ORIGINAL ARTICLE

Invariant Synapse Density and Neuronal Connectivity Scaling in Primate Neocortical Evolution

Chet C. Sherwood¹, Sarah B. Miller², Molly Karl¹, Cheryl D. Stimpson¹, Kimberley A. Phillips³, Bob Jacobs⁴, Patrick R. Hof⁵, Mary Ann Raghanti⁶ and Jeroen B. Smaers^{7,8}

¹Department of Anthropology, Center for the Advanced Study of Human Paleobiology, The George Washington University, Washington, DC 20052, USA, ²Department of Psychological and Brain Sciences, Dartmouth College, Hanover, NH 03755, USA, ³Department of Psychology, Trinity University, San Antonio, TX 78212, USA, ⁴Department of Psychology, Laboratory of Quantitative Neuromorphology, Colorado College, Colorado Springs, CO 80946, USA, ⁵Nash Family Department of Neuroscience, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA, ⁶Department of Anthropology, School of Biomedical Sciences, Brain Health Research Institute, Kent State University, Kent, OH 44242, USA, ⁷Department of Anthropology, Stony Brook University, Stony Brook, NY 11794, USA and ⁸Division of Anthropology, American Museum of Natural History, New York, NY 10024, USA

Address correspondence to Chet C. Sherwood. Email: sherwood@gwu.edu

Abstract

Synapses are involved in the communication of information from one neuron to another. However, a systematic analysis of synapse density in the neocortex from a diversity of species is lacking, limiting what can be understood about the evolution of this fundamental aspect of brain structure. To address this, we quantified synapse density in supragranular layers II–III and infragranular layers V–VI from primary visual cortex and inferior temporal cortex in a sample of 25 species of primates, including humans. We found that synapse densities were relatively constant across these levels of the cortical visual processing hierarchy and did not significantly differ with brain mass, varying by only 1.9-fold across species. We also found that neuron densities decreased in relation to brain enlargement. Consequently, these data show that the number of synapses per neuron significantly rises as a function of brain expansion in these neocortical areas of primates. Humans displayed the highest number of synapses per neuron, but these values were generally within expectations based on brain size. The metabolic and biophysical constraints that regulate uniformity of synapse density, therefore, likely underlie a key principle of neuronal connectivity scaling in primate neocortical evolution.

Key words: brain evolution, neocortex, primate, synapse

Introduction

Synapses are the site of interneuronal communication and consume the majority of metabolic energy in the brain (Karbowski 2012; Magistretti and Allaman 2018). The density of synapses in a region of the brain, therefore, may be functionally

significant and reflect the integrative capacity of local neurons. For example, synaptic pathology has been associated with various brain disorders in humans, with synapse loss in the cerebral cortex and hippocampus characterizing Alzheimer's disease, autism spectrum disorder, depression, and schizophrenia

(Terry et al. 1991; Scheff and Price 1993; Glantz and Lewis 2000; Akram et al. 2008; Kang et al. 2012; Ross et al. 2020). In primates, cortical pyramidal neurons generally harbor a greater density of dendritic spines, the sites of excitatory synaptic contacts, in higher-order association areas than in primary sensory and motor regions (Elston et al. 2001, 2005, 2011; Jacobs et al. 2001; Bianchi et al. 2013a, 2013b). In addition, pyramidal neurons in prefrontal and temporal cortices of primates have been shown to display increased dendritic branching and greater synaptic spine numbers in correlation with brain enlargement (Elston et al. 2001, 2006; Bianchi et al. 2013a; Mohan et al. 2015).

Although such comparative data on pyramidal neuron dendritic morphology and spine numbers provide important insight into regional specializations of cortical processing, synapse density itself has not yet been systematically evaluated in the cerebral cortex. In particular, previous comparative research to quantify synapse density in mammals has considered a small number of species and a limited sample of cortical regions (Cragg 1967; O'Kusky and Colonnier 1982; Bourgeois 1997; Miki et al. 1997; Benavides-Piccione et al. 2002; DeFelipe et al. 2002; Gutiérrez-Ospina et al. 2004; Hassiotis et al. 2005; Hsu et al. 2017). Although some authors have claimed that there is relative invariance of synapse density per unit tissue volume in the adult mammalian neocortex (e.g., Changizi 2001; Herculano-Houzel 2014; Karbowski 2014), this conclusion is uncertain given differences in methodologies for synapse quantification from study to study and the sparse number of species analyzed to date. Here we examined synapse and neuron density in primates in relation to brain size to provide a more complete understanding of connectivity scaling in neocortical evolution.

We quantified synapse and neuron densities in supra-granular layers II–III and infragranular layers V–VI of primary visual cortex (V1) and inferior temporal cortex (IT) across 25 phylogenetically diverse species of primates. Synapses were labeled using immunohistochemical staining against synaptophysin (SYP), a protein that is ubiquitously present in vesicles of presynaptic terminals (Calhoun et al. 1996; Li et al. 2002; Eastwood et al. 2006; Derksen et al. 2007; Glantz et al. 2007). Area V1 and IT cortex represent two distinct hierarchical levels of functional processing within the ventral visual stream. Area V1 (Brodman's area 17) is located in the occipital lobe and is the first stage of cortical visual processing, whereas IT cortex (Brodman's areas 20 or von Economo's area TE2) is located on the convexity of the inferior temporal lobe and is involved in higher-order visual integration important for object recognition (Desimone et al. 1985; Gross 1992; Kravitz et al. 2013). Supragranular and infragranular cortical layers contain neurons that project predominantly to other cortical regions and to non-cortical brain structures, respectively (Gilbert and Kelly 1975; Barbas 1986; Nudo and Masterton 1990). Accordingly, the present study allows for a comprehensive investigation of synapse distributions in primates across variation in phylogeny, brain size, levels of cortical processing, and connectivity profile.

Materials and Methods

Specimens

We used formalin-fixed brain samples of 27 individuals from 25 primate species ($n=1$ for all species, except $n=2$ for *Homo sapiens* and *Pan troglodytes*; Figure 1, Supplementary Table 1). All brains were from adults above the age of species-typical

sexual maturity. Reference data on age of sexual maturity and maximum longevity for each species were taken from the Human Aging Genomic Resources AnAge database (Tacutu et al. 2018). On average, individuals in this sample were from ages at the $41 \pm 27\%$ (mean $\pm$ standard deviation ([SD])) point of the adult lifespan (i.e., time from sexual maturity to maximum longevity). Only two individuals in the sample were from the upper quartile of their species-specific adult lifespan (*Ateles belzebuth* and *Tarsius bancanus*). Nonhuman primate specimens came from individuals that lived in zoos and research centers. Animals were housed according to each institution's Animal Care and Use Committee guidelines and died for reasons unrelated to the current study. Human brain samples were provided by the El Paso County coroner's office in Colorado from a 29-year-old female who died of sudden myocardial infarction and a 19-year-old male who died from sepsis secondary to surgery. Neither of the human subjects had a reported history of neurological or psychiatric disorders. Although human prefrontal cortex neurons undergo prolonged synaptic overproduction in development and are not pruned to adult-like levels until the third decade of life (Petanjek et al. 2011, 2019), area V1 synaptogenesis occurs earlier, and pruning is largely complete by adolescence (Huttenlocher and Dabholkar 1997). Therefore, we considered the 19-year-old male to be generally representative of a typical adult for the regions we analyzed here.

Within 14 h of each individual's death, the brain was removed and immersed in 10% formalin. Postmortem brains appeared normal upon routine neuropathology evaluation. All experimental procedures with postmortem tissue were carried out according to the National Institutes of Health guidelines for animal research and were approved by the Institutional Animal Care and Use Committee at the George Washington University. Individual brain mass was measured at necropsy/autopsy or immediately upon receiving them from the donating institution. After fixation, brains were then transferred to 0.1 M phosphate buffered saline (PBS) with 0.1% sodium azide solution and stored at 4 °C.

All nonhuman primate brains used in the present study had previously been sectioned in the coronal plane at 40 μ m on a Leica SM2000 R sliding microtome, and every 10th section was Nissl-stained with 0.5% cresyl violet. Unstained sections were maintained in freezer storage in numbered tubes containing 30% distilled water, ethylene glycol, and glycerol and 10% 0.244 M PBS at 20 °C. The ranges of sections containing area V1 and IT cortex were identified by comparing the Nissl-stained sections to publications that illustrate the location of the relevant cortical areas in primates (Figure 2A) (Preuss and Goldman-Rakic 1991; Fang et al. 2006; Solomon and Rosa 2014; Bryant et al. 2019). Area V1 and IT cortex are both considered to be homologous across primate phylogeny (Rosa and Tweedale 2005; Kaas 2012). Three equidistantly spaced sections from freezer storage were selected from each cortical region of interest for synapse labeling. For human brain samples, area V1 and IT cortex were dissected out based on anatomical location and sectioned at 40 μ m as described above.

Immunohistochemistry for Synaptophysin

Free-floating sections of the regions of interest were stained with rabbit polyclonal IgG₁ antibodies against synaptophysin (SYP), which is an acidic, homo-oligomeric integral membrane glycoprotein localized in presynaptic vesicles (1:100 dilution,

Figure 1. Phylogenetic tree of the primate species included in this study.

A0010, DakoCytomation, Glostrup, Denmark). SYP concentrations in human and rat cortex have been shown to be unaffected by postmortem intervals up to 72 h (Siew et al. 2004). Prior to immunostaining, sections were rinsed thoroughly in PBS and pretreated for antigen retrieval by incubation in 10 mM sodium citrate buffer (pH 3.5) at 37 °C in an oven for 30 min. Sections were rinsed and immersed in a solution of 0.75% hydrogen peroxide in 75% methanol to eliminate endogenous peroxidase activity and then incubated in the primary antiserum diluted in PBS with 2% normal goat serum and 0.1% Triton X-100 for approximately 24 h on a rotator at 4 °C. After rinsing in PBS, sections were incubated in biotinylated anti-rabbit IgG (1:200 dilution, BA-2000, Vector Laboratories, Burlingame, CA) and processed with the avidin-biotin-peroxidase method using a Vectastain Elite ABC kit (pk-6100, Vector Laboratories). Sections were rinsed again in PBS, followed by a rinse in sodium acetate buffer. Immunoreactivity was revealed using 3,3'-diaminobenzidine and nickel enhancement according to a modification of methods described previously (Shu et al. 1988). After SYP immunostaining of sections, we reviewed slides for quality control before inclusion in our study. To be used in further quantitative analyses, the sections needed to show a punctate distribution of immunostaining surrounding unstained ovoid and pyramidal shapes putatively corresponding to cell somata, which is consistent with the localization of presynaptic boutons (Figure 2). Employing these strict quality control criteria meant that several brain specimens that were initially processed and stained had to be excluded from further analysis.

Quantification of SYP-Immunoreactive Puncta

The density of SYP-immunoreactive puncta and neurons from adjacent Nissl-stained sections was estimated using a Zeiss Axioplan 2 photomicroscope equipped with a Ludl XY motorized stage, Heidenhain z-axis encoder, Optronics MicroFire color video camera, a Dell PC, and StereoInvestigator software (version 11, MBF Bioscience, Williston, VT). Three equidistantly spaced sections were chosen for stereologic analysis. Adjacent Nissl-stained sections of regions of interest were used to confirm the cytoarchitecture, to outline layer boundaries and to obtain counts of neurons. The contouring tool was used to outline layers II–III and layers V–VI at 1.25× magnification. Because precise boundaries of layer IV may be difficult to discern, it was not included as a separate region of interest in the analysis. To count SYP-immunoreactive puncta, each region of interest was investigated through a set of optical disector frames of 3 × 3 μm with a square scan grid size of 250 × 250 μm. Disector analysis was performed under Koehler illumination using a 100× oil objective (Zeiss Plan-Apochromat, N.A. 1.4). The thickness of optical disectors was set to 1 μm, with a 1-μm guard zone at the top of the section. These sampling parameters were chosen because SYP immunostaining does not penetrate the entire section. Measurement of mounted section thickness was collected at every fifth sampling site. Synapse counting was performed blind to species identity of the specimen. We counted all puncta that had a spherical appearance with clear boundaries irrespective of staining intensity, following the methods of previous studies (Calhoun et al. 1996; Sherwood et al. 2010b; Bianchi et al. 2013b). Synapse density in each region of interest was calculated by

Figure 2. (A) The locations of primary visual cortex (V1) and inferior temporal (IT) cortex in three representative primate species of different brain sizes. (B) Boundaries of supragranular layers II–III and infragranular layers V–VI in V1 and IT cortex from a rhesus macaque (*Macaca mulatta*); scale bar = 500 μm . (C) SYP immunostaining and (D) Nissl-staining from IT cortex layer III of a chimpanzee (*Pan troglodytes*). We counted all SYP-immunoreactive puncta that had a spherical appearance with clear boundaries. Examples of stained puncta are indicated with arrowheads. Scale bar = 20 μm in C and 40 μm in D.

dividing the total puncta counted by the product of the disector volume ($9 \mu\text{m}^3$) and the number of sampling sites, correcting for tissue shrinkage from histological processing as described previously (Sherwood et al. 2005). Across all individuals and regions of interest in the study, this sampling design yielded on average 70.5 ± 19.2 optical disector frames, 156.4 ± 64.2 markers counted, and a coefficient of error (Schmitz-Hof) of 0.08 ± 0.019 . Synapse density counts from the two older individuals in the sample (*Ateles belzebuth* and *Tarsius bancanus*) were not outliers. Inter-rater reliability was determined by recounting SYP-immunoreactive puncta in 14 different regions of interest, which yielded an acceptable level of agreement with an intraclass correlation coefficient of 0.80 (two-way mixed model, $F_{13} = 4.88$, $P = 0.004$).

To determine total neuron density with Nissl-stained sections, disector frames of $30 \times 30 \mu\text{m}$ with a scan grid size of $250 \times 250 \mu\text{m}$ were used. The optical disector analysis was performed under Koehler illumination using a $63\times$ objective lens (Zeiss Plan-Apochromat, N.A. 1.4). The thickness of optical disectors was set to 6 μm with a 2- μm guard zone at the top of the section, and measurement of mounted section thickness was collected at every fifth sampling site. Neurons (including large pyramidal cells and smaller interneurons) were identified as distinct from glia by the presence of a visible cytoplasm surrounding a round or ovoid lightly stained nucleus and the

frequent appearance of lightly stained proximal segments of dendritic processes. Often a distinct nucleolus was evident, although small neurons may have small nucleoli surrounded by thick perinucleolar heterochromatin clumps. Neuronal densities were derived from these stereological counts as described above. Across all individuals in the study, this sampling design yielded 95.0 ± 41.7 optical disector frames, 176.6 ± 82.4 markers counted, and a coefficient of error of 0.07 ± 0.013 .

Electron Microscopy

Sections of area V1 from adult individuals of five different primates (*Lemur catta* [female, 16 years old], *Saimiri sciureus* [male, 25 years old], *Papio anubis* [male, 11 years old], *Pan troglodytes* [female, 23 years old], and *Homo sapiens* [male, 19 years old]) were postfixed with 1% OsO_4 and stained with 1% uranyl acetate. All samples came from the right hemisphere, with the exception of *Pan troglodytes*, which came from the left hemisphere. After embedding in Epon, 80-nm ultrathin sections were collected on silicon wafers. Quantification of synapse density was then measured on digital images obtained from a Helios FIBSEM (FEI) with a concentric back scatter detector, at a working distance of approximately 4 mm. Scanning montage images of vertical strips of cortex from the pial surface to the white matter junction were acquired at $65,000\times$ magnification, with a

dwell time of 5 μ s and a resolution of 1536×1024 pixels using MAPS software, with independent pixel sizes recorded for all images. Image quantification was performed using ImageJ (NIH). Sampling fields covering the entire cortical width were chosen by using a systematic-random sampling method. A uniformly spaced grid (500 000 square pixels) was overlaid on each image, and counting grids were investigated. Three montage images of the cortical width were quantified per individual.

Criteria for identification of synapses included the presence of a synaptic junction and the appearance of at least two synaptic vesicles in the presynaptic component. Symmetric and asymmetric synapses were pooled in this analysis. The number of synapses per unit volume was calculated through the following formula, $N_V = N_A/d$, where N_V is the number of synapses per unit volume, N_A is the number of synaptic junctions per unit area of an electron micrograph, and d is the median length of postsynaptic densities associated with the synaptic junctions (Colonnier and Beaulieu 1985). Each cortical area was investigated with 415 ± 155 sampling sites. Measures of synapse length (d) were obtained from 328 ± 156 randomly chosen synapses in each cortical region of each individual.

Data Analysis

All species mean data used in analyses are presented in [Supplementary Table 2](#). To account for the expected error structure due to the relatedness among species in this comparative sample, we employed phylogenetic generalized least-squares procedures (pGLS). The pGLS procedures allow performing all tests of standard least-squares analysis but additionally account for phylogenetic relatedness by including a variance-covariance matrix of shared ancestry in the error term of the standard GLS equation (Rohlf 2001). The variance-covariance matrix of phylogenetic relatedness is further adjusted for the extent to which the data adhere to a pure gradual model of evolution using a likelihood-fitted lambda transformation (Pagel 1999). Lambda varies between 0 and 1, where 0 indicates that traits covary independently of their degree of shared ancestry and 1 indicates that traits covary in a manner accurately described by their degree of shared ancestry.

We obtained a phylogeny of the primate species in the sample from the 10 k Trees website (Arnold et al. 2010). To determine the scaling relationships in our dataset, we employed pGLS regression analysis on log-transformed data. pGLS confidence intervals (CIs) were calculated according to Smaers and Rohlf (2016). In these scaling analyses, both dependent and independent variables contain similar error from any shrinkage fixation artifact that might be present in the sample. As a result, individual data points might shift along the major axis of the regression, but slope and intercept calculations are not significantly altered. To test for differences in slope and intercept among regions of interest, and to test for differences in intercepts of human data points for particular regions of interest, standard extensions of pGLS procedures were employed (Smaers and Rohlf 2016).

Within the pGLS framework, the strength of fit across different regressions was calculated using the Brownian motion rate parameter (σ^2) of the residuals. This rate parameter calculates accumulation of variance over time. Given that variables are measured in the same sample with a common phylogenetic tree, differences in this rate parameter indicate differences among measures in the accumulation of residual variance and the strength of allometric integration (i.e., similar to what r^2 would signify for a standard regression). Low σ^2

rates indicate little residual variation (high strength of allometric integration), and high rates indicate high residual variation (low strength of allometric integration).

We used brain mass as an independent scaling variable in our analyses because it was recorded from the same individuals as histological measures of synapse and neuron densities. Notably, interspecific variation in overall brain mass of primates has been shown to be closely associated with neocortical size and neuron number (Charvet and Finlay 2012; Herculano-Houzel et al. 2015). To confirm this correspondence, we examined the correlation of brain mass measurements from the individuals in our study with neocortical gray matter volumes in the published literature from the same species ($n = 13$ species; Navarrete et al. 2018; DeCasien and Higham 2019) and found a strong positive association ($r^2 = 0.976$, $P < 0.001$). Therefore, the scaling relationships related to brain mass we observe here are likely to reflect scaling to the neocortex more specifically.

Results

[Figure 3](#) provides an overview of synapse densities, neuron densities, and numbers of synapses per neuron across the phylogeny of primates in our sample. Synapse densities in supragranular and infragranular layers of area V1 and IT cortex were not associated with brain size ([Figure 4A](#)). The pGLS regression of synapse densities in each cortical area and layer against brain mass was not significant, and the 95% CIs of all regression slopes included 0 ([Table 1](#)). Furthermore, pGLS analyses showed that slopes and intercepts did not significantly differ among cortical regions ([Supplementary Table 3](#)). The range of synapse density observed across the total sample was 1.9-fold, from a minimum of 195 million synapses/ mm^3 in area V1 infragranular layers of *Ateles belzebuth* to a maximum of 362 million synapses/ mm^3 in IT cortex supragranular layers of *Homo sapiens*. The overall average synapse density across all species and cortical regions was 256 million synapses/ mm^3 ($SD = 37.5$ million). Although scaling analyses showed relative invariance of synapse densities, paired-sample t-tests between supragranular versus infragranular layers within each cortical area indicated significant differences (area V1: $t_{24} = 2.33$, $P = 0.029$; IT cortex: $t_{24} = 2.36$, $P = 0.027$). On average, supragranular layers in both cortical areas have approximately 13.9 million more synapses per mm^3 than infragranular layers.

Independent validation using electron microscopy to quantify synapses in area V1 of five phylogenetically diverse primate species also supports the conclusion that synaptic densities are relatively constant (Spearman's rho correlation with brain mass: 0.60, $P = 0.285$; [Figure 5](#)).

By contrast, neuron densities displayed a significant negative relationship with brain mass ([Figure 4B](#)). In each cortical area and layer, neuron densities decreased as a function of increasing brain mass ([Table 1](#)). pGLS analyses showed that scaling slopes were generally similar among cortical areas and layers, with significant differences in intercepts between area V1 and IT cortex ([Supplementary Table 3](#)). Area V1 tended to have higher neuron density than IT cortex in this sample of primate species.

We tested whether synapse densities within each cortical area and layer were associated with neuron densities and found that they were independent of each other ([Table 1](#); [Figure 6](#)). Next, we calculated the number of synapses per neuron (S/N) for each region of interest. In all cortical areas and layers, S/N increased as a function of brain mass enlargement ([Figure 4C](#)).

Figure 3. Overview of synapse densities, neuron densities, and numbers of synapses per neuron across the phylogeny of primates in our sample.

Figure 4. Scatterplots and best fit lines of pGLS scaling regressions against brain mass for (A) synapse density, (B) neuron density, and (C) synapses per neuron (S/N).

pGLS analyses showed that slopes did not differ among cortical areas and layers; however, intercepts significantly varied (Supplementary Table 3). This indicates that S/N scales with a common allometric relationship across these levels of the cortical visual processing hierarchy. S/N was highest in IT cortex infragranular layers of *Homo sapiens* (5081 synapses/neuron) and lowest in area V1 supragranular layers of *Tarsius bancanus* (671 synapses/neuron). This range represents a 7.6-fold difference

across all species. Pairwise comparisons of sigma2 rates among all S/N regressions on brain mass showed only one significant contrast, with a 3.9 higher rate of residual variance accumulated in supragranular layers relative to infragranular layers of IT cortex ($P = 0.011$). This rate difference between the layers of IT cortex is driven by cross-species residual variation in neuron density (sigma2 rate difference ratio = 2.8, $P = 0.056$) rather than synapse density (sigma2 rate difference ratio = 1.1, $P = 0.781$).

Table 1 Results from phylogenetic analysis of scaling relationships

Y	X	95% CI of the intercept		95% CI of the slope		sigma2	Lambda	P
Synapse density in V1 layers II–III	Brain mass	19.10	19.51	−0.04	0.06	0.000967	0.34	0.63
Synapse density in V1 layers V–VI	Brain mass	19.18	19.53	−0.05	0.03	0.001163	0.00	0.73
Synapse density in IT layers II–III	Brain mass	19.22	19.57	−0.04	0.04	0.001616	0.00	0.82
Synapse density in IT layers V–VI	Brain mass	19.11	19.47	−0.03	0.05	0.001462	0.00	0.69
Neuron density in V1 layers II–III	Brain mass	12.57	13.09	−0.19	−0.07	0.002773	0.00	0.00
Neuron density in V1 layers V–VI	Brain mass	12.25	13.03	−0.25	−0.06	0.002482	0.54	0.00
Neuron density in IT layers II–III	Brain mass	12.11	12.71	−0.22	−0.09	0.004247	0.00	0.00
Neuron density in IT layers V–VI	Brain mass	12.25	12.75	−0.26	−0.15	0.001522	0.00	0.00
Synapses per neuron in V1 layers II–III	Brain mass	6.23	6.77	0.08	0.20	0.003277	0.00	0.00
Synapses per neuron in V1 layers V–VI	Brain mass	6.30	7.11	0.05	0.25	0.003164	0.54	0.00
Synapses per neuron in IT layers II–III	Brain mass	6.65	7.33	0.07	0.23	0.006455	0.00	0.00
Synapses per neuron in IT layers V–VI	Brain mass	6.65	7.02	0.16	0.27	0.001666	0.00	0.00
Synapse density in V1 layers II–III	Neuron density in V1 layers II–III	15.76	21.05	−0.14	0.29	0.001013	0.34	0.47
Synapse density in V1 layers V–VI	Neuron density in V1 layers V–VI	15.97	20.55	−0.10	0.28	0.001150	0.00	0.34
Synapse density in IT layers II–III	Neuron density in IT layers II–III	16.92	21.22	−0.16	0.21	0.001632	0.00	0.77
Synapse density in IT layers V–VI	Neuron density in IT layers V–VI	16.55	20.44	−0.10	0.24	0.001449	0.00	0.39

Figure 5. Electron microscopy images of primary visual cortex (V1) from (A) ring-tailed lemur (*Lemur catta*) and (B) chimpanzee (*Pan troglodytes*) showing synaptic junctions labeled with asterisks; scale bar = 500 nm in A and B. (C) The density of synapses as measured from electron microscopy in V1 from selected primate species, with their respective brain masses arranged in increasing size order. Electron microscopy evaluation of synapses directly from ultrastructure showed agreement with SYP immunostaining in finding invariant synapse density across species, although the densities observed from electron microscopy were higher overall, which is consistent with other studies (DeFelipe et al. 1999; Alonso-Nanclares et al. 2008).

To determine whether humans have S/N values that differ from what would be expected for their brain mass, pANCOVA was performed to test for significant human-specific shifts in intercept. Overall, these tests showed that human S/N values were positive departures from the primate-wide scaling trends, but generally did not cross the threshold of conventional statistical significance (area V1 supragranular layers: $F = 4.15$, $P = 0.05$; area V1 infragranular layers: $F = 2.22$, $P = 0.15$; IT cortex supragranular layers: $F = 2.53$, $P = 0.13$; IT cortex infragranular layers: $F = 1.53$, $P = 0.23$).

Discussion

We found that synapse density is relatively uniform in the primate neocortex, as observed across variation in brain size and levels of the processing hierarchy in supragranular and infragranular layers of primary visual cortex (V1) and inferior

temporal association cortex (IT). These results provide a more comprehensive context to understand the scaling of synaptic integration by cortical neurons and energetic constraints in primate brain evolution.

Invariance of synapse density is congruent with data indicating that other aspects of synapse biology are highly conserved in neocortical evolution. The length of the postsynaptic density and overall size of synapses vary minimally with developmental stage, aging, and brain size among mammals (reviewed in Karbowski 2012, 2014). The ratio of excitatory (asymmetric) to inhibitory (symmetric) synapses also appears to be fairly constant among rodents and primates, with about 80–90% of all synapses in the neocortex being excitatory and 10–20% inhibitory (DeFelipe et al. 2002). This aligns with the finding that 15–20% of cortical neurons in primates are inhibitory interneurons (Sherwood et al. 2007, 2010a), suggesting an important role for balancing neuronal activity in maintaining stable function of cortical circuits (Vogels et al. 2011).

Figure 6. Scatterplot of synapse density versus neuron density.

Although synapse density was found to be relatively invariant in the neocortical areas of the primates we examined, neuron density decreased as a function of brain enlargement, leading to positive scaling of S/N in primate neocortical evolution. This likely impacts aspects of cellular and systems function, such as discharge properties and memory storage capacity (Ashford and Fuster 1985; Murayama et al. 1997; Stepanyants et al. 2002). Theoretically, neocortical pyramidal neurons with expansive dendritic trees and thousands of synapses are modeled to be capable of recognizing hundreds of independent patterns of cellular activity, even in the presence of large amount of noise and signal variability (Hawkins and Ahmad 2016).

Increased complexity of dendritic structure and greater numbers of synapses determines the biophysical properties of neurons and the potential for plasticity (Chklovskii et al. 2004). Differences in the number of synapses incorporated by neurons may influence local summation, the degree of dendritic compartmentalization of processing, and the inhibitory control of inputs (White 1989; Koch 1999). Smaller neurons with fewer synapses have a higher input resistance and greater evoked action potential firing rate as compared to neurons with more synapses (Amatrudo et al. 2012). Area V1 neurons, accordingly, have physiological properties of being highly excitable, which is optimal for signal transformations with high fidelity (Olshausen and Field 2004). This contrasts with higher-order association cortex, such as IT cortex, which contains neurons with many synapses that integrate a greater diversity of inputs along more complex dendritic arbors (Elston et al. 2001, 2005, 2006; Jacobs et al. 2001). This neuronal morphology is consistent with excitatory events of larger amplitude and longer decay time, making them better suited for facilitating sustained activation, coincidence detection, and spike-timing dependent plasticity for a wider dynamic range of information integration (Constantinidis and Wang 2004).

The functional relationship between synapses and neurons in the neocortex is limited by energy availability. The consistency of synapse structure and distributions may be associated with constant glial density, which has also been reported in adult mammalian neocortex (Herculano-Houzel 2012, 2014). The

formation, maintenance, turnover, and elimination of synaptic connections have been shown to depend crucially on glia, particularly astrocytes (De Pittà et al. 2016). Glucose taken up by astrocytes is coupled to neuronal energetics through glutamate-mediated synaptic transmission (Magistretti and Allaman 2018), suggesting that synapses and glia are anatomically and functionally interrelated in a manner that is governed by energetic constraints per unit cortical tissue volume.

Synapse distribution differences across cortical layers are likely to also be functionally significant. Neurons in supragranular layers generally furnish corticocortical projections, whereas infragranular layer neurons tend to form connections between cortex and other brain areas (Gilbert and Kelly 1975; Barbas 1986; Nudo and Masterton 1990). Across the sample of primates, we found a higher density of synapses and a greater S/N in supragranular layers. Additionally, we found a higher rate of residual variance in S/N values accumulated in supragranular layers relative to infragranular layers of IT cortex. Neocortical histological organization develops in a sequence with pyramidal neurons of the deepest layers generated first and neurons exiting the stem cell pool later migrating to the more superficial layers of the cortical plate (McConnell 1995). Furthermore, in humans and macaques, there is evidence that there is a higher degree of synaptic overproduction prior to the pruning phase in supragranular layers versus infragranular layers in postnatal development, with especially pronounced dendritic spine overproduction of pyramidal neurons in cortical association areas compared to primary sensory regions (Bourgeois et al. 1994; Huttenlocher and Dabholkar 1997; Jacobs et al. 1997; Petanjek et al. 2008, 2019; Goulas et al. 2014; Oga et al. 2017). Thus, extended synaptic formation in supragranular layers may lend a further channel for the development of complex corticocortical networks that are shaped by experience and learning in primate brain evolution.

These comparative data from 25 primate species allows us to view human neocortical synapse distributions in a phylogenetic context. We found that humans have among the highest synapse densities, lowest neuron densities, and the greatest S/N in the sample. Notably, the human S/N values were generally elevated compared to brain size scaling trends from other primates, but represent only moderate positive deviations from what would be expected. Consistent with this finding, compared to other primates, pyramidal neurons of the human neocortex, especially prefrontal association cortex, have been demonstrated to display more complex dendritic branching, greater neuropil fraction, and wider spacing between minicolumns (Elston et al. 2001; Jacobs et al. 2001; Semendeferi et al. 2011; Spocter et al. 2012; Bianchi et al. 2013a, 2013b). Among the genes identified as differentially expressed at a higher level in human cortex than in chimpanzees and macaques, there is an enrichment of those involved in energy metabolism, synaptic maintenance, and plasticity (Cáceres et al. 2003; Uddin et al. 2004; Fu et al. 2011; Bauernfeind et al. 2015).

Furthermore, since the last common ancestor shared by humans-chimpanzees-bonobos, the gene *SRGAP2* underwent a series of duplications in the human lineage (Dennis et al. 2012). The human-specific paralog interferes with the ancestral copy of *SRGAP2* and regulates synapse development, resulting in protracted synapse maturation and increased dendritic spine density of neocortical pyramidal neurons (Charrier et al. 2012; Fossati et al. 2016). Additionally, comparative gene expression profiling from human prefrontal cortex has revealed developmental delays in synapse-associated transcripts, which

may prolong the period of plasticity of higher-order cortical networks (Liu et al. 2012, 2016). Molecular modifications of synaptic strength, which occurs during the period of developmental overproduction and elimination of synapses, are thought to be important for incorporating environmental influences in circuit reorganization because they occur when the magnitude of plasticity of dendritic spines is greatest (Rakic et al. 1994; Bourgeois 1997; Harris 1999; Elston et al. 2009; Petanjek et al. 2011, 2019).

Our research approach allowed for data collection across a large diversity of primate species; however, the current study was not designed to address certain parameters of synaptic organization that might also be relevant to the evolution of cortical connectivity. First, although we examined different regions within occipital and temporal cortex to model increasing complexity of visual information processing, the extent to which these results generalize across the entire neocortex remains to be fully tested. The ratio of S/N within cortical layers is also a relatively coarse measure of general connectivity because the dendrites of neurons, particularly pyramidal cells, may span across many other layers and axon terminals may originate from multiple sources, including local interneurons, neurons from other layers of cortex, and subcortical structures (DeFelipe 2011). Layers II and III were grouped together in our analysis to represent the supragranular portion of the cortex, and layers V and VI were combined to represent the infragranular cortex. Across all species of primates and both cortical regions of interest, we found this to be the most reliable approach given significant variation in the differentiation of lamination. Notably, in various cortical areas of humans, synapse densities in supragranular layers II and III have been reported to overlap in distributions, as do synapse densities in infragranular layers V and VI (Masliyah et al. 1990; DeFelipe et al. 1999). Furthermore, we did not characterize differences in the morphology of dendritic spines or the structure of synapses, which may be important for determining the strength and stability of signal transmission (Medalla and Luebke 2015). Finally, aside from numerical and morphological differences among species, it is likely that pre- and postsynaptic structures show variation in their proteomes and expression levels of signaling molecules, which may have significance for dendritic and synaptic plasticity (Bayés et al. 2012).

Our aim was to examine general principles of synaptic scaling across primate neocortical evolution. To address this question, we prioritized sampling diversity across phylogeny rather than investigating intraspecific variation. Using one or two representative individuals per species is common in broadly comparative studies of brain macro- and microstructure (e.g., Stephan et al. 1981; Herculano-Houzel et al. 2015; DeCasien and Higham 2019). In addition, for many of the species in the current analysis, few brains of non-geriatric adults were available that fulfilled our quality control requirements for reliable SYP immunolabeling of synapses and electron microscopy. It should be noted that intraspecific variation of synapse density has been documented in adult neocortex (e.g., sex differences in humans; Alonso-Nanclares et al. 2008), but the degree of variation reported within a species is similar to what we observe across this sample of primates that differ in brain mass by 500-fold.

Synapses are the underlying substrate of learning, memory, perception, action, and cognition. Our findings suggest that properties of neuronal activity scale in a predictable manner across cortical regions, layers, and species according to brain size in primates and are constrained by a relatively constant

synaptic density per unit cortical tissue volume. Comparative data from diverse species advances our understanding of the principles that govern synapse distribution in the neocortex and how neuronal connectivity evolves in humans and other primates.

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

Funding

James S. McDonnell Foundation (220020293); NSF INSPIRE (SMA-1542848).

Notes

We acknowledge Cleveland Metroparks Zoo, Smithsonian National Zoological Park, Milwaukee County Zoo, and the National Chimpanzee Brain Resource (funded by NIH NS092988) for contributing brain specimens used in this study. We thank Yong Do Kim and the GW Nanofabrication and Imaging Center for expert assistance with electron microscopy.

References

- Akram A, Christoffel D, Rocher AB, Bouras C, Kövari E, Perl DP, Morrison JH, Herrmann FR, Haroutunian V, Giannakopoulos P, et al. 2008. Stereologic estimates of total spinophilin-immunoreactive spine number in area 9 and the CA1 field: relationship with the progression of Alzheimer's disease. *Neurobiol Aging*. 29:1296–12307.
- Alonso-Nanclares L, Gonzalez-Soriano J, Rodriguez JR, DeFelipe J. 2008. Gender differences in human cortical synaptic density. *Proc Natl Acad Sci USA*. 105:14615–14619.
- Amatrudo JM, Weaver CM, Crimins JL, Hof PR, Rosene DL, Luebke JI. 2012. Influence of highly distinctive structural properties on the excitability of pyramidal neurons in monkey visual and prefrontal cortices. *J Neurosci*. 32:13644–13660.
- Arnold C, Matthews LJ, Nunn CL. 2010. The 10k trees website: a new online resource for primate phylogeny. *Evol Anthropol*. 19:114–118.
- Ashford JW, Fuster JM. 1985. Occipital and inferotemporal responses to visual signals in the monkey. *Exp Neurol*. 90:444–466.
- Barbas H. 1986. Pattern in the laminar origin of corticocortical connections. *J Comp Neurol*. 252:415–422.
- Bauernfeind AL, Soderblom EJ, Turner ME, Moseley MA, Ely JJ, Hof PR, Sherwood CC, Wray GA, Babbitt CC. 2015. Evolutionary divergence of gene and protein expression in the brains of humans and chimpanzees. *Genome Biol Evol*. 7:2276–2288.
- Bayés A, Collins MO, Croning MD, van de Lagemaat LN, Choudhary JS, Grant SG. 2012. Comparative study of human and mouse postsynaptic proteomes finds high compositional conservation and abundance differences for key synaptic proteins. *PLoS One*. 7:e46683.
- Benavides-Piccione R, Ballesteros-Yáñez I, DeFelipe J, Yuste R. 2002. Cortical area and species differences in dendritic spine morphology. *J Neurocytol*. 31:337–346.
- Bianchi S, Stimpson CD, Bauernfeind AL, Schapiro SJ, Baze WB, McArthur MJ, Bronson E, Hopkins WD, Semendeferi K, Jacobs B, et al. 2013a. Dendritic morphology of pyramidal neurons

- in the chimpanzee neocortex: regional specializations and comparison to humans. *Cereb Cortex*. 23:2429–2436.
- Bianchi S, Stimpson CD, Duka T, Larsen MD, Janssen WG, Collins Z, Bauernfeind AL, Schapiro SJ, Baze WB, McArthur MJ, et al. 2013b. Synaptogenesis and development of pyramidal neuron dendritic morphology in the chimpanzee neocortex resembles humans. *Proc Natl Acad Sci USA*. 110(Suppl 2):10395–10401.
- Bourgeois JP. 1997. Synaptogenesis, heterochrony and epigenesis in the mammalian neocortex. *Acta Paediatr Suppl*. 422:27–33.
- Bourgeois JP, Goldman-Rakic PS, Rakic P. 1994. Synaptogenesis in the prefrontal cortex of rhesus monkeys. *Cereb Cortex*. 4:78–96.
- Bryant KL, Glasser MF, Li L, Jae-Cheol Bae J, Jacquez NJ, Alarcón L, Fields A 3rd, Preuss TM. 2019. Organization of extrastriate and temporal cortex in chimpanzees compared to humans and macaques. *Cortex*. 118:223–243.
- Cáceres M, Lachuer J, Zapala MA, Redmond JC, Kudo L, Geschwind DH, Lockhart DJ, Preuss TM, Barlow C. 2003. Elevated gene expression levels distinguish human from non-human primate brains. *Proc Natl Acad Sci USA*. 100:13030–13035.
- Calhoun ME, Jucker M, Martin LJ, Thinakaran G, Price DL, Mouton PR. 1996. Comparative evaluation of synaptophysin-based methods for quantification of synapses. *J Neurocytol*. 25:821–828.
- Charvet CJ, Finlay BL. 2012. Embracing covariation in brain evolution: large brains, extended development, and flexible primate social systems. *Prog Brain Res*. 195:71–87.
- Changizi MA. 2001. Principles underlying mammalian neocortical scaling. *Biol Cybern*. 84:207–215.
- Charrier C, Joshi K, Coutinho-Budd J, Kim JE, Lambert N, de Marchena J, Jin WL, Vanderhaeghen P, Ghosh A, Sassa T, et al. 2012. Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation. *Cell*. 149:923–935.
- Chklovskii DB, Mel BW, Svoboda K. 2004. Cortical rewiring and information storage. *Nature*. 431:782–788.
- Colonnier M, Beaulieu C. 1985. An empirical assessment of stereological formulae applied to the counting of synaptic disks in the cerebral cortex. *J Comp Neurol*. 231:175–179.
- Constantinidis C, Wang XJ. 2004. A neural circuit basis for spatial working memory. *Neuroscientist*. 10:553–565.
- Cragg BG. 1967. The density of synapses and neurones in the motor and visual areas of the cerebral cortex. *J Anat*. 101:639–654.
- DeCasien AR, Higham JP. 2019. Primate mosaic brain evolution reflects selection on sensory and cognitive specialization. *Nat Ecol Evol*. 3:1483–1493.
- DeFelipe J. 2011. The evolution of the brain, the human nature of cortical circuits, and intellectual creativity. *Front Neuroanat*. 5:29.
- DeFelipe J, Marco P, Busturia I, Merchán-Pérez A. 1999. Estimation of the number of synapses in the cerebral cortex: methodological considerations. *Cereb Cortex*. 9:722–732.
- DeFelipe J, Alonso-Nanclares L, Arellano JI. 2002. Microstructure of the neocortex: comparative aspects. *J Neurocytol*. 31:299–316.
- Dennis MY, Nuttle X, Sudmant PH, Antonacci F, Graves TA, Nefedov M, Rosenfeld JA, Sajjadian S, Malig M, Kotkiewicz H, et al. 2012. Evolution of human-specific neural SRGAP2 genes by incomplete segmental duplication. *Cell*. 149:912–922.
- De Pittà M, Brunel N, Volterra A. 2016. Astrocytes: orchestrating synaptic plasticity? *Neuroscience*. 323:43–61.
- Derkksen MJ, Ward NL, Hartle KD, Ivanco TL. 2007. MAP2 and synaptophysin protein expression following motor learning suggests dynamic regulation and distinct alterations coinciding with synaptogenesis. *Neurobiol Learn Mem*. 87:404–415.
- Desimone R, Schein SJ, Moran J, Ungerleider LG. 1985. Contour, color and shape analysis beyond the striate cortex. *Vision Res*. 25:441–452.
- Eastwood SL, Weickert CS, Webster MJ, Herman MM, Kleinman JE, Harrison PJ. 2006. Synaptophysin protein and mRNA expression in the human hippocampal formation from birth to old age. *Hippocampus*. 16:645–654.
- Elston GN, Benavides-Piccione R, DeFelipe J. 2001. The pyramidal cell in cognition: a comparative study in human and monkey. *J Neurosci*. 21:RC163.
- Elston GN, Benavides-Piccione R, DeFelipe J. 2005. A study of pyramidal cell structure in the cingulate cortex of the macaque monkey with comparative notes on inferotemporal and primary visual cortex. *Cereb Cortex*. 15:64–73.
- Elston GN, Benavides-Piccione R, Elston A, Zietsch B, DeFelipe J, Manger P, Casagrande V, Kaas JH. 2006. Specializations for cognitive processing. *Anat Rec A Discov Mol Cell Evol Biol*. 288:26–35.
- Elston GN, Oga T, Fujita I. 2009. Spinogenesis and pruning scales across functional hierarchies. *J Neurosci*. 29:3271–3275.
- Elston GN, Benavides-Piccione R, Elston A, Manger PR, DeFelipe J. 2011. Pyramidal cells in prefrontal cortex of primates: marked differences in neuronal structure among species. *Front Neuroanat*. 5:2.
- Fang PC, Stepniewska I, Kaas JH. 2006. The thalamic connections of motor, premotor, and prefrontal areas of cortex in a prosimian primate (*Otolemur garnetti*). *Neuroscience*. 143:987–1020.
- Fossati M, Pizzarelli R, Schmidt ER, Kupferman JV, Stroebel D, Polleux F, Charrier C. 2016. SRGAP2 and its human-specific paralog co-regulate the development of excitatory and inhibitory synapses. *Neuron*. 91:356–369.
- Fu X, Gavalisco P, Liu X, Catchpole G, Fu N, Ning ZB, Guo S, Yan Z, Somel M, Pääbo S, et al. 2011. Rapid metabolic evolution in human prefrontal cortex. *Proc Natl Acad Sci USA*. 108:6181–6186.
- Gilbert CD, Kelly JP. 1975. The projections of cells in different layers of the cat's visual cortex. *J Comp Neurol*. 163:81–105.
- Glantz LA, Lewis DA. 2000. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry*. 57:65–73.
- Glantz LA, Gilmore JH, Hamer RM, Lieberman JA, Jarskog LF. 2007. Synaptophysin and postsynaptic density protein 95 in the human prefrontal cortex from mid-gestation into early adulthood. *Neuroscience*. 149:582–591.
- Goulas A, Uylings HB, Stiers P. 2014. Mapping the hierarchical layout of the structural network of the macaque prefrontal cortex. *Cereb Cortex*. 24:1178–1194.
- Gross CG. 1992. Representation of visual stimuli in inferior temporal cortex. *Philos Trans R Soc Lond B Biol Sci*. 335:3–10.
- Gutiérrez-Ospina G, Uribe-Querol E, Sánchez N, Geovannini H, Padilla P, Hernández-Echeagaray E. 2004. Similar synapse density in layer IV columns of the primary somatosensory cortex of transgenic mice with different brain size: implications for mechanisms underlying the differential allocation of cortical space. *Brain Behav Evol*. 64:61–69.

- Harris KM. 1999. Structure, development, and plasticity of dendritic spines. *Curr Opin Neurobiol.* 9:343–348.
- Hassiotis M, Paxinos G, Ashwell KW. 2005. Cyto- and chemoarchitecture of the cerebral cortex of an echidna (*Tachyglossus aculeatus*). II. Laminar organization and synaptic density. *J Comp Neurol.* 482:94–122.
- Hawkins J, Ahmad S. 2016. Why neurons have thousands of synapses, a theory of sequence memory in neocortex. *Front Neural Circuits.* 10:23.
- Herculano-Houzel S. 2012. The remarkable, yet not extraordinary, human brain as a scaled-up primate brain and its associated cost. *Proc Natl Acad Sci USA.* 109(Suppl 1):10661–10668.
- Herculano-Houzel S. 2014. The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. *Glia.* 62:1377–1391.
- Herculano-Houzel S, Catania K, Manger PR, Kaas JH. 2015. Mammalian brains are made of these: a dataset of the numbers and densities of neuronal and nonneuronal cells in the brain of Glires, Primates, Scandentia, Eulipotyphlans, Afrotherians and artiodactyls, and their relationship with body mass. *Brain Behav Evol.* 86:145–163.
- Hsu A, Luebke JI, Medalla M. 2017. Comparative ultrastructural features of excitatory synapses in the visual and frontal cortices of the adult mouse and monkey. *J Comp Neurol.* 525:2175–2191.
- Huttenlocher PR, Dabholkar AS. 1997. Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol.* 387:167–178.
- Jacobs B, Driscoll L, Schall M. 1997. Life-span dendritic and spine changes in areas 10 and 18 of human cortex: a quantitative Golgi study. *J Comp Neurol.* 386:661–680.
- Jacobs B, Schall M, Prather M, Kapler E, Driscoll L, Baca S, Jacobs J, Ford K, Wainwright M, Trembl M. 2001. Regional dendritic and spine variation in human cerebral cortex: a quantitative golgi study. *Cereb Cortex.* 11:558–571.
- Kaas JH. 2012. The evolution of neocortex in primates. *Prog Brain Res.* 195:91–102.
- Kang HJ, Voleti B, Hajsan T, Rajkowska G, Stockmeier CA, Licznarski P, Lepack A, Majik MS, Jeong LS, Banasr M, et al. 2012. Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. *Nat Med.* 18:1413–1417.
- Karbowski J. 2012. Approximate invariance of metabolic energy per synapse during development in mammalian brains. *PLoS One.* 7:e33425.
- Karbowski J. 2014. Constancy and trade-offs in the neuroanatomical and metabolic design of the cerebral cortex. *Front Neural Circuits.* 8:9.
- Koch C. 1999. *Biophysics of Computation. Information Processing of Single Neurons.* New York: Oxford University Press.
- Kravitz DJ, Saleem KS, Baker CI, Ungerleider LG, Mishkin M. 2013. The ventral visual pathway: an expanded neural framework for the processing of object quality. *Trends Cogn Sci.* 17:26–49.
- Li S, Reinprecht I, Fahnstock M, Racine RJ. 2002. Activity-dependent changes in synaptophysin immunoreactivity in hippocampus, piriform cortex, and entorhinal cortex of the rat. *Neuroscience.* 115:1221–1229.
- Liu X, Somel M, Tang L, Yan Z, Jiang X, Guo S, Yuan Y, He L, Oleksiak A, Zhang Y, et al. 2012. Extension of cortical synaptic development distinguishes humans from chimpanzees and macaques. *Genome Res.* 22:611–622.
- Liu X, Han D, Somel M, Jiang X, Hu H, Guijarro P, Zhang N, Mitchell A, Halene T, Ely JJ, et al. 2016. Disruption of an evolutionarily novel synaptic expression pattern in autism. *PLoS Biol.* 14:e1002558.
- Magistretti PJ, Allaman I. 2018. Lactate in the brain: from metabolic end-product to signalling molecule. *Nat Rev Neurosci.* 19:235–249.
- Masliah E, Terry RD, Alford M, DeTeresa R. 1990. Quantitative immunohistochemistry of synaptophysin in human neocortex: an alternative method to estimate density of presynaptic terminals in paraffin sections. *J Histochem Cytochem.* 138:837–844.
- McConnell SK. 1995. Constructing the cerebral cortex: neurogenesis and fate determination. *Neuron.* 15:761–768.
- Medalla M, Luebke JI. 2015. Diversity of glutamatergic synaptic strength in lateral prefrontal versus primary visual cortices in the rhesus monkey. *J Neurosci.* 35:112–127.
- Miki T, Fukui Y, Itoh M, Hisano S, Xie Q, Takeuchi Y. 1997. Estimation of the numerical densities of neurons and synapses in cerebral cortex. *Brain Res Protoc.* 2:9–16.
- Mohan H, Verhoog MB, Doreswamy KK, Eyal G, Aardse R, Lodder BN, Goriounova NA, Asamoah B, AB BB, et al. 2015. Dendritic and axonal architecture of individual pyramidal neurons across layers of adult human neocortex. *Cereb Cortex.* 25:4839–4853.
- Murayama Y, Fujita I, Kato M. 1997. Contrasting forms of synaptic plasticity in monkey inferotemporal and primary visual cortices. *Neuroreport.* 8:1503–1508.
- Navarrete AF, Blezer ELA, Pagnotta M, de Viet ESM, Todorov OS, Lindenfors P, Laland KN, Reader SM. 2018. Primate brain anatomy: new volumetric MRI measurements for neuroanatomical studies. *Brain Behav Evol.* 91:109–117.
- Nudo RJ, Masterton RB. 1990. Descending pathways to the spinal cord. III: Sites of origin of the corticospinal tract. *J Comp Neurol.* 296:559–583.
- Oga T, Elston GN, Fujita I. 2017. Postnatal dendritic growth and spinogenesis of layer-V pyramidal cells differ between visual, inferotemporal, and prefrontal cortex of the macaque monkey. *Front Neurosci.* 11:118.
- O’Kusky J, Colonnier M. 1982. A laminar analysis of the number of neurons, glia, and synapses in the visual cortex (area 17) of adult macaque monkeys. *J Comp Neurol.* 210:278–290.
- Olshausen BA, Field DJ. 2004. Sparse coding of sensory inputs. *Curr Opin Neurobiol.* 14:481–487.
- Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature.* 401:877–884.
- Petanjek Z, Judas M, Kostović I, Uylings HB. 2008. Lifespan alterations of basal dendritic trees of pyramidal neurons in the human prefrontal cortex: a layer-specific pattern. *Cereb Cortex.* 18:915–929.
- Petanjek Z, Judas M, Šimic G, Rasin MR, Uylings HB, Rakic P, Kostovic I. 2011. Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proc Natl Acad Sci USA.* 108:13281–13286.
- Petanjek Z, Sedmak D, Džaja D, Hladnik A, Rašin MR, Jovanov-Milosevic N. 2019. The protracted maturation of associative layer II/III pyramidal neurons in the human prefrontal cortex during childhood: a major role in cognitive development and selective alteration in autism. *Front Psychiatry.* 10:122.
- Preuss TM, Goldman-Rakic PS. 1991. Architectonics of the parietal and temporal association cortex in the strepsirrhine primate *Galago* compared to the anthropoid primate *Macaca*. *J Comp Neurol.* 310:475–506.

- Rakic P, Bourgeois JP, Goldman-Rakic PS. 1994. Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. *Prog Brain Res.* 102:227–243.
- Rohlf FJ. 2001. Comparative methods for the analysis of continuous variables: geometric interpretations. *Evolution.* 55:2143–2160.
- Rosa MG, Tweeddale R. 2005. Brain maps, great and small: lessons from comparative studies of primate visual cortical organization. *Philos Trans R Soc Lond B Biol Sci.* 360:665–691.
- Ross PJ, Zhang WB, Mok RSF, Zaslavsky K, Deneault E, D'Abate L, Rodrigues DC, Yuen RKC, Faheem M, Muftuev M, et al. 2020. Synaptic dysfunction in human neurons with autism-associated deletions in PTCHD1-AS. *Biol Psychiatry.* 87:139–149.
- Scheff SW, Price DA. 1993. Synapse loss in the temporal lobe in Alzheimer's disease. *Ann Neurol.* 33:190–199.
- Semendeferi K, Teffer K, Buxhoeveden DP, Park MS, Bludau S, Amunts K, Travis K, Buckwalter J. 2011. Spatial organization of neurons in the frontal pole sets humans apart from great apes. *Cereb Cortex.* 21:1485–1497.
- Sherwood CC, Raghanti MA, Wenstrup JJ. 2005. Is humanlike cytoarchitectural asymmetry present in another species with complex social vocalization? A stereologic analysis of mustached bat auditory cortex. *Brain Res.* 1045:164–174.
- Sherwood CC, Raghanti MA, Stimpson CD, Bonar CJ, de Sousa AA, Preuss TM, Hof PR. 2007. Scaling of inhibitory interneurons in areas V1 and V2 of anthropoid primates as revealed by calcium-binding protein immunohistochemistry. *Brain Behav Evol.* 69:176–195.
- Sherwood CC, Raghanti MA, Stimpson CD, Spocter MA, Uddin M, Boddy AM, Wildman DE, Bonar CJ, Lewandowski AH, Phillips KA, et al. 2010a. Inhibitory interneurons of the human prefrontal cortex display conserved evolution of the phenotype and related genes. *Proc Biol Sci.* 277:1011–1020.
- Sherwood CC, Duka T, Stimpson CD, Schenker NM, Garrison AR, Schapiro SJ, Baze WB, McArthur MJ, Erwin JM, Hof PR, et al. 2010b. Neocortical synaptophysin asymmetry and behavioral lateralization in chimpanzees (*Pan troglodytes*). *Eur J Neurosci.* 31:1456–1464.
- Shu SY, Ju G, Fan LZ. 1988. The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. *Neurosci Lett.* 85:169–171.
- Siew LK, Love S, Dawbarn D, Wilcock GK, Allen SJ. 2004. Measurement of pre- and post-synaptic proteins in cerebral cortex: effects of post-mortem delay. *J. Neurosci Meth.* 139:153–159.
- Smaers JB, Rohlf FJ. 2016. Testing species' deviation from allometric predictions using the phylogenetic regression. *Evolution.* 70:1145–1149.
- Solomon SG, Rosa MG. 2014. A simpler primate brain: the visual system of the marmoset monkey. *Front Neural Circuits.* 8:96.
- Spocter MA, Hopkins WD, Barks SK, Bianchi S, Hehmeyer AE, Anderson SM, Stimpson CD, Fobbs AJ, Hof PR, Sherwood CC. 2012. Neuropil distribution in the cerebral cortex differs between humans and chimpanzees. *J Comp Neurol.* 520:2917–2929.
- Stepanyants A, Hof PR, Chklovskii DB. 2002. Geometry and structural plasticity of synaptic connectivity. *Neuron.* 34:275–288.
- Stephan H, Frahm H, Baron G. 1981. New and revised data on volumes of brain structures in insectivores and primates. *Folia Primatol.* 35:1–29.
- Tacutu R, Thornton D, Johnson E, Budovsky A, Barardo D, Craig T, Diana E, Lehmann G, Toren D, Wang J, et al. 2018. Human ageing genomic resources: new and updated databases. *Nucleic Acids Res.* 246:D1083–D1090.
- Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. 1991. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol.* 30:572–580.
- Uddin M, Wildman DE, Liu G, Xu W, Johnson RM, Hof PR, Kapatos G, Grossman LI, Goodman M. 2004. Sister grouping of chimpanzees and humans as revealed by genome-wide phylogenetic analysis of brain gene expression profiles. *Proc Natl Acad Sci USA.* 101:2957–2962.
- Vogels TP, Sprekeler H, Zenke F, Clopath C, Gerstner W. 2011. Inhibitory plasticity balances excitation and inhibition in sensory pathways and memory networks. *Science.* 334:1569–1573.
- White EL. 1989. *Cortical Circuits: Synaptic Organization of the Cerebral Cortex. Structure, Function and Theory.* Boston: Birkhauser.