeck tissue grinders (Fisher Scientific). The resulting suspensions contained cellular nuclei, with very few cell membranes remaining intact. Because tissue samples from the present experiment were immersed in fixative solution for at least 4 wk before dissociation and processing, all went through an epitope retrieval step, which consisted of 60 min in 0.2 M boric acid solution in an oven set at 70 °C, before processing for immunocytochemistry. Samples were spun down in a centrifuge and resuspended in a mixture of PBS and DAPI. All suspension volumes were between 3 and 15 mL and were determined based on approximate cellular density. DAPI-stained nuclei were counted on a fluorescence microscope (Nikon E-800, Nikon Instruments, Inc.) using a Neubauer counting chamber; then a sample was taken from the main suspension and stained for NeuN (Millipore, Inc.), an antibody that stains neuronal nuclear antigen expressed only in the cytoplasm and nuclei of neurons. A fluorescent secondary antibody, goat anti-mouse IgG-Alexa Fluor 594 (Invitrogen), was used to visualize stained nuclei, and the ratio of neurons to nonneuron cells was determined using a fluorescence microscope. For each sample, a minimum of 500 DAPI-positive nuclei were evaluated for labeling with NeuN/AF594.

Data Analysis. Percentages of neurons relative to nonneuron cells and neuron densities were calculated for all tissue samples. For the galago cortex (07-104) dissected into a grid of samples, surface areas were measured using ImageJ software (National Institutes of Health), and x and y centroids were determined for each tissue piece in the cortical grid (Fig. 1*B*). This process allowed us to produce contour plots of the cellular and neuron density data depicted in Fig. 1 *C* and *D*, respectively, using the "Akima" package (6) of R statistical software (http://www.r-project.org/). (See plot of neuronal density in the galago cortex, case 07–104; Fig. 1*D*.) Larger samples from the macaque monkey cortex were related to the estimated locations of primary sensory and motor areas as well as to several other previously described subdivisions of cortex.

1. Huerta MF, Krubitzer LA, Kaas JH (1987) Frontal eye field as defined by intracortical microstimulation in squirrel monkeys, owl monkeys, and macaque monkeys. II. Cortical connections. *J Comp Neurol* 265:332–361.
2. Killackey HP, Gould HJ 3rd, Cusick CG, Pons TP, Kaas JH (1983) The relation of corpus callosum connections to architectonic fields and body surface maps in sensorimotor cortex of new and old world monkeys. *J Comp Neurol* 219:384–419.
3. Sincich LC, Adams DL, Horton JC (2003) Complete flatmounting of the macaque cerebral cortex. *Vis Neurosci* 20:663–686.
4. Herculano-Houzel S, Lent R (2005) Isotropic fractionator: A simple, rapid method for the quantification of total cell and neuron numbers in the brain. *J Neurosci* 25: 2518–2521.
5. Collins CE, Young NA, Flaherty DK, Airey DC, Kaas JH (2010) A rapid and reliable method of counting neurons and other cells in brain tissue: A comparison of flow cytometry and manual counting methods. *Frontiers in Neuroanatomy* 4:5.
6. Akima H (1978) A method of bivariate interpolation and smooth surface fitting for irregularly distributed data points. *ACM Trans Math Softw* 4:148–159.

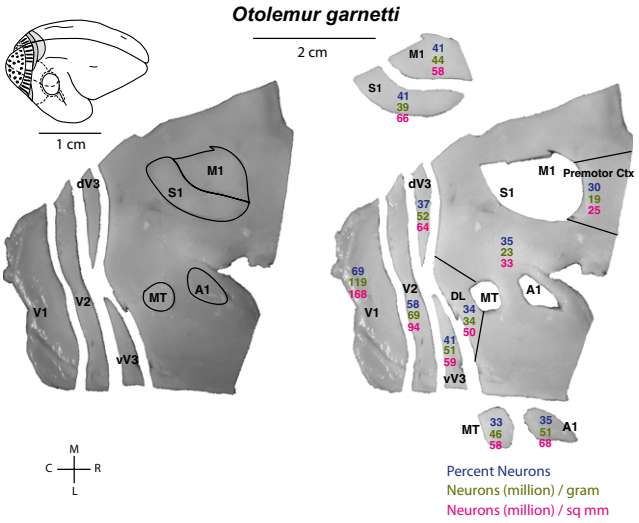

Fig. S1. A right hemisphere from a prosimian galago (*Otolemur garnetti*, galago #2; case 08–07) was flattened and backlit to reveal the locations of densely myelinated areas for dissection. The primary visual cortex (V1)/V2 border was clearly visible, and V1 was dissected into a single, large sample. The second visual area, V2, and the ventral and dorsal portions of the third visual area, V3v and V3d, were dissected by approximating the width of the area relative to the V1/V2 border. All other areas—the middle temporal area (MT), primary auditory cortex (A1), primary somatosensory cortex (S1), and primary motor cortex (M1)—were easily identified and dissected. The undissected, remaining cortex was processed as a single sample and was found to have 35% neurons and about 23 million neurons/g of tissue. The flat cortex on the right shows all the dissected areas with the percent neurons, neuron densities by weight (millions/g), and neuron densities per square millimeter of cortical surface. V1 has the highest percentage of neurons and the highest neuron density (119 million neurons/g), followed by V2. Raw data for this case are given in Dataset S1.

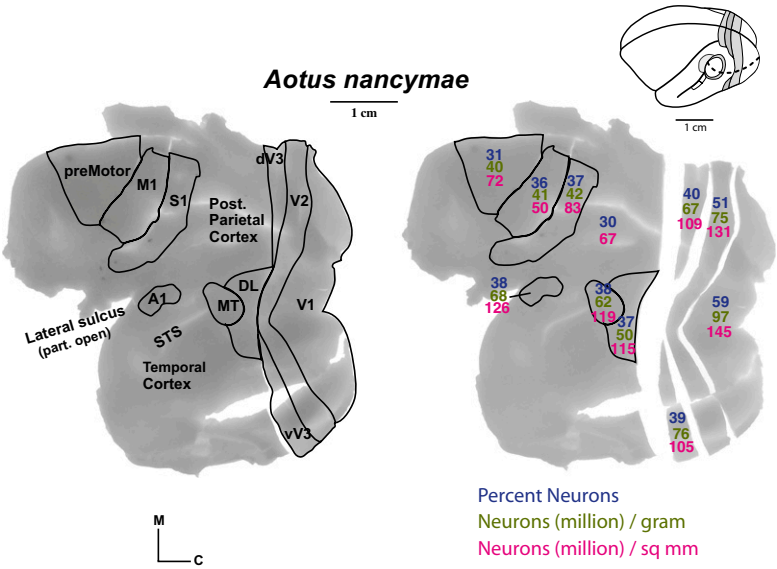

Fig. S2. A left hemisphere from a New World owl monkey (*Aotus nancymae*, case 07–78) was dissected into identifiable areas as detailed in Fig. S1. STS, superior temporal sulcus. The pattern of neuron densities and distributions is similar to the nocturnal galago pattern but is less variable across the cortical sheet than in the Old World monkeys. Raw data for this case are given in Dataset S1.

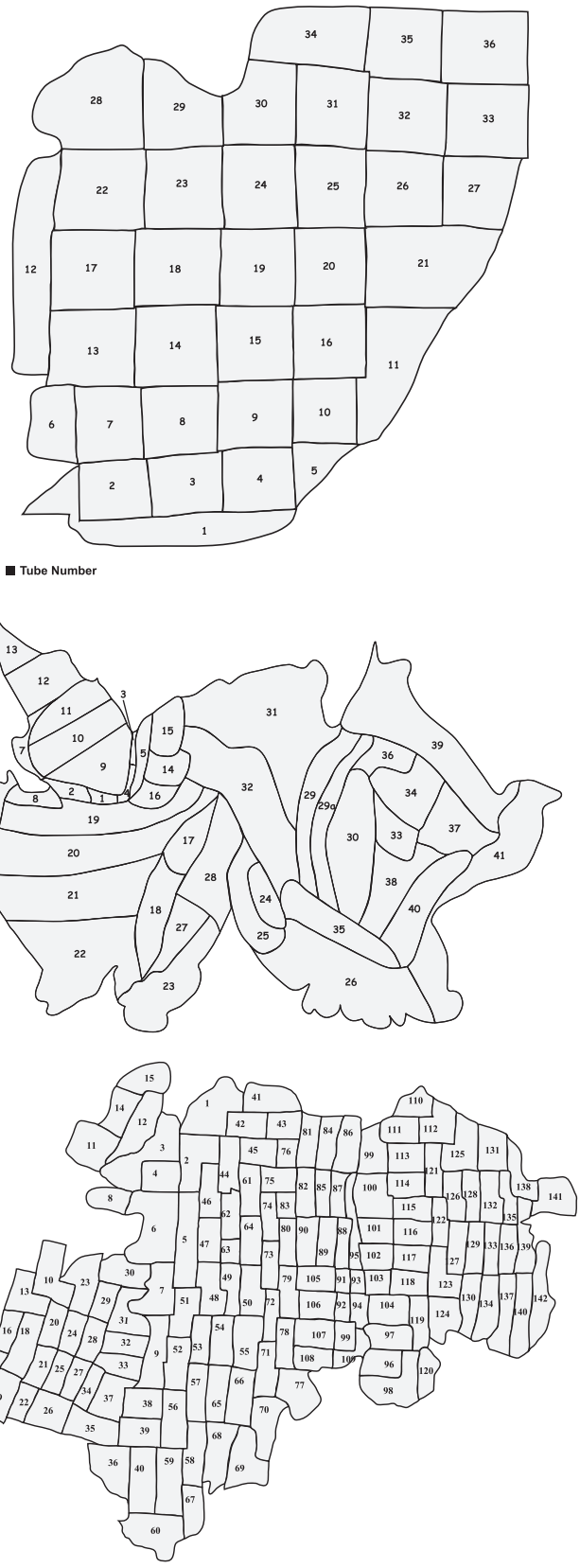

Fig. S3. Sample arrangement on cortical sheets from galago 07-104 (*Top*), macaque 08-59 (*Middle*), and baboon 09-27 (*Bottom*).

Other Supporting Information Files

Dataset S1 (XLS)