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ORIGINAL ARTICLE

Neuronal morphology in the African elephant (*Loxodonta africana*) neocortex

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Abstract Virtually nothing is known about the morphology of cortical neurons in the elephant. To this end, the current study provides the first documentation of neuronal morphology in frontal and occipital regions of the African elephant (*Loxodonta africana*). Cortical tissue from the perfusion-fixed brains of two free-ranging African elephants was stained with a modified Golgi technique. Neurons of different types (N = 75), with a focus on superficial (i.e., layers II–III) pyramidal neurons, were quantified on a computer-assisted microscopy system using Neurolucida software. Qualitatively, elephant neocortex exhibited large, complex spiny neurons, many of which differed in morphology/orientation from typical primate and rodent pyramidal neurons. Elephant cortex exhibited a V-shaped arrangement of bifurcating apical dendritic bundles. Quantitatively, the dendrites of superficial pyramidal neurons in elephant frontal cortex were more complex than in occipital cortex. In comparison to human supragranular pyramidal neurons, elephant superficial pyramidal neurons exhibited similar overall basilar dendritic length, but the dendritic segments tended to be longer in the elephant with less intricate branching. Finally, elephant aspiny interneurons appeared to be morphologically consistent with other eutherian mammals. The current results thus elaborate on the evolutionary roots of Afrotherian brain organization and highlight unique aspects of neural architecture in elephants.

Keywords Dendrite · Morphometry · Dendritic spine · Golgi method · Brain evolution

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Introduction

In recent years, elephants have received considerable attention because of their complex social structure and cognitive abilities (Poole and Moss 2008; Hart and Hart, in press). Nevertheless, despite elephants' status as the largest living terrestrial mammal, very little is known about the brain of this species. Prior to 2001, only a few original articles had specifically focused on the elephant central nervous system (Cozzi et al. 2001). Although the gross anatomy of the ~5 kg adult elephant brain has been investigated in the last decade (Kupsky et al. 2001; Hakeem et al. 2005, 2009; Shoshani et al. 2006; Manger et al. 2010), virtually nothing is known about the microstructural morphology of its neurons. Indeed, until recently, the only apparent insight into elephant cortical neuromorphology was a single camera lucida drawing from an Indian elephant (*Elephas maximus*; Barasa and Shochatovitz 1961). Reasons for this poverty of data are many (Cozzi et al. 2001), but include a propensity for neuroscientists to focus on the rodent and primate species commonly used in biomedical

research (Manger et al. 2008), and a lack of well-preserved elephant brain tissue suitable for histological analysis. This latter issue was recently addressed by Manger et al. (2009), who were able to fix the brains of wild African elephants (*Loxodonta africana*) by postmortem, carotid cannulation-perfusion. The quality of this tissue facilitated the present investigation, which qualitatively and quantitatively explores, for the first time, the neuromorphology of frontal and occipital cortices in the African elephant.

Elephants belong to the order Proboscidea, which emerged approximately 60 million years ago from within the eutherian superorder Afrotheria (Sukumar 2003; Shoshani and Tassy 2005; Gheerbrant and Tassy 2009; Fig. 1). Extant afrotherian species (e.g., elephants, manatees, dugongs, hyraxes, aardvarks, golden moles, tenrecs, and elephant shrews) exhibit significant diversity in terms of body and brain mass, neural organization, and life history (Gravett et al. 2009; Pieters et al. 2010). Because the afrotherian clade represents a major adaptive radiation within Eutheria, it is in a position to provide insight into mammalian brain evolution, with current evidence suggesting that

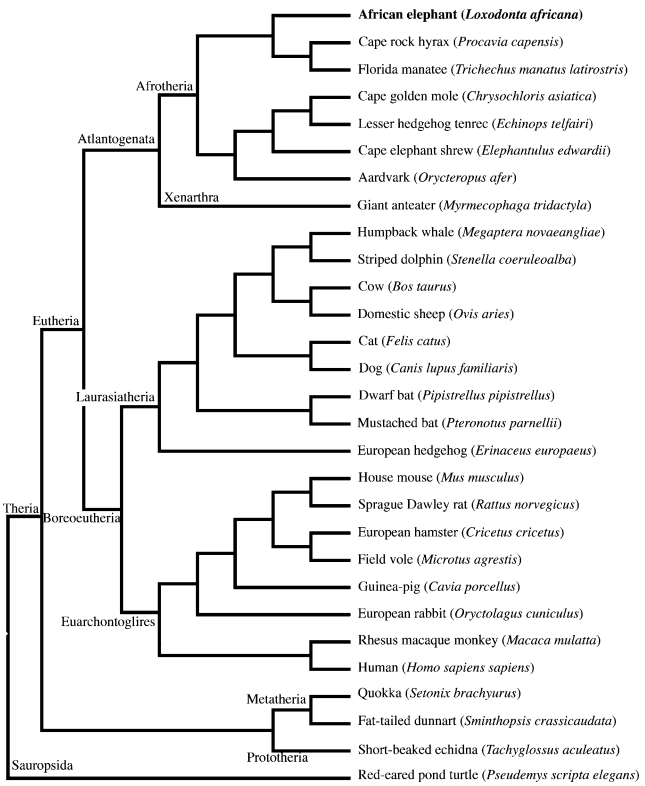


Fig. 1 Taxonomy of the elephant (*Loxodonta africana*) and a sampling of related species mentioned in the present paper. During the late Cenozoic era, Eutheria (placental mammals) diverged into two phylogenetic groups: Atlantogenata and Boreoeutheria (Wildman et al. 2007). Atlantogenata subsequently diverged into two sister clades, Xenarthra (e.g., sloths, anteaters, and armadillos) and Afrotheria, whereas Boroeutheria diverged into Laurasiatheria (e.g., cetaceans, carnivores, and ungulates) and Euarchontoglires (e.g., primates and rodents)

Afrotheria contains many neural plesiomorphies in comparison with other placental mammal species (Sherwood et al. 2009). Thus, examination of neuronal morphology in elephants is essential for understanding broader phylogenetic patterns of neuromorphological evolution in Afrotherians and provides data concerning the scaling of neuronal somatodendritic geometry with increasing body and brain size (Tower 1954; Haug 1987; Hart et al. 2008).

Both cognitive and sociobehavioral investigations of elephants reinforce the view that such a large brain is associated with extensive abilities (Roth and Dicke 2005; Hart and Hart, in press). The cognitive repertoire of elephants includes elementary tool construction/usage (Hart et al. 2001), impressive spatial and temporal memory (Markowitz et al. 1975; Hart et al. 2008), creative problem solving skills (Bates et al. 2008b), the ability to classify human ethnic groups by odor and garment color (Bates et al. 2007a), and the ability to locate out-of-site family members by smell (Bates et al. 2007b). Social interactions in elephants are underscored by several documented abilities: ultra-low frequency sound communication over both local and long distances (Poole 1999; Garstang 2004; Soltis 2009), vocal learning (Poole et al. 2005), long-term care of their young (Bates et al. 2008b), individual identification of conspecifics (Bates et al. 2007b), and targeted helping behavior (Hart et al. 2008). These abilities suggest elephants may be capable of empathy (Bates et al. 2008a, b) and self-recognition, including theory of mind (Plotnik et al. 2006), all of which crucially contribute to their complex social networks (McComb et al. 2000, 2001).

Unfortunately, besides the recent documentation of von Economo neurons in the anterior insular cortex of elephants (Hakeem et al. 2009), one can only extrapolate the neural foundations for these cognitively demanding abilities. For example, based on the low density of neurons in their cerebral cortex, it has been suggested that the elephants' cognitive faculties may be concentrated in long-term processing (Hart and Hart 2007; Hart et al. 2008), although fine sensorimotor integration is certainly required to guide the trunk (Hoffmann et al. 2004). Apart from such speculation, there are currently no data that allow for direct examination of the computational biophysics of elephant cortical neurons. However, in the prototherian echidna, whose ancestry is close to the root of the mammalian phylogenetic tree (Fig. 1), a large proportion of pyramidal neurons contain so-called "atypical" features, such as bifurcated apical dendrites, inverted somata, poorly developed basilar skirts, and a lack of terminal bouquets in layer I (Hassiotis et al. 2003, 2005). This suggests that there may be significant phylogenetic diversity in the morphology of spiny projection neurons of the cerebral cortex. As such, based on its afrotherian origin, we expect a variety of spiny neuron morphologies in the elephant that differs from the euarchontoglire branch of

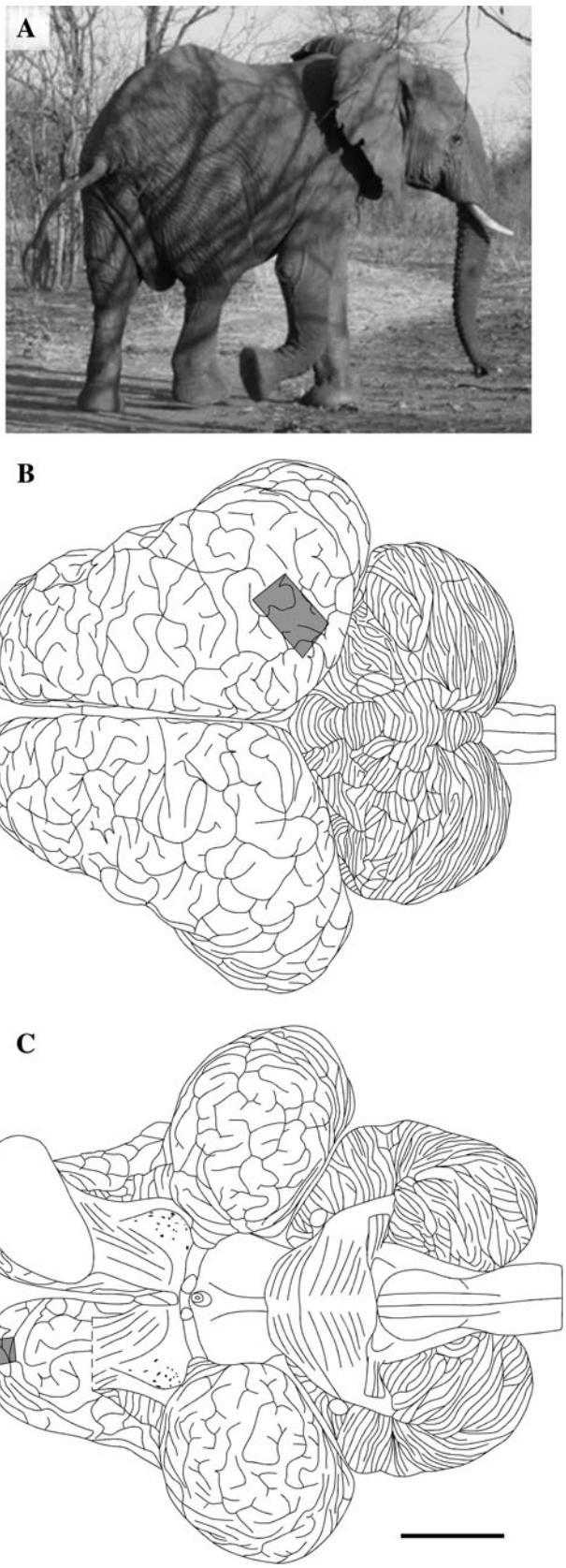


Fig. 2 **a** Photograph of elephant LA1; **b** superior and **c** inferior views ▶ of the elephant brain illustrating the relative position of sampled tissue blocks (*shaded areas*) for the occipital and frontal regions, respectively. Note that, in **c**, the right hemisphere's olfactory bulb has been removed in order to view the orbital gyri and facilitate removal of the tissue block. *Scale bar* 5 cm

placental mammals, mostly typified by anthropoid primate and murid rodent models, and thus appear "atypical". In contrast, the morphological classes for aspiny interneurons are more uniform across eutherian mammals (Hof et al. 1996, 1999; DeFelipe et al. 2002; Hassiotis and Ashwell 2003; Hof and Sherwood 2005). Thus, we expect aspiny interneurons in elephants to resemble more closely the types that are common in the mammalian cerebral cortex.

By extension, neuromorphological diversity in the elephant implies a diverse cytoarchitecture, which would contradict the long-held, but problematic claim that large brains—such as in cetaceans—lack histological complexity (Kesarev 1975; Glezer et al. 1988). In fact, recent studies on cetacean cerebral cortices have revealed a complex cytoarchitecture, comparable to that observed in many mammals, including primates (Hof et al. 2005; Hof and Van der Gucht 2007; Butti et al. 2009; Butti and Hof 2010). The present study examines such neuromorphological diversity by exploring regional variation in neuron morphologies across all layers of the elephant cortex and by examining quantitative differences in superficial (i.e., layers II–III) pyramidal neurons between frontal and occipital cortical regions. As is the case with human supragranular pyramidal neurons, these superficial pyramidal neurons also tended to stain clearly with the rapid Golgi technique (Jacobs et al. 2001). Both functional (Foxe and Simpson 2002) and morphological data in primates (Elston and Rosa 1997; Jacobs et al. 2001) suggest regional variation in cortical processing, generally with successively more complex processing as one moves rostrally. Greater processing demands on neurons appear to be reflected in the increasing complexity of their dendritic arrays (Elston 2003, 2007; Jacobs and Scheibel 2002). Although it is not possible to know which functional cortical areas are contained in the frontal and occipital samples in the present study, examination of dendritic complexity, as revealed by superficial pyramidal neurons, may indicate regional variation of integrative functions in the elephant.

Materials and methods

Specimens

Two (LA1 and LA3; Fig. 2a), free ranging, solitary male African elephants (*Loxodonta Africana*; approximate age

20–30 years) scheduled for population management culling were humanely killed as described in Manger et al. (2009). In situ perfusion-fixation of the brains was conducted by removal of the head, flushing of the cranium with cold saline, and intra-carotid perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer, resulting in relatively short autolysis times (LA1: 160 min; LA3: 110 min). The brains were then removed from the skull, placed in the same cold fixative, weighed (LA1: 5,145 g, LA3: 4,835 g), and stored in 4% paraformaldehyde in 0.1 M phosphate buffer for 72 h. Small tissue blocks containing the cortical regions of interest were removed and stored in 0.1% sodium azide in 0.1 M phosphate buffer saline at 4°C for 8 months before staining. The present research protocol was approved by the Colorado College Institutional Review Board (#H94-004) and by the University of the Witwatersrand Animal Ethics Committee (Manger et al. 2009).

Tissue selection

Frontal and occipital tissue blocks (1–2 cm, perpendicular to the long axis of the sampled gyri) were removed from the right hemisphere. Because no cytoarchitectural criteria exist in the elephant for identification of these cortical regions, localization was based solely on gross examination of elephant brains (Haug 1970; Hakeem et al. 2005; Shoshani et al. 2006) and on reports from other species (e.g., giraffes: Badlangana et al. 2007; cetaceans: Hof and Van der Gucht 2007). Occipital tissue was removed from the parieto-occipital contour close to the midline, approximately 3–5 cm immediately anterior to the cerebellum, although it should be noted that the elephant occipital lobe is not as clearly demarcated as in humans (Shoshani et al. 2006; Fig. 2b). Frontal tissue was removed from the anterior portion of what appeared to be the orbital gyri, 3–5 cm from the midline, in a position superior to the anterior third of the olfactory bulb (Fig. 2c).

Tissue blocks were trimmed to 3–5 mm in thickness, coded to prevent experimenter bias, and processed by a modified rapid Golgi technique (Scheibel and Scheibel 1978b). To be consistent with previous studies (Jacobs et al. 1997, 2001), processed tissue was serially sectioned at 120 μm with a vibratome. Tissue blocks adjacent to those removed for Golgi analysis were examined with a routine Nissl stain to establish laminar and cortical depth. Thirty depth measurements for each layer were averaged across 12 sections from each cortical region.

Neuron selection and quantification

Analyses qualitatively and quantitatively characterized elephant neocortical neurons (N = 75), with a particular focus on superficial (i.e., layers II–III) pyramidal neurons (mean soma depth = 794 ± 131 μm) in the two cortical regions. Because the Golgi impregnation was not uniform, more neurons appropriate for quantification were sampled from LA1 ($n = 46$) than from LA3 ($n = 29$). A total of 40 superficial pyramidal neurons were traced in the occipital cortex (15 from LA1; 5 from LA3) and in the frontal cortex (20 from LA1); of these, 16 had relatively complete apical dendrites, which were analyzed only descriptively. Although pooling data across animals was not an optimal solution, an ANOVA comparing occipital total dendritic length in superficial neurons between LA3 and LA1 found no significant difference, suggesting this as an acceptable compromise for subsequent analyses. A broader sampling of neuron morphology resulted in 16 additional, deep layer III to layer VI pyramidal neurons being traced in occipital cortex (10 from LA1; 6 from LA3), and 19 in frontal cortex (1 from LA1; 18 from LA3).

Previously established selection criteria for human supragranular pyramidal neurons (Jacobs et al. 1997, 2001; Anderson et al. 2009) were adapted in the present study. Briefly, selected neurons were required to be relatively isolated and unobscured, to appear fully impregnated with a soma roughly centered within the 120 μm-thick section, and to have as complete dendritic arbors as possible. In order to create a relatively homogeneous cell population for superficial pyramidal neurons, a running average of soma depth from the pial surface was maintained and cells were chosen to preserve similar averages across both cortical areas.

Cells were quantified along *x*-, *y*-, and *z*-coordinates using an Olympus BH-2 microscope under a Planachromat 40× (NA = 0.70) dry objective interfaced with a Neurolucida software system (MBF Bioscience, Williston, VT, USA). The system utilized a MicroFire Digital CCD 2-Megapixel camera (Optronics, Goleta, CA, USA), which was mounted on a trinocular head. The camera image was viewed on a Dell E248WFP 24-inch LCD monitor with 1,920 × 1,200 resolution. First, the soma was traced at its widest point in the two-dimensional plane in order to provide an estimate of its cross-sectional area. Dendrites were then traced somatofugally in their entirety, accounting for dendritic diameter and quantifying all visible spines, regardless of spine type or differences in spine length. Dendritic processes were not followed into adjacent sections; as such, broken tips and unclear terminations were identified as incomplete endings.

Neurons were traced by three observers (BJ, JL, and KA). Intrarater reliability was assessed by having each rater trace the same dendritic branch, with somata and spines, 10 times. Tracings varied little, as indicated by the relatively low coefficient of variation for soma size (2.8%), total dendritic length (TDL 2.8%), and dendritic

spine number (DSN 3.9%). Intrarater reliability was also tested with a split plot design ($\alpha = 0.05$), which revealed no significant difference within tracers between the first five tracings and the last five tracings of a dendritic system. For interrater reliability, tracers were normed by comparing their tracings of 10 dendritic systems to the same tracings completed by the primary investigator (BJ). Pearson's product correlations across soma size, TDL, and DSN (defined below) averaged 0.97, 0.96, and 0.96, respectively. An analysis of variance (ANOVA; $\alpha = 0.05$) indicated that there was no significant difference among tracers in the three measures. Finally, the primary investigator reexamined all final tracings to ensure accuracy.

Cell descriptions and dependent dendritic/spine measures

Deep pyramidal neurons were qualitatively described according to nomenclature found in previous neuromorphological research (e.g., Ngowyang 1932; Ferrer et al. 1986a, b). Descriptions for each neuron used qualitative criteria, such as cortical location, size, dendritic field patterns, presence of spines, and soma characteristics. Nevertheless, because axons were not visible and because intermediates between different neuronal types exist, these designations remain tentative.

For quantitative measures, a centrifugal nomenclature was used (Bok 1959; Uylings et al. 1986): dendritic branches arising from the soma are first-order segments until they bifurcate into second-order segments, which branch into third-order segments, and so on. In addition to noting soma size (as apparent surface area in μm^2) and soma depth from the pial surface, five previously established measures (Jacobs et al. 2001) were used to analyze each neuron. Total dendritic length (TDL, μm) refers to the summed length of all dendritic segments. Mean segment length (MSL, μm) is the average length of each dendritic segment. Dendritic segment count (DSC) represents the number of dendritic segments. Dendritic spine number (DSN) refers to the total number of spines on dendritic segments. Dendritic spine density (DSD) is the average number of spines per micron of dendritic length. Additionally, dendritic diameter throughout the cell was determined, thus providing a sixth measure: dendritic volume (Vol, μm^3). Although tested independently, many of these measures are inherently interrelated. Finally, dendritic branching patterns of all traced neurons were examined using Sholl analysis (Sholl 1953), which quantified the number of dendritic intersections at 20-μm intervals radiating centrifugally from the soma. This analysis assisted in the characterization of apical and basilar dendrites.

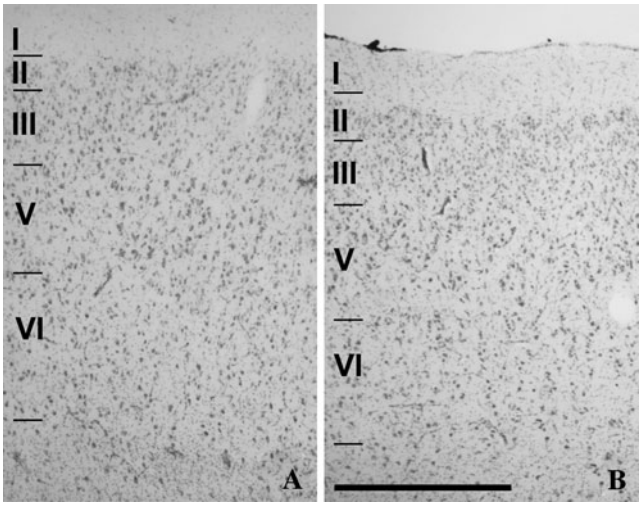


Fig. 3 Photomicrographs of Nissl-stained cortex from frontal (**a**) and occipital (**b**) cortex with labeled layers. Note that the elephant appears to lack a well-developed layer IV. *Scale bar* 1 mm

Table 1 Laminar and cortical depth from the pial surface in elephant cortex (μm)

	Frontal region	Occipital region
Layer I/II junction	560 ± 14	519 ± 20
Layer II/III junction	730 ± 13	675 ± 19
Layer III/V junction	1,495 ± 14	1,643 ± 38
Layer V/VI junction	2,034 ± 21	2,098 ± 42
Gray/white matter junction	2,828 ± 41	2,737 ± 55

Values represent mean ± SEM

Independent variables and statistical analyses

For superficial pyramidal neuron comparisons, only basilar dendrites were examined inferentially because apical dendrites were often incomplete. Basilar dendritic data were aggregated by neuron (CELL), and brain region (AREA). The resulting dataset was analyzed using a one-way analysis of variance (ANOVA; $\alpha = 0.05$) to investigate the effects of area (i.e., frontal and occipital) on each dependent measure for the basilar skirt.

Results

Overview

Nissl-stained sections indicated that the frontal cortex was slightly thicker than the occipital cortex, with both regions exhibiting a relatively thick layer III and lacking a distinct layer IV (Fig. 3; Table 1). The majority of cells traced resided in layers III and V. In Golgi preparations, the dominant characteristic across both occipital and frontal

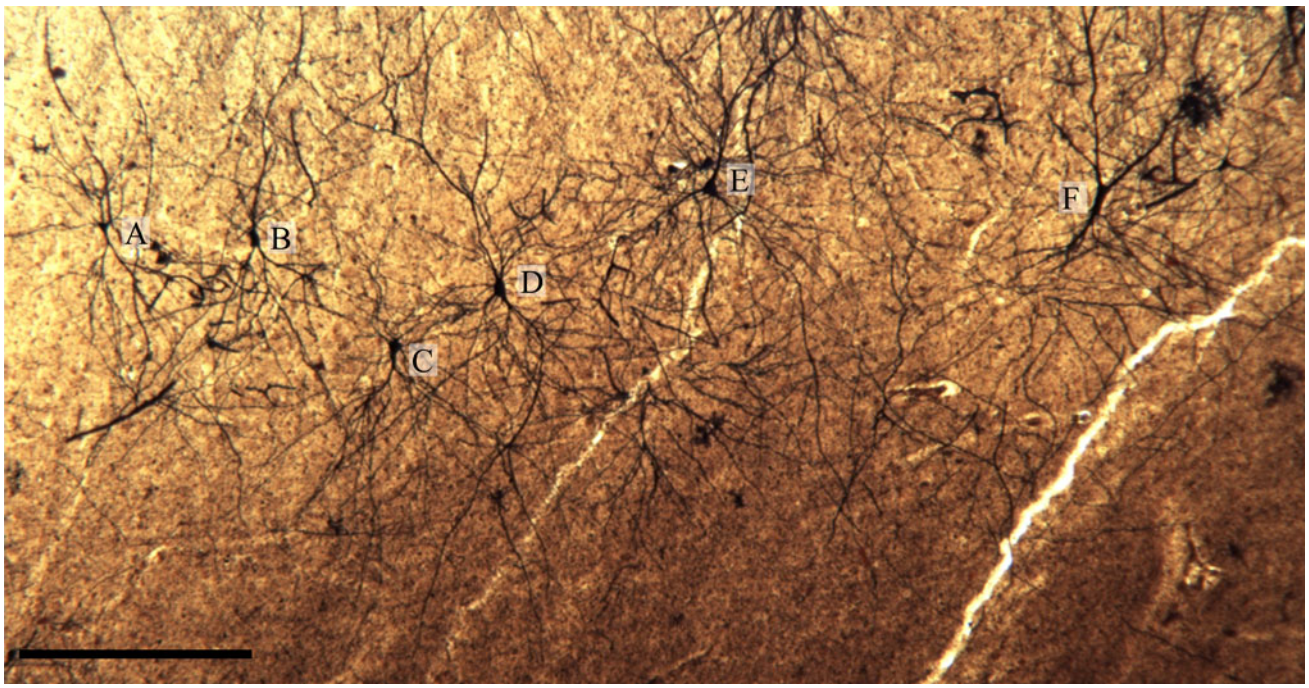


Fig. 4 Low magnification photomicrograph of elephant frontal cortex illustrating a wide diversity of neuronal morphologies: inverted pyramidal neurons (*A*, *C*); superficial pyramidal neurons (*B*, *E*); magnopyramidal-taproot or matriarch neuron (*D*, also seen in Fig. 5J); and fork neuron (*F*, also seen in Fig. 5*F*). Pial surface is at the top of the photomicrograph. *Scale bar* 500 μm

regions was the vast heterogeneity of spiny cell types (Fig. 4). Generally, superficial layers contained many small, bifurcating variations of the pyramidal cell with thick apical dendrites that ramified as they ascended toward the pial surface. Deeper neurons possessed broad basilar skirts and often extended multiple widely diverging apical dendrites. In some cells, a prominent basilar dendrite or taproot penetrated the white matter. Aspiny neurons tended to possess long dendrites that bifurcated near the soma or not at all, and were located in upper layer III or lower layer V. Tracings of individual cell types are provided in Figs. 5, 6, and 7 with quantitative data provided in Table 2. It should be noted that laminar boundaries are not indicated in Figs. 5, 6, and 7 because, given the considerable variability across individual sections, neurons of similar depth may not necessarily be located in the same cortical layer across different sections. These descriptions, based on examination of all Golgi-stained sections as well as the quantified neurons themselves, are presented below with superficial pyramidal neurons being considered separately because they were more extensively analyzed.

Spiny neurons

Deeper pyramidal neurons with ascending apical dendrites exhibited several variations: magnopyramidal (with and without a taproot), multiapical, and fork neurons. *Magnopyramidal neurons* ($n = 3$, Fig. 6F–H), located at a depth of approximately 1,500 μm in layer III of the occipital lobe, projected an ascending, typically bifurcating, apical process, often exceeding 1,200 μm in length. Their somata varied in size (Table 2) and shape, from small and triangular to very large and fusiform. The basilar skirt extended in all directions to produce a circular receptive area. The magnopyramidal cells were generally less spiny than their superficial counterparts, with a DSD between 0.33 and 0.47 (Table 2). Sholl analysis revealed a high density of basilar dendrites similar in size and distribution to those of superficial pyramidal cells, although the apical dendrite was always longer (Fig. 8a).

An apparent variation of the above was the *magnopyramidal-taproot neurons* ($n = 4$, Figs. 4D, 5I–L, 9), which were located in layers III and V of the frontal lobe. Because of their prominent appearance in Golgi-stained sections and their trunk-like taproot, we named these "*matriarch*" neurons (referring to the matriarch's prominent role in elephant society). They exhibited extensive dendritic branching, which suggests a broad sampling of cortical information (Brown et al. 2008). The matriarch neuron's fusiform soma extended an apical process for up to 900 μm that often bifurcated during its ascent to the pial surface. A basilar skirt projected densely and uniformly in all directions. Unique to this neuron was the addition of a complex, descending taproot that tended to bifurcate widely into smaller segments while the main branch extended for long distances (up to 1 mm) toward the underlying white matter.

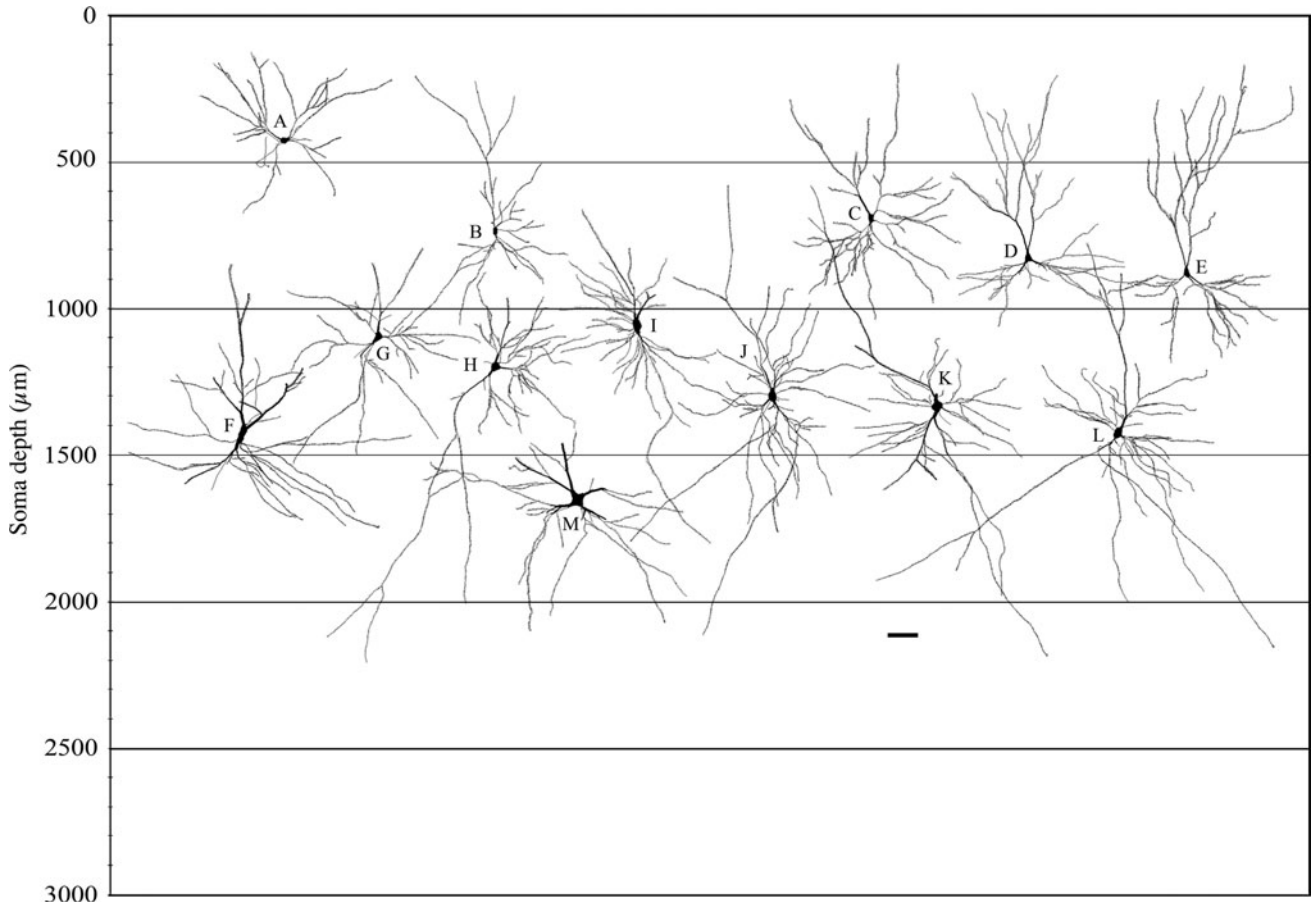


Fig. 5 Neurolucida tracings of spiny neurons in the elephant frontal cortex presented to indicate their relative soma depths from the pial surface (in μm): crab-like neuron (*A*); superficial pyramidal neurons (*B–E*); fork neuron (*F*); inverted pyramidal neuron (*G*, *H*); magnopyramidal-taproot or matriarch neurons (*I–L*); multiapical pyramidal neuron (*M*). The quantitative dependent measures for all neurons are provided in Table 2. *Scale bar* 100 μm

DSD ranged from 0.32 to 0.54 (Table 2). In the Sholl analysis, the matriarch neuron (Fig. 8c) was second only to the neurogliaform cell (Fig. 7L) in terms of overall dendritic density.

Multiapical pyramidal neurons ($n = 3$, Figs. 5M, 6K–L), present both in frontal and in occipital cortex, were among the deepest of the spiny cells, located predominantly in layer V at a depth of 1,500–1,700 μm (Table 2). Although often incomplete due to sectioning, multiple, prominent apical dendrites extended from a spherical cell body to ascend symmetrically toward the pial surface. Basilar dendrites extended with some variability from the soma, either favoring radial processes that descended toward the white matter or more lateral, tufted projections. Dendrites possessed a relatively low density of spines, with a DSD ranging from 0.30 to 0.36 (Table 2). Sholl analysis revealed two apical dendritic peaks, with the second peak being somewhat larger (Fig. 8e). Basilar dendritic density peaked closer to the soma than did the apical dendrite.

A *fork neuron* (or Gabelzelle; $n = 1$, Figs. 4F, 5F), originally described by Ngowyang (1932) in the human frontoinsular cortex, was found at the boundary between layers III and V of the frontal cortex. It possessed a split soma that tapered into two thick, ascending apical dendrites 600 μm in length. Basilar projections extended obliquely as fine, parallel dendritic processes. DSD was relatively high at 0.58 (Table 2). Its axon emerged proximally from a basilar dendrite and appeared to descend. The fork neuron was unusual in that, like the horizontal pyramidal cell, its apical and basilar branches were similar in length (Fig. 8g), although the apical dendrites were clearly truncated by sectioning.

Greater variation in apical dendritic orientation was observed with horizontal and inverted pyramidal neurons. *Horizontal pyramidal neurons* ($n = 2$, Fig. 6I–J), found in layer III of the occipital lobe, possessed a narrow, elongated soma from which a quickly ramifying apical dendrite extended laterally or obliquely (<45°). Basilar dendrites

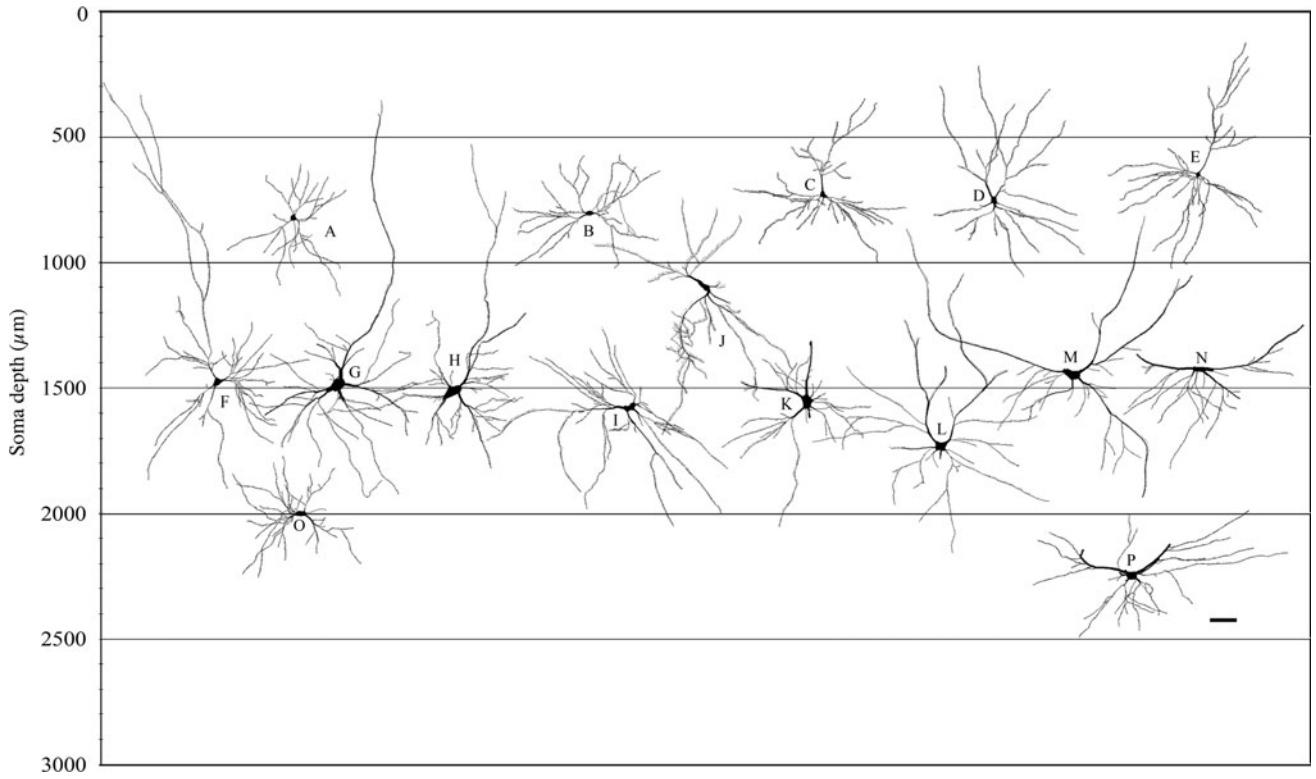


Fig. 6 Neurolucida tracings of spiny neurons in the elephant occipital cortex presented to indicate their relative soma depths from the pial surface (in μm): inverted pyramidal neuron (*A*); crab-like neurons (*B*, *O*); superficial pyramidal neurons (*C–E*); magnopyramidal neurons (*F–H*); horizontal pyramidal neurons (*I–J*); multiapical pyramidal neurons (*K–L*); flattened pyramidal neurons (*M–N*, *P*). The quantitative dependent measures for all neurons are provided in Table 2. *Scale bar* 100 μm

branched into distinct, parallel collaterals or extended numerous secondary and tertiary dendrites. DSD was between 0.44 and 0.47 for the two cells (Table 2). In the Sholl analysis, the apical and basilar dendrites were roughly equal in density and length (Fig. 8f). *Inverted pyramidal neurons* ($n = 3$, Figs. 4A, C, 5G–H, 6A), found in layer III of frontal and occipital cortices, possessed apical dendrites that descended and bifurcated from a roughly triangular soma. In the frontal lobe, the length of the apical process varied from 600 to 1,100 μm and possessed few collaterals. The basilar skirt projected toward the pial surface, producing a circular receptive area. The smallest and most superficial of these cells (Fig. 6A) possessed a short apical shaft that descended obliquely for less than 400 μm in length, and an axon that emerged from the base of the soma and bifurcated, one branch ascending and the other descending. DSD ranged from 0.39 to 0.46 (Table 2). Sholl analyses confirmed the inverted pyramidal neuron's (Fig. 8b) structural similarity to the magnopyramidal neurons.

Finally, two "atypical" spiny neuron variants appeared to have horizontally oriented dendritic arbors: flattened pyramidal and "crab-like" neurons. *Flattened pyramidal neurons* ($n = 3$, Fig. 6M–N, P) were found in deep layer III and upper layer VI of the occipital cortex, where they appeared to be fairly common. Flattened pyramidal neurons sent multiple, thick apical dendrites from opposing ends of the soma to ascend outward and then gradually toward the pial surface. Somata varied from elongated to globular. Dendrites extended further laterally than vertically, with basilar dendrites often descending toward the white matter alongside apical dendrite collaterals. Their thick dendrites made the cells, by volume, among the largest in the present sample. Their DSD was similar to other large pyramidal cells, ranging from 0.37 to 0.41 (Table 2). Sholl analysis revealed the maximum density of apical dendrites to be further from the soma than that of the basilar dendrites (Fig. 8d). *Crab-like neurons* ($n = 3$, Figs. 5A, 6B, O) were so designated because their dendritic branches emerged from opposite ends of a rounded soma to ramify symmetrically, producing claw-like shapes. These were located in layers II and III and at the junction between layer V and VI of the frontal and occipital lobes. DSD varied greatly, ranging from 0.39 to 0.67 (Table 2). Sholl analysis indicated that crab-like neurons (Fig. 8j) exhibited a relatively small dendritic receptive area.

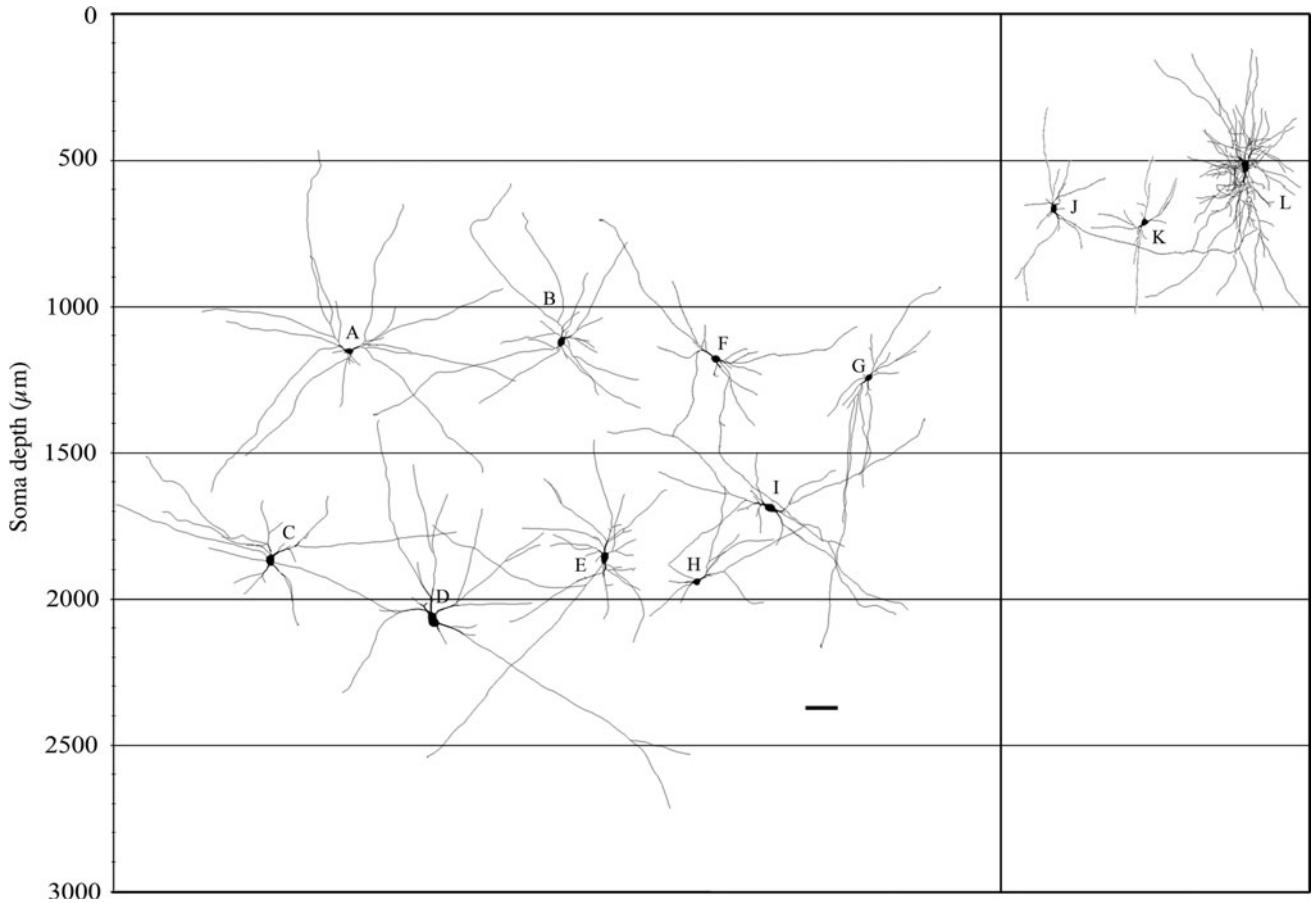


Fig. 7 Neurolucida tracings of aspiny neurons found in elephant frontal (*A–I*) and occipital (*J–L*) cortices presented to indicate their relative soma depths from the pial surface (in μm): multipolar neurons (*A–D*, *J–K*); bipolar neurons (*E–I*); neurogliaform neuron (*L*). The quantitative dependent measures for all neurons are provided in Table 2. *Scale bar* 100 μm

Aspiny neurons

Aspiny multipolar neurons ($n = 6$, Figs. 7A–D, J–K) were found throughout all layers of frontal and occipital cortices. They varied in appearance, but generally exhibited a small, globular soma from which a few (2–6) primary, straight dendritic projections extended to 1,000 μm in length. In the Sholl analysis, some multipolar cells (Fig. 8h) exhibited dendritic projections that were longer than any other cells, excluding the magnopyramidal and inverted pyramidal neurons.

Bipolar neurons ($n = 5$, Fig. 7E–I), similar in size and shape to the multipolar neurons, were found in layers III and V of frontal cortex. They possessed a small, globular soma from which two main, thick dendritic segments extended in opposite directions. Thin collaterals, up to 900 μm in length, extended outward from these segments and ramified to produce a roughly symmetrical bitufted appearance. According to the Sholl analysis, the dendrites of these cells (Fig. 8i) were, on average, shorter and less dense than the comparable multipolar neurons.

The *neurogliaform neuron* ($n = 1$, Fig. 7L), also called a spiderweb cell by Ramón y Cajal (1922), was an aspiny, diffusely branching multipolar cell in layers II and III of occipital cortex. Three main dendritic branches emerged from an elongated soma and gave rise to numerous, approximately 200 μm dendritic projections that bifurcated densely in all directions to produce a spherical, net-like receptive area with a TDL that far surpassed that of any other cell (Table 2). A few longer dendrites (up to 600 μm in length) extended along the cell's vertical axis. This cell had by far the densest dendritic arbor (DSC = 337), with over twice as many ring intersections as any other neuron in the Sholl analysis (Fig. 8k).

Quantitatively, three general observations about aspiny interneurons and spiny neurons in the elephant emerge (excluding the neurogliaform neuron, which was a clear dendritic outlier): (1) aspiny neurons in frontal cortex ($M_{Vol} = 11{,}870 \pm 5{,}337$ μm^3; $M_{TDL} = 4{,}650 \pm 1{,}462$ μm) appeared somewhat larger than those in occipital cortex ($M_{Vol} = 7{,}514 \pm 2{,}441$ μm^3; $M_{TDL} = 2{,}558 \pm 599$ μm), although the small sample size severely limits the value of

Table 2 Summary statistics for elephant neurons in frontal and occipital cortex

Type	Cell[a]	Vol.[b]	TDL[c]	MSL[c]	DSC[d]	DSN[e]	DSD[f]	Soma size[g]	Soma depth[h]
Frontal									
Crab-like	5A	5,732	4,249	83	51	2,863	0.67	439	424
Superficial pyramidal	5B	8,233	4,574	82	56	2,036	0.45	286	741
Superficial pyramidal	5C	17,880	6,740	95	71	4,162	0.62	478	691
Superficial pyramidal	5D	30,300	7,603	104	98	4,839	0.64	479	818
Superficial pyramidal	5E	17,250	8,233	106	78	5,379	0.65	506	875
Fork	5F	55,256	8,050	117	69	4,685	0.58	1,154	1,436
Inverted pyramidal	5G	13,937	5,634	95	59	2,608	0.46	681	1,101
Inverted pyramidal	5H	18,055	7,370	93	79	3,012	0.41	706	1,203
Matriarch	5I	27,149	7,716	100	77	2,489	0.32	1,061	1,066
Matriarch	5J	33,500	10,838	111	97	5,856	0.54	766	1,299
Matriarch	5K	43,904	8,269	115	72	3,977	0.48	973	1,317
Matriarch	5L	28,360	10,377	113	92	5,430	0.52	799	1,423
Multiapical pyramidal	5M	54,261	6,742	98	69	2,340	0.35	1,552	1,662
Multipolar	7A	10,843	6,879	186	37	–	–	387	1,124
Multipolar	7B	9,311	5,152	152	34	–	–	611	1,069
Multipolar	7C	11,905	3,778	114	33	–	–	801	1,811
Multipolar	7D	24,569	5,839	195	30	–	–	1,375	2,027
Bipolar	7E	13,497	5,883	115	51	–	–	757	1,811
Bipolar	7F	9,186	4,129	118	35	193	0.05	494	1,132
Bipolar	7G	8,510	3,996	125	32	–	–	351	1,203
Bipolar	7H	5,851	1,946	97	20	–	–	411	1,893
Bipolar	7I	13,160	4,248	112	38	–	–	737	1,641
Occipital									
Inverted pyramidal	6A	5,173	3,465	96	36	1,346	0.39	398	823
Crab-like	6B	4,596	2,500	104	24	1,214	0.49	407	806
Superficial pyramidal	6C	10,897	5,921	82	72	3,346	0.57	505	722
Superficial pyramidal	6D	16,264	5,509	115	48	3,630	0.66	643	746
Superficial pyramidal	6E	11,312	6,752	83	81	3,914	0.58	256	650
Magnopyramidal	6F	18,924	8,752	115	76	4,094	0.47	780	1,470
Magnopyramidal	6G	76,591	7,925	113	70	3,148	0.40	2,015	1,487
Magnopyramidal	6H	46,090	6,895	90	77	2,296	0.33	2,004	1,525
Horizontal pyramidal	6I	29,131	7,554	113	67	3,521	0.47	988	1,571
Horizontal pyramidal	6J	28,811	6,411	88	73	2,853	0.44	941	1,090
Multiapical pyramidal	6K	35,055	4,428	84	53	1,311	0.30	1,562	1,536
Multiapical pyramidal	6L	32,114	6,375	133	48	2,291	0.36	1,284	1,734
Flattened pyramidal	6M	58,109	6,187	115	54	2,293	0.37	1,694	1,456
Flattened pyramidal	6N	36,137	4,346	92	47	1,706	0.39	1,222	1,407
Crab-like	6O	13,679	4,938	60	83	1,949	0.39	689	2,003
Flattened pyramidal	6P	57,494	6,476	93	70	2,674	0.41	1,092	2,233
Multipolar	7J	9,241	2,981	110	27	210	0.07	479	672
Multipolar	7K	5,788	2,134	71	30	34	0.02	423	704
Neurogliaform	7L	26,959	14,818	44	337	–	–	857	528

[a] Refers to tracings of individual cells as indentified in Figs. 5, 6, 7

[b] Volume in μm^3

[c] Length in μm

[d] Number of segments per neuron

[e] Number of spines per neuron

[f] Number of spines per μm of dendritic length

[g] Soma size in μm^2

[h] Soma depth in μm from the pial surface

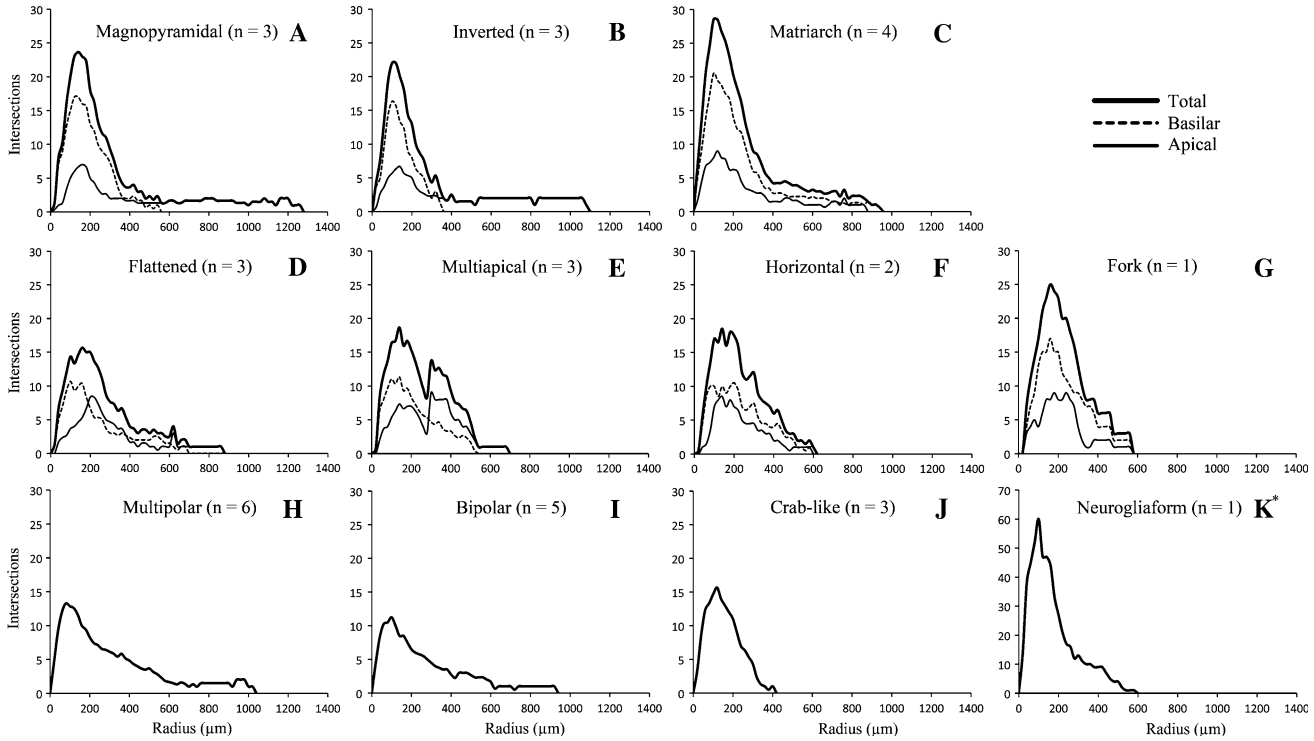


Fig. 8 Sholl analyses of 11 cell types assessing the relative complexity of basilar, apical, and total dendritic branching patterns. Concentric rings, separated by 20 μm increments and centered on the soma, were used to measure dendritic intersections. Neurons **a**–**g** were spiny. Neurons **h**–**k** were aspiny. **a**–**c** exhibited relatively long apical dendrites, **d**–**g** had shorter apical dendrites. For cells with basilar dendrites, the density of basilar intersections peaked before 200 μm, as was the case for the total dendritic density of all aspiny neurons. The apical dendrite either tended to extend for a much larger distance than the basilar dendrites (**a**, **b**) or was nearly equal to them (**c**–**g**). Aspiny neurons (**h**–**j**) generally had low dendritic densities, the notable exception being the neurogliaform neuron (**k**), which had the highest dendritic density of all neurons. *Asterisks* represents that the *y*-axis for the neurogliaform graph (**k**) is set to a larger scale than other cells to compensate for its particularly high dendritic density

this comparison; (2) the aspiny interneurons (M_{Vol} = 11,078 ± 5,147 μm^3; M_{TDL} = 4,270 ± 1,569 μm) exhibited substantially less overall dendritic extent than the spiny neurons (M_{Vol} = 28,765 ± 11,078 μm^3; M_{TDL} = 6,580 ± 1,906 μm); and (3) the aspiny interneurons exhibited considerably longer (M_{MSL} = 127 ± 37 μm), but fewer (M_{DSC} = 33 ± 8) dendritic branches than did the spiny neurons (M_{MSL} = 100 ± 15 μm; M_{DSC} = 67 ± 17).

Superficial pyramidal neurons

Superficial pyramidal neurons (n = 40; Figs. 4B, E, 5B–E, 6C–E) were well impregnated across both occipital and frontal cortices (Fig. 10), facilitating a more detailed quantitative analysis. These neurons were sampled primarily from upper cortical layer III with minimal variation of soma depth across cortical areas (frontal 818 ± 108 μm; occipital 769 ± 149 μm). Measurements of soma size indicated minimal variation between cortical areas (frontal 515 ± 80 μm^2; occipital 517 ± 156 μm^2). There was a significant positive correlation between soma depth and soma size ($r_{(40)}$ = 0.451, $p < 0.01$), as well as between soma size and volume ($r_{(40)}$ = 0.353, $p < 0.05$), which underscored the importance of maintaining similar soma depths between areas for subsequent comparisons.

In terms of appearance, superficial pyramidal neurons exhibited typical basilar skirts, which projected radially for a short distance from the soma. They were characterized by apical dendrites that generally bifurcated around 50 μm from the soma to form two, ascending apical shafts (Figs. 5D–E, 6C, E, 10a, d), or by two diverging apical dendrites (Figs. 5C, 6D) that emerged directly from the soma. These resulting apical trunks traveled obliquely to the pial surface, thus creating a large apical arbor for each cell and a crisscross or "V" appearance in the upper layers (Fig. 11b–c). Generally, these branches appeared to bundle with other branches traveling at similar angles toward the pial surface (Fig. 11a, d).

Dendritic morphology-dependent measures

Selected dependent measures for basilar data were first analyzed using a MANOVA test statistic. Due to direct

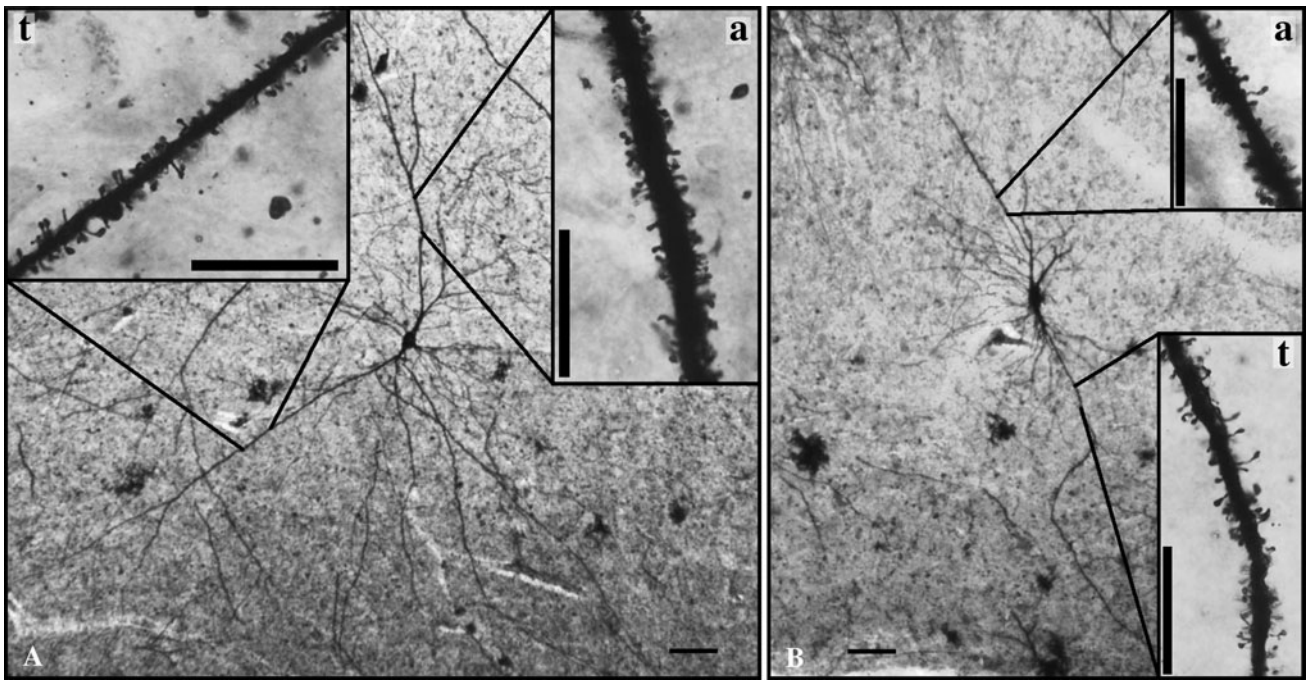


Fig. 9 Photomicrographs of two magnopyramidal-taproot or matriarch neurons (**a**, **b**) with higher magnification insets of their spine-rich ascending apical (*a*) and descending taproot (*t*) dendrites. Neurolucida tracings can be seen in Fig. 5L for **a**, and in Fig. 5I for **b**. *Scale bar* 100 μm for **a** and **b**, and 25 μm for the insets

associations between DSD and DSC with other measures, these variables were not used in the MANOVA. The MANOVA was significant ($F_{(4,\ 35)} = 3.125$, $p < 0.05$, $\eta_p^2 = 0.263$). Post hoc analyses, consisting of six one-way ANOVAs, were then conducted for each dependent variable. All dependent measures, except MSL and DSD, were significantly higher in frontal cortex, indicating the presence of more complex basilar dendrites. Although there were not enough complete apical branches to warrant statistical tests, descriptive data indicated a similar pattern for apical dendrites. The differences in complexity can be seen visually in Fig. 12, which contains representative tracings from each region.

Volume

Basilar dendritic volume was significantly greater ($F_{(1,\ 38)} = 5.80$, $p < 0.05$, $\eta_p^2 = 0.132$) in frontal than in occipital cortex (by 25.2%; Fig. 13a). On average, neurons from frontal cortex contained 29.5% thicker apical dendrites and 23.3% more overall volume than neurons from occipital cortex.

Dendritic length

Basilar dendrites in frontal cortex exhibited significantly greater TDL ($F_{(1,\ 38)} = 7.15$, $p < 0.05$, $\eta_p^2 = 0.158$) than basilar dendrites in occipital cortex (Fig. 13b). The trend was in the opposite direction for MSL, but was not significant (Fig. 13c). Both TDL (by 19.0%) and MSL (by 5.0%) were greater for apical dendrites in the frontal cortex than in the occipital cortex. Descriptive data from complete neurons (i.e., basilar and apical dendrites combined) showed that, overall, TDL was greater (by 13.5%) and MSL was shorter (by 9.2%) in the frontal cortex than in the occipital cortex.

Dendritic segment count

There was a significant difference in basilar DSC across both areas ($F_{(1,\ 38)} = 10.93$, $p < 0.05$, $\eta_p^2 = 0.223$), with basilar dendrites in frontal cortex exhibiting 25.1% higher DSC than occipital cortex (Fig. 13d). Apical dendrites also appeared to have higher DSC in frontal cortex (by 14.8%), and complete neurons in frontal cortex had 20.7% higher DSC than those in occipital cortex.

Dendritic spine number and density

Basilar dendrites of neurons in the frontal cortex displayed a significantly higher DSN (by 25.1%; Fig. 13e) than neurons in the occipital cortex ($F_{(1,\ 38)} = 7.95$, $p < 0.01$, $\eta_p^2 = 0.173$). DSD was not significant (Fig. 13f). Apical dendrites in frontal cortex contained 29.4% more spines and had a 13.4% higher DSD than those in occipital cortex. Evidence from complete neurons indicated that frontal

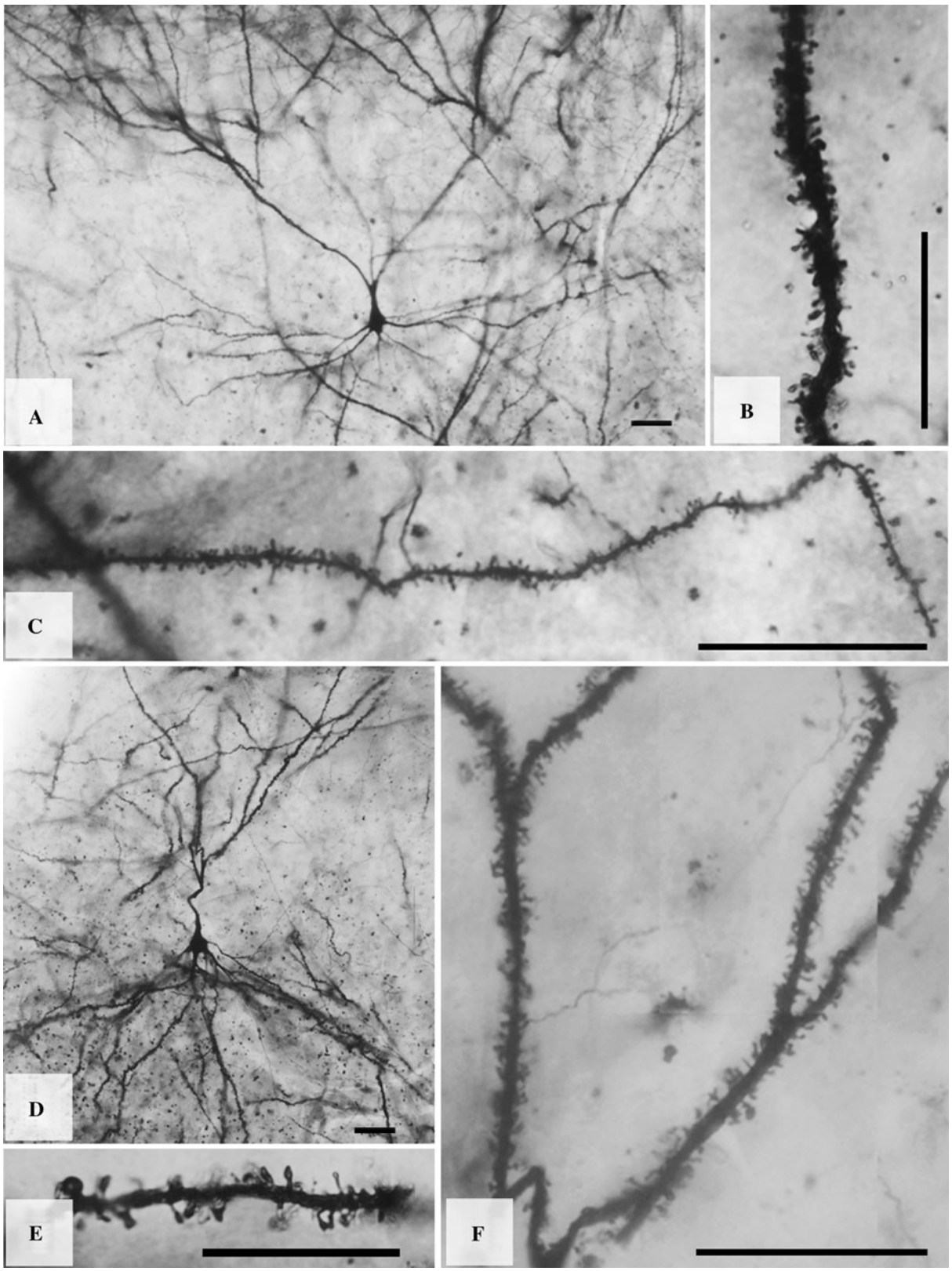


Fig. 10 Photomicrographs of Golgi-impregnated superficial pyramidal neurons in occipital (**a**) and frontal (**d**) cortices. Associated photomicrographs of apical (occipital: **b**; frontal **f**) and basilar (occipital: **c**; frontal **e**) dendrites are also shown at higher magnifications. Note in both cortical regions the presence of bifurcating apical dendrites. For **a**, **b**, **d**, **f** *scale bars* 50 μm. For **c**, **e** *scale bars* 25 μm

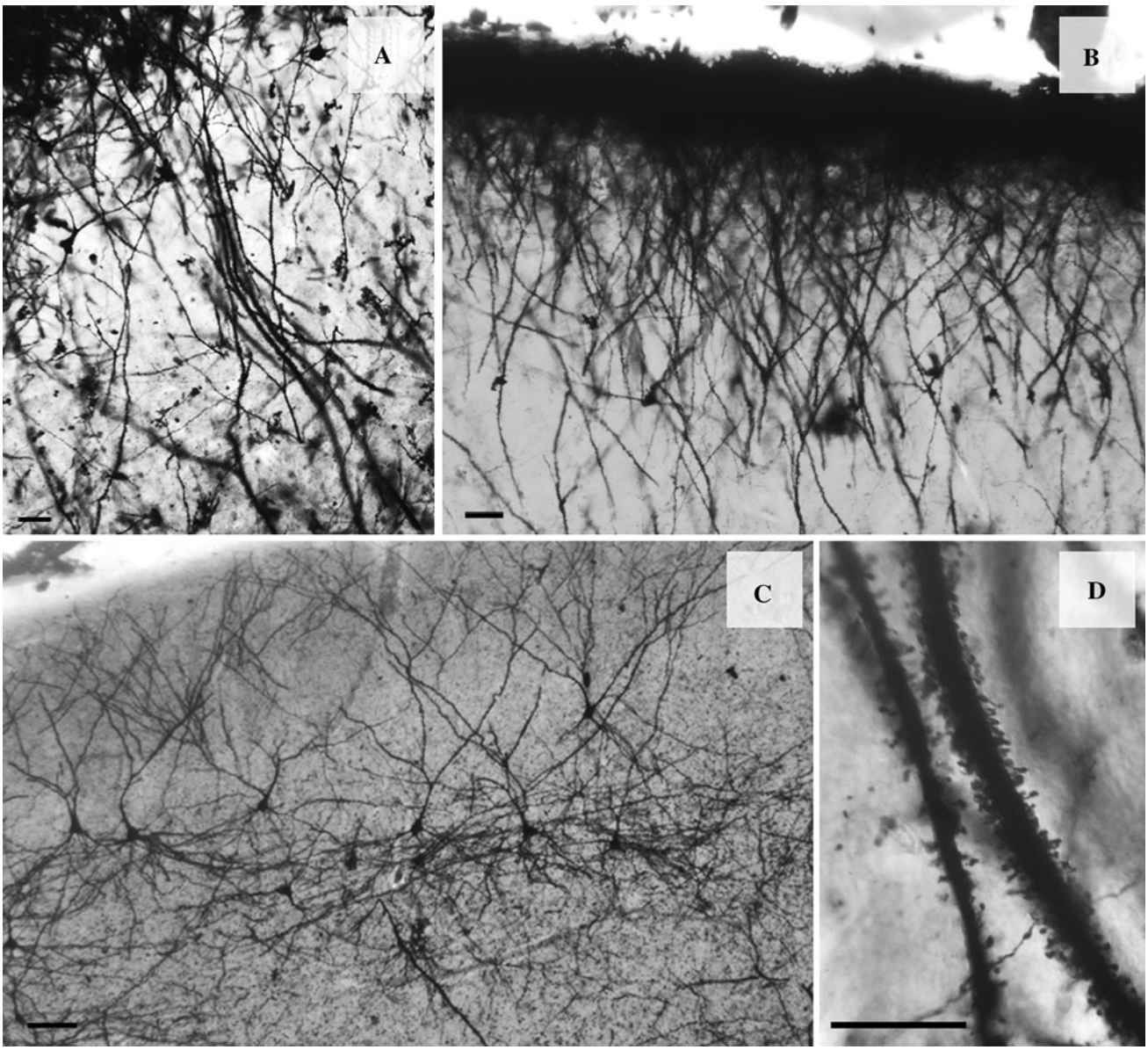


Fig. 11 Photomicrographs of V-apical bundling within elephant occipital cortex. At lower magnifications (**b** and **c**), a V-shaped crossing pattern resulting from bifurcating apical dendrites is apparent. At higher magnifications (**a** and **d**), apical dendrites appear to bundle together as they obliquely approach the pial surface. For **a** and **b** *scale bars* 50 μm. For **c** *scale bar* 100 μm. For **d** *scale bar* 25 μm

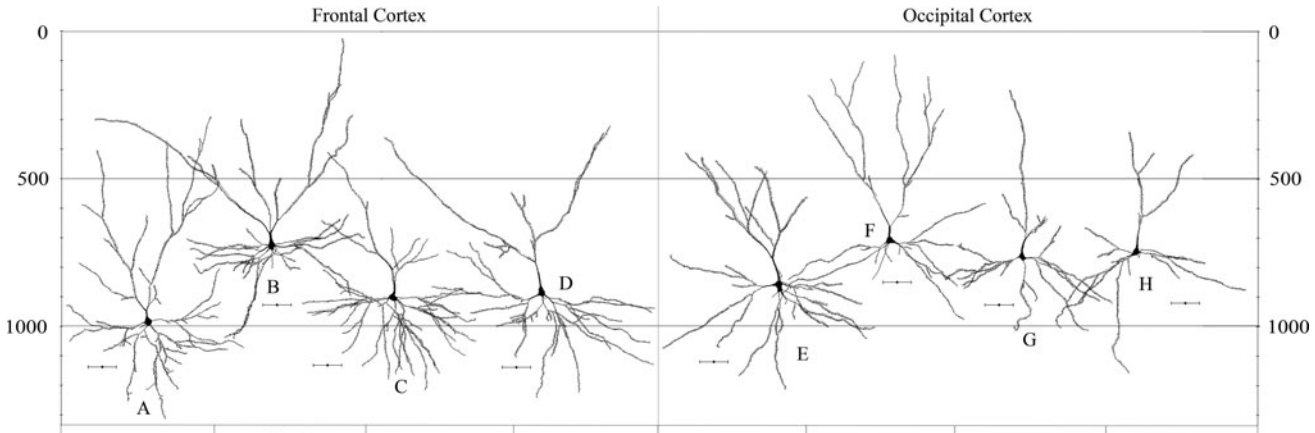
superficial neurons contained 20.2% more spines and had a 7.6% higher DSD than did occipital neurons.

Sholl analysis

Frontal and occipital regions had similar shapes in Sholl analysis profiles, but frontal results were slightly higher on average (Fig. 14a, b). Basilar dendrites in both areas reached their maximum complexity (frontal 16.45 ± 0.99 intersections; occipital 12.80 ± 0.86 intersections) 120 μm from the soma, and apical dendritic complexity peaked around 200 μm from the soma (frontal 4.88 ± 0.50 intersections; occipital 4.10 ± 0.48 intersections).

Comparison to human supragranular pyramidal neurons

Elephant superficial pyramidal neurons were compared to supragranular pyramidal neuron data gathered with similar methodology in human inferior frontal (specifically, area 11, from Jacobs et al. 2001) and occipital regions (area 18, from Jacobs et al. 1997). These regions were chosen to be a relatively close topographic match for those examined in the elephant. The human supragranular pyramidal neurons



Cell A		Cell B		Cell C		Cell D	
Basilar		*Basilar*		*Basilar*		*Basilar*	
Vol	5,526.80	Vol	8,553.40	Vol	10,705.16	Vol	7,545.27
TDL	4,349.60	TDL	4,478.20	TDL	6,762.50	TDL	5,461.10
MSL	61.26	MSL	84.49	MSL	107.34	MSL	88.08
DSC	71.00	DSC	53.00	DSC	63.00	DSC	62.00
DSN	2,004.00	DSN	2,907.00	DSN	3,080.00	DSN	3,032.00
DSD	0.46	DSD	0.65	DSD	0.46	DSD	0.56
Apical		*Apical*					
Vol	6,286.73	Vol	13,189.63				
TDL	3,101.80	TDL	3,153.50				
MSL	114.88	MSL	165.97				
DSC	27.00	DSC	19.00				
DSN	1,864.00	DSN	2,536.00				
DSD	0.60	DSD	0.80				

Cell E		Cell F		Cell G		Cell H	
Basilar		*Basilar*		*Basilar*		*Basilar*	
Vol	10,362.15	Vol	5,820.19	Vol	4,429.22	Vol	4,038.70
TDL	4,221.20	TDL	2,629.10	TDL	3,123.60	TDL	2,935.80
MSL	111.08	MSL	114.31	MSL	80.09	MSL	104.85
DSC	38.00	DSC	23.00	DSC	39.00	DSC	28.00
DSN	2,804.00	DSN	1,470.00	DSN	2,102.00	DSN	1,816.00
DSD	0.66	DSD	0.56	DSD	0.67	DSD	0.62
Apical		*Apical*					
Vol	11,995.62	Vol	9,254.41				
TDL	2,128.90	TDL	2,795.10				
MSL	141.93	MSL	133.10				
DSC	15.00	DSC	21.00				
DSN	1,779.00	DSN	1,444.00				
DSD	0.84	DSD	0.52				

Fig. 12 Sample tracings of superficial pyramidal neurons, and their corresponding dendritic measurements, from frontal (cells *a–d*) and occipital (cells *e–h*) cortices. Neurons are presented to indicate their relative soma depths from the pial surface (in μm). Within each cortical area, the two neurons with relatively complete apical dendrites appear on the *left*. Note also the bifurcation of all apical dendrites. In general, frontal neurons appeared more dendritically complex than occipital neurons. *Scale bars* 100 μm

($n = 116$) were traced in 6 neurologically normal subjects (4 men, 23–69 years of age; 2 women, 32 and 34 years of age).

Dendritic length and segment count

On average, elephant superficial pyramidal neurons demonstrated only slightly higher TDL than human neurons both in frontal (by 6.8%) and in occipital (by 2.9%) cortices (Fig. 15a). However, TDL was manifested differently in humans and elephants, as revealed by MSL and DSC values. Basilar dendrites in the elephant exhibited greater MSL both in frontal (by 23.3%) and in occipital cortices (by 35.6%; Fig. 15b) than they did in humans. In contrast, elephant DSC was considerably lower than human DSC (by 16.0% in frontal cortex, and by 33.5% in occipital cortex; Fig. 15c).

Dendritic spines

Elephant superficial pyramidal neurons exhibited greater values for DSN and DSD than did human neurons. In elephant frontal cortex, basilar dendrites exhibited 63.6% higher DSN and 61.7% higher DSD than in humans. In elephant occipital cortex, basilar dendrites exhibited 52.3% higher DSN and 49.9% higher DSD than in humans.

Sholl analysis

Although Sholl analysis profiles were similar across both area 11 and area 18 in human, they varied considerably from those in the elephants (Fig. 14). On average, elephant basilar dendrites extended 760 μm from the soma in frontal cortex and 740 μm from the soma in occipital cortex. In contrast, human basilar dendrites extended 380 μm from the soma in the frontal cortex and 400 μm in occipital cortex. At the high point of dendritic intersections, human supragranular pyramidal neurons contained more intersections than did elephant superficial pyramidal neurons. In frontal cortex, human basilar dendrites peaked at 22.90 ± 0.90 intersections, whereas elephant dendrites peaked at 16.45 ± 0.99 intersections. In occipital cortex, human basilar dendrites peaked at 21.47 ± 0.65 intersections, whereas elephant dendrites peaked at 12.80 ± 0.86 intersections. Overall, the Sholl analysis profiles confirm that human supragranular pyramidal neurons were more condensed and contained more branch points than the elephant superficial pyramidal neurons.

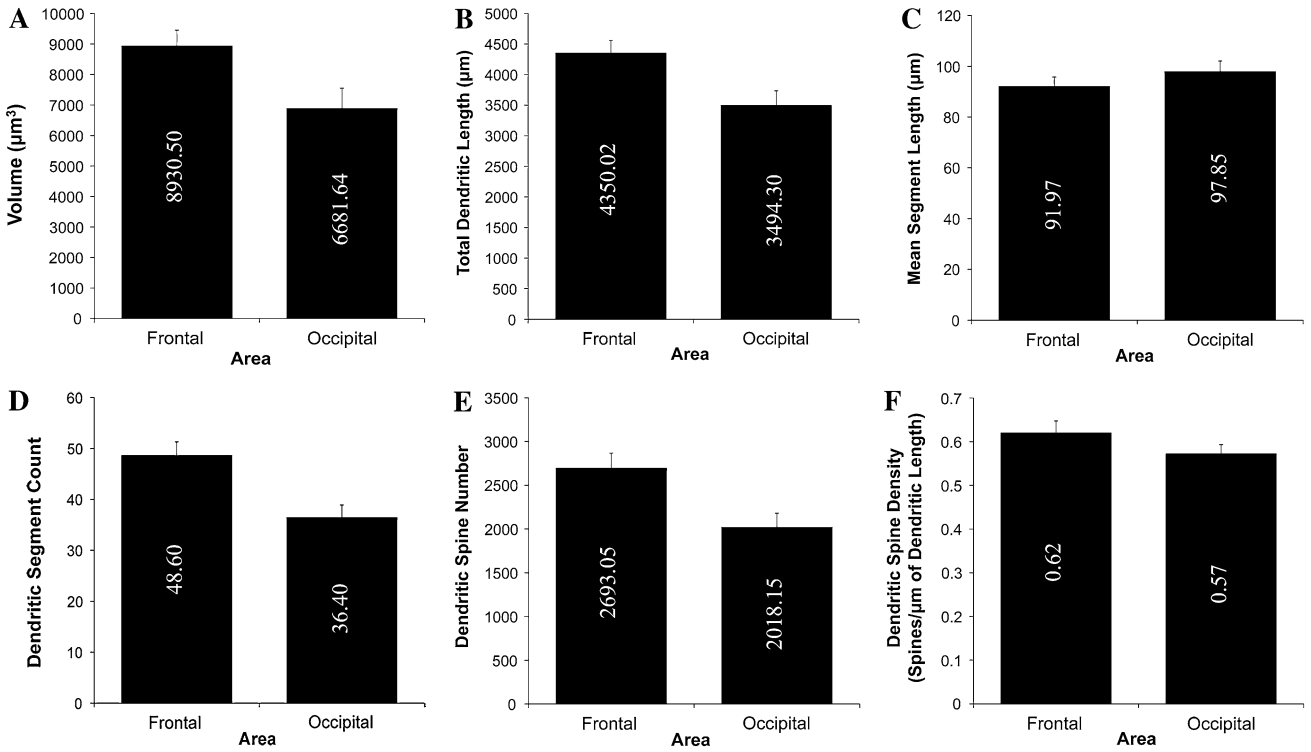


Fig. 13 Bar graphs of relative volume (**a**), total dendritic length (**b**), mean segment length (**c**), dendritic segment count (**d**), dendritic spine number (**e**), and dendritic spine density (**f**) of superficial pyramidal neuron basilar dendrites in elephant frontal and occipital cortex. The basilar dendrites in frontal cortex had significantly greater volume, TDL, DSC, DSC, and DSN than did those in occipital cortex. *Error bars* represent SEM

Discussion

The present study is the first to document neuronal morphology in the African elephant cortex. In terms of cytoarchitecture, the elephant neocortex is characterized by the presence of only five cortical layers as it altogether lacks a visible layer IV, which is quite different from what is observed in primates. Although it has not been studied in much detail to date, especially in terms of regional differences, we can make several generalizations. Layer I appears to be relatively thick and more cellular than in most terrestrial species. Layer II is clearly visible, densely packed, and displays cellular clustering in many cortical regions, particularly in the insula (Hakeem et al. 2009), as has been reported in cetaceans (Manger et al. 1998; Hof and Van der Gucht 2007). Layer II also contains occasional large pyramid-like neurons. Layer III is relatively thick and populated by large pyramidal neurons, the density and size of which clearly vary among different cortical domains; this fact deserves a detailed investigation. Layer V presents as a relatively thin row of very large pyramidal cells usually distributed in small groups, presumably representing principal efferent neurons, and a thicker deep portion containing smaller pyramidal cells. Layer VI is of variable thickness among cortical regions and contains a variety of pyramidal and non-pyramidal neurons as seen in many other mammalian species. This cortical lamination pattern with lack of an internal granular layer IV may reflect a particular organization of cortical connectivity in elephants, perhaps comparable to that in cetaceans, where agranularity is also a neocortical characteristic (Hof et al. 2005; Hof and Van der Gucht 2007). Although, we are currently exploring the cytoarchitecture of elephant cortex in detail, it remains clear that complex neocortical gyrification and distinct laminar organization represent remarkable features of the elephant brain.

In terms of neuromorphology, elephant cortex featured a great diversity of large, complex neurons, with considerable variety exhibited by "atypical" spiny neurons. A prominent characteristic in elephant cortex was the V-shaped arrangement of bifurcating apical dendrites. Quantitatively, the dendrites of superficial pyramidal neurons in the elephant frontal cortex were more complex than those in occipital cortex. In comparison to humans, elephant superficial pyramidal neurons exhibited similar overall basilar dendritic length, but individual dendrites tended to be longer in the elephant with less intricate branching. Before considering the implications of these findings, methodological issues must be addressed.

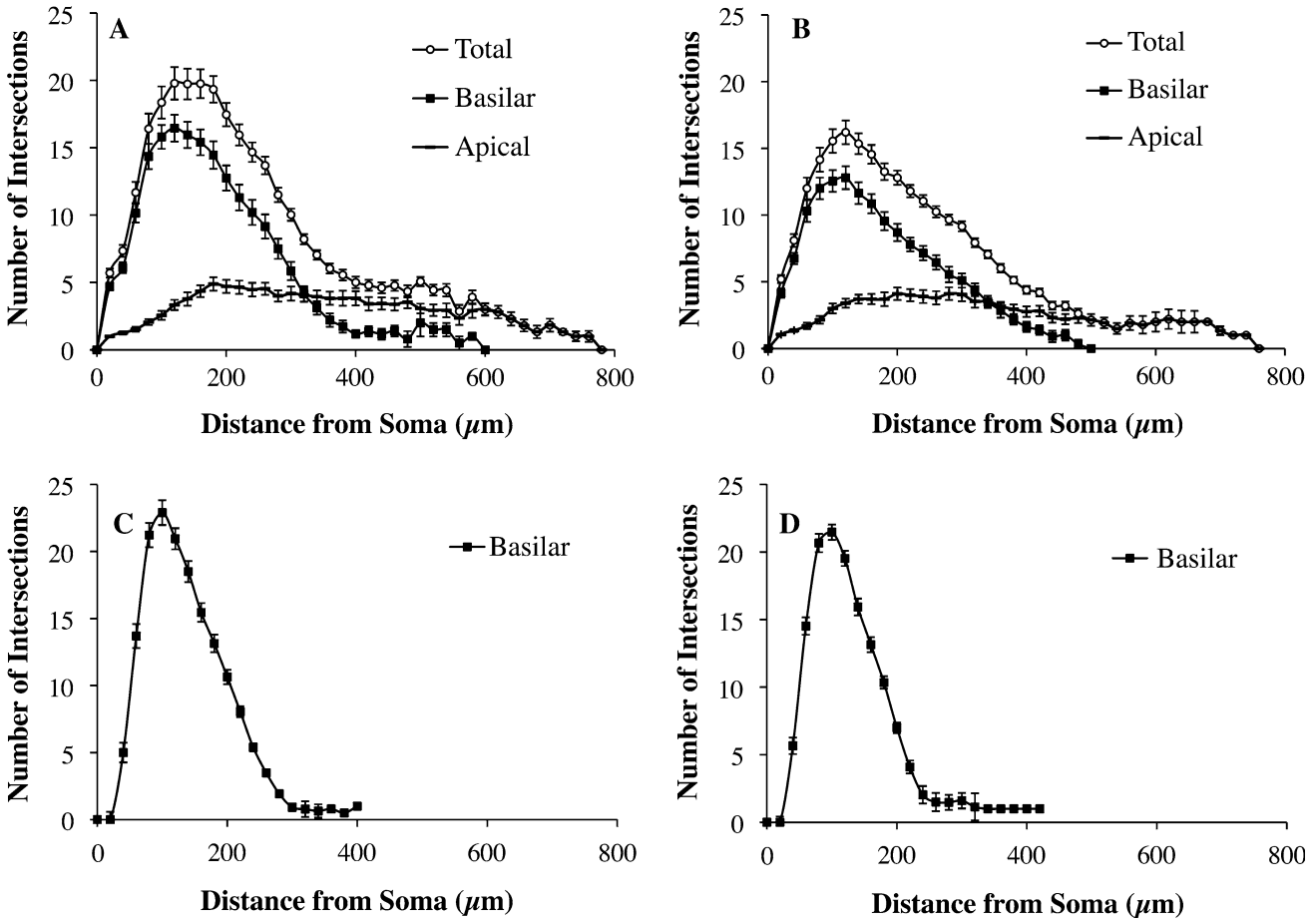


Fig. 14 Graphic representation of Sholl analysis results for elephant superficial and human supragranular pyramidal neurons, showing mean numbers of dendritic intersections in elephant frontal (**a**), elephant occipital (**b**), human frontal (**c**), and human occipital (**d**) cortices. Although dendritic extent between areas and species is similar, elephant dendrites are distributed across a greater distance from the soma and exhibit fewer intersections at the peak than observed in humans. *Error bars* represent SEM

Methodological considerations

In general, the same constraints that apply to Golgi-stained human tissue also apply here (Jacobs and Scheibel 2002): (1) a small sample size in terms of subjects and sampled neurons (Jacobs and Scheibel 1993); (2) lack of historical information on the subjects (Jacobs et al. 1993); (3) underestimation of spines in light microscopy (Horner and Arbuthnott 1991); and (4) inherent issues with Golgi impregnations (Braak and Braak 1985). These general limitations are accepted and we focus here on three broader issues.

Functional classification of elephant cortex and regional specialization

Functional characteristics of the sampled regions could not be determined. The frontal cortex, although located in what appear to be anterior orbital gyri, could subserve prefrontal executive functions (Barbas 1995) or, equally likely, premotor activities. The function of the occipital cortex is even more problematic, as this region could support visual or even auditory processes. As such, it is impossible to correlate dendritic/spine measure with the functional attributes of these regions, as has been done in primates (Elston and Rosa 1998a, b; Jacobs et al. 2001). Moreover, it is not possible to make definitive statements about the regional distribution of traced neurons in the present study despite observing that some neuron types appeared only in one cortical region.

Neuromorphological nomenclature

Unambiguously classifying neurons is problematic when the criteria are not clearly defined (Bota and Swanson 2007), are too restrictive (Germroth et al. 1989), or when cellular morphologies form a continuum rather than distinct categories, as may be the case for extraverted telencephalic neurons (Sanides and Sanides 1972; Ferrer and Perera 1988). Further classification issues emerge when definitions

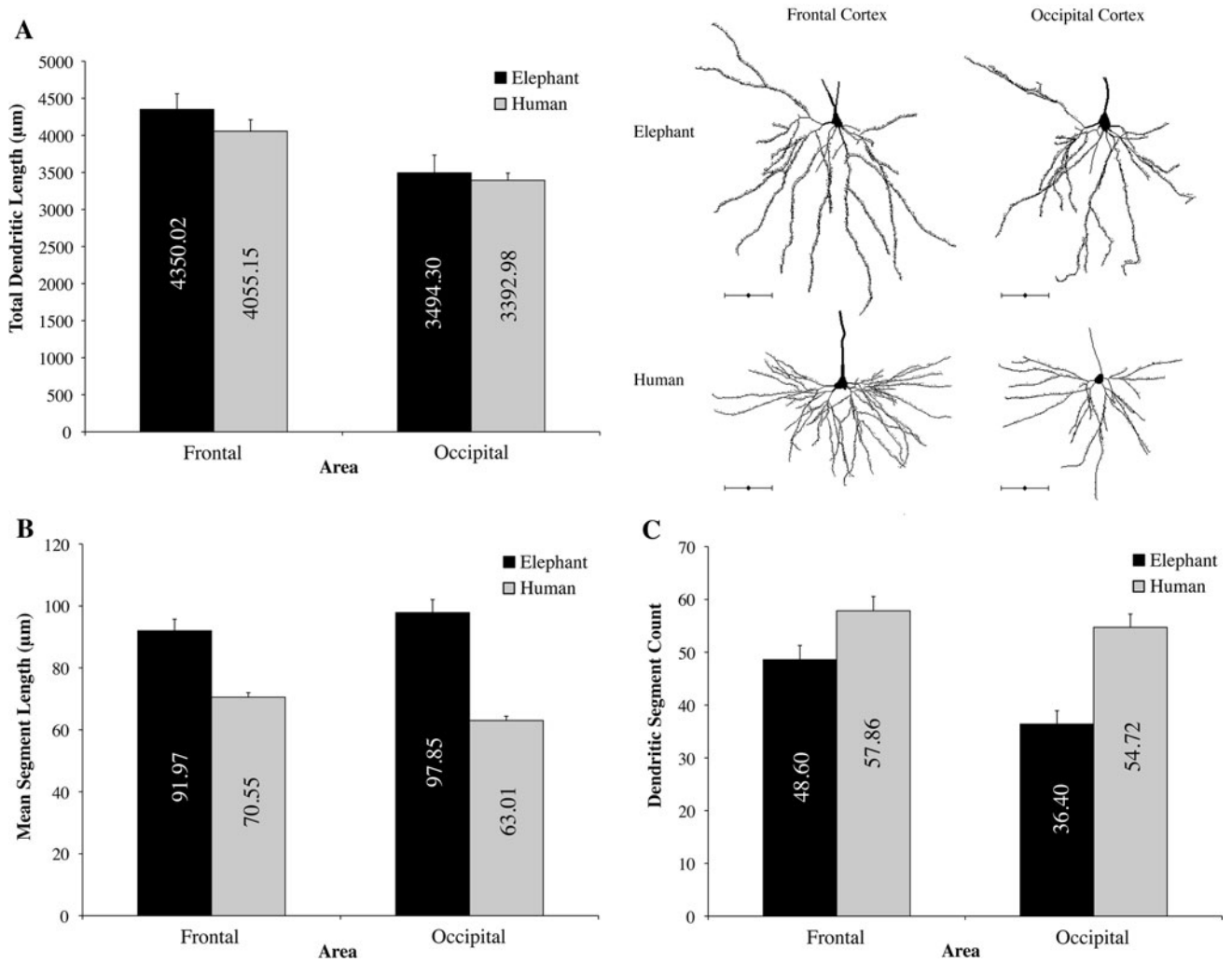


Fig. 15 Bar graphs displaying the differences in relative total dendritic length (**a**), mean segment length (**b**), and dendritic segment count (**c**) for basilar dendrites in elephant superficial pyramidal neurons and human supragranular pyramidal neurons across frontal and occipital cortex. Sample tracings of elephant superficial and human supragranular pyramidal neurons are also provided. Although elephant and human pyramidal neurons show similar TDL values, human neurons contain shorter segments and more intricate branching. All human data were obtained from Jacobs et al. (1997) for occipital cortex (specifically area 18), and Jacobs et al. (2001) for frontal cortex (specifically area 11). *Error bars* represent SEM

change over time, as typified by the term "pyramidal," which now applies to a much broader category of neurons than it did originally (Masland 2004). Compounding the classification problem is an essentially Euarchontoglires-centric nomenclature in the literature, whereby the (anthropoid) primate together with the (murid) rodent brain constitutes the template against which all other brains are compared, thus dictating what is "typical" versus "atypical" (Manger et al. 2008). In the present study, we have attempted to follow existing nomenclature where possible or, when appropriate, named cells based loosely on their dendritic morphology (e.g., crab-like neuron). Although we observed many examples of the presented neurons in our examination of the Golgi-stained sections, we only traced those that were relatively complete and unobscured, which limits our overall conclusions regarding neuronal types. Finally, the present study was also limited because it described neurons based only on somatodendritic measures.

Comparison of human and elephant dendritic/spine measures

With regard to dendritic measures, only portions captured in the 120-µm thick section could be compared. Because the basilar dendrites in elephants exhibited greater TDL than in humans, the observed TDL difference between the two species was probably attenuated (Jacobs et al. 1997). Nevertheless, sampling within an equal volumetric "slice" provides useful relative measures for comparison. With regard to spine measures, several issues prohibit a

meaningful interpretation of the relative DSN and DSD values. First, the human brains were immersion-fixed, whereas the elephant brains were perfusion-fixed, the latter revealing greater detail (i.e., spines; Morest and Morest 2005). Second, autolysis time in the humans was longer (~14 h) than in the elephants (~2 h), which potentially decreased the number of impregnated spines in the human samples (de Ruiter 1983). Finally, although data were collected in a methodologically similar manner, human neurons were traced using a Neurolucida Lucivid system, whereas elephant neurons were traced on a Neurolucida camera system. It was recently discovered that the higher magnification in the camera system increases the number of spines identified by about 17% (Anderson et al. 2010), clearly making accurate spine comparisons between the two species problematic.

Spiny neurons

The elephant cortex is characterized by an even greater variety of spiny neurons than the morphologically heterogeneous inferior temporal cortex of macaque monkeys and rats (Germroth et al. 1989; De Lima et al. 1990). Although several of these neuron types have been observed in other eutherian species, what remains noteworthy in the elephant is their arrangement, which represents a striking departure from the vertical apical dendrites and cortical columns that have been deemed the fundamental, if not canonical building block of the cerebral cortex (Mountcastle 1997; Innocenti and Vercelli 2010).

These spiny cortical neurons exist along a continuum from those that appear more pyramid-like to those that radically differ from the "typical" pyramidal neuron in terms of morphology and/or orientation. In the elephant, three neuron types, in addition to superficial pyramidal neurons, approximate general pyramidal neuron morphology: magnopyramidal, multiapical, and fork neurons. The only exact comparison point in the literature is the single Indian elephant neuron depicted in Barasa and Shochatovitz (1961), which is consistent with the present layer V magnopyramidal neurons. Although pyramidal neurons in the cat, dog, pig, and sheep resemble those in the elephant, the most striking similarities are found in the cow, horse, two-toed sloth, and anteaters (Barasa 1960; Ferrer et al. 1986b; Sherwood et al. 2009), which exhibit prominent, bifurcating apical dendrites much the same as those observed in elephant magnopyramidal and multiapical neurons. Unfortunately, size comparisons with cow and horse neurons are not possible because Barasa (1960) did not provide scale bars. The fork neuron has been observed in the insula and hippocampus of humans, chimpanzees, and orangutans (Ngowyang 1936; de Crinis 1934). Although originally considered a unique type of neuron, subsequent investigations in several mammals (e.g., primates, carnivores, ungulates, and rodents) have concluded that it is a type of enveloping neuron (or Umfassungszelle) and thus is merely a variant of pyramidal neurons (Juba 1934; Syring 1956). Finally, elephant magnopyramidal, multiapical, and fork neurons appear at least as dendritically complex as infragranular pyramidal neurons in the cat parietal cortex (Yamamoto et al. 1987), human cingulate, and motor cortices (Meyer 1987; Schlaug et al. 1993).

Within a more polymorphic subgroup of pyramidal neurons are the horizontal and inverted pyramidal neurons (Ramón y Cajal 1891; Van der Loos 1965). In terms of morphology, these are similar to magnopyramidal and multiapical neurons, but are slightly smaller in dendritic extent and differ in their specific orientations. Although horizontal pyramidal neurons have typically been noted in superficial and deep cortical layers in primates and rodents (Meyer 1987; Miller 1988), elephant horizontal pyramidal neurons were located in layer III. These strongly resembled one variant of the asymmetrical pyramidal neurons in inferior temporal cortex (Fig. 8e of De Lima et al. 1990), although one could argue that what De Lima et al. refer to as a prominent, laterally oriented, basilar dendrite is actually the apical dendrite. The elephant variant appears to be a more dendritically complex version than what has been noted in layer VI of both lissencephalic and gyrencephalic brains (Ferrer et al. 1986a, b, 1987). Although the function of these neurons remains unclear, it is possible that they contribute to the lateral integration of synaptic input (Van Brederode et al. 2000), which has been suggested as a major step in neocortical evolution (Ferrer et al. 1986b). Much the same as horizontal pyramidal neurons, elephant inverted pyramidal neurons were located in layer III. In human temporal cortex, they have been documented in layer V (Ong and Garey 1990). In chimpanzees, inverted pyramidal neurons are located in layers III, V and especially VI of sensorimotor cortices, constituting a small percentage (<1%) of all pyramidal neurons (Qi et al. 1999). In rats, rabbits, cats, sheep, sloths, anteaters, rock hyrax, and elephant shrews, they also tend to be located in infragranular layers and constitute a small (1–8.5%) percentage of all neurons in the cortex (Parnavelas et al. 1977; Mendizabal-Zubiaga et al. 2007; Sherwood et al. 2009). These excitatory neurons have been shown to project to ipsi- and contra-lateral cortical regions, as well as to the claustrum and striatum (Bueno-López et al. 1991; Mendizabal-Zubiaga et al. 2007). In the elephant, however, the extent to which inverted pyramidal neurons share connectional and/or functional attributes with their counterparts in other species remains unclear.

One type of elephant neuron that does not have a clear counterpart in the literature is the magnopyramidal-taproot or "matriarch" neuron of the frontal cortex. Although they

resemble, because of the descending taproot, some of the layer VI vertical fusiform projection and asymmetric pyramidal neurons described in macaque inferior temporal gyrus (De Lima et al. 1990) and some of the layer VI pyramidal neurons in human motor cortex (Meyer 1987), they exhibited a much more extensive dendritic system. Indeed, these were among the most complex neurons in the present sample, approaching the dendritic extent of human Betz or Meynert neurons. Although morphologically very idiosyncratic, some Betz cells, like matriarch neurons, possess a bifurcating apical shaft with branches that spread laterally (Braak and Braak 1976), and may also exhibit a long, descending taproot dendrite (Scheibel and Scheibel 1978a). However, unlike Betz cells, matriarch neurons were consistently very spiny along both thin and thick dendrites, including the taproot. Although the function of matriarch neurons remains unknown, they may represent extreme adaptations of pyramidal neurons, much the same as other large cortical neurons in primates (e.g., Betz, Meynert, and von Economo's spindle cells; Nimchinsky et al. 1995; Sherwood et al. 2003; Wittenberg and Wang 2007). Their morphology and potential regional localization in the frontal cortex suggests that, like Betz cells, matriarch neurons may sample a wide range of cortical input and exert modulatory field effects in the surrounding neuropil, potentially contributing to associative cognitive processes (Kaiserman-Abramof and Peters 1972; Scheibel and Scheibel 1978a).

Among the more "atypical" spiny neurons in elephant cortex were the horizontally oriented crab-like neurons and the flattened pyramidal neurons. One could also describe the three crab-like neurons as horizontally oriented bitufted neurons; similarly, one cannot rule out that the deeper of these, in layer VI of occipital cortex (Fig. 6O), may even have been an unusual, apical-less variant of the solitary cell of Meynert or of the Meynert–Cajal neuron (von Economo and Koskinas 1925). Both the crab-like and flattened pyramidal neurons appear to be much larger variants of neurons described in the dog and sheep as pyramidal neurons with multiple horizontal collaterals or horizontal pyramidal neurons (Figs. 6 and 9 of Ferrer et al. 1986b). The flattened pyramidal neuron is also strongly reminiscent of the large, widely bifurcating neuron observed in the cow (Fig. 7 in Barasa 1960), but with a more pronounced lateral extension of the apical dendrites (up to 1 mm, Fig. 6M). The horizontal nature of these two neuronal types may argue against a strict vertical, columnar arrangement of cortex (Lübke et al. 2000, 2003), and again suggests a role for lateral integration of information, perhaps related to the low density of elephant cortical neurons (Hart et al. 2008). What remains unclear, however, is why flattened pyramidal neurons appeared to be more prominent in the occipital cortex.

Aspiny neurons

Classification of aspiny interneurons typically incorporates axonal morphology (Lund and Lewis 1993; Petilla Interneuron Nomenclature Group 2008; Druga 2009), which can be further supplemented with electrophysiological and molecular properties (DeFelipe 1997; Zeitsev et al. 2009). Aspiny interneurons in the present study, however, could only be described according to their general somatodendritic characteristics because axonal arbors were not revealed by the Golgi stain. Despite this limitation, it appeared that frontal aspiny neurons exhibited greater dendritic spread than did those in occipital cortex. Apart from this size difference, all elephant aspiny interneurons could be categorized as either multipolar, bipolar, or neurogliaform neurons. With the exception of one aspiny interneuron (Fig. 7G), which had vertically oriented dendritic branches, all other aspiny interneurons contained a sparse number of laterally or radially extending dendrites.

Dendritic spread in elephant aspiny interneurons, at least in frontal cortex (with a radius of up to 1,000 μm from the soma) was extensive. Comparatively, dendritic radii for local circuit neurons have been reported to be approximately 50–400 μm in the rat (Kawaguchi 1995) and the cat (Peters and Regidor 1981; Somogyi et al. 1983), 100–300 μm in the striped dolphin (*Stenella coeruleoalba*, Ferrer and Perera 1988), 100–400 μm in the echidna (Hassiotis and Ashwell 2003), and up to 500 μm in macaque monkey (Lund and Lewis 1993) and humans (Kisvárday et al. 1990), although aspiny interneurons in human motor cortex have been reported with radii of up to 800 μm (Meyer 1987). Similarly, the elephant's neurogliaform neuron was morphologically similar to what has been reported in other species, but was approximately 2–3 times larger in terms of its dendritic field (Jones 1984; Povysheva et al. 2007). Thus, although aspiny interneuron morphology appears to be highly conserved among eutherian mammals (Ferrer 1986; Sherwood et al. 2009) and in the monotreme echidna (Hassiotis et al. 2005), the elephant variants, at least in the frontal lobe, appear to be relatively large in terms of dendritic extent. This large size may be required for these interneurons to exert the widespread inhibitory influences required to shape the temporal flow of information during cortical processing (Constantinidis et al. 2002).

Elephant superficial pyramidal neurons and human supragranular pyramidal neurons

Among the most spiny of cortical neurons in the elephant, superficial pyramidal neurons were "atypical" insofar as they possessed an apical dendrite with a primary bifurcation at or near the soma, resulting in two obliquely

ascending secondary branches. In the literature, there appears to be little agreement on whether these bifurcating apical branches are characteristic of bitufted (Bartesaghi et al. 2003), multiapical (Ferrer et al. 1986a, b), or even extraverted pyramidal neurons (Fitzpatrick and Henson 1994). Although resembling layer II extraverted pyramidal neurons in several other species (hedgehog, bat, and opossum: Sanides and Sanides 1972; dolphin: Glezer and Morgane 1990; quokka: Tyler et al. 1998; humpback whale: Hof and Van der Gucht 2007; giant elephant shrew, giant and lesser anteater: Sherwood et al. 2009), elephant superficial pyramidal neurons did not meet the strict definition of extraversion, whereby apical dendritic extent exceeds that of basilar dendrites (Sanides and Sanides 1972). In general, the elephant superficial pyramidal neurons possessed a very well-developed basilar skirt, the TDL of which exceeded that of the apical dendrite in 35 of the 40 traced neurons. Because the basilar array is considered to be the most progressive feature of the pyramidal neuron (Sanides and Sanides 1972), it is possible that elephant superficial pyramidal neurons represent an intermediary between extraverted neurons and "typical" primate pyramidal neurons. This could be an adaptation that accompanies a large brain as an extensive basilar skirt could compensate for the relatively low neuron density in elephant cerebral cortex (Tower 1954) by increasing the neuron's receptive surface area.

Similarly, the bifurcating apical dendrite greatly increases the lateral spread of apical branches and could represent an adaptation to the cytoarchitecture of the elephant neocortex which, as in the hedgehog (Valverde and Facal-Valverde 1986), bat (Ferrer 1987), and cetaceans (Garey et al. 1985), does not contain a developed layer IV. Such laminar organization is associated with an increase in thalamic inputs into the molecular layer over the deeper layers (Ferrer and Perera 1988; Glezer and Morgane 1990). Bifurcating apical dendrites formed V-shaped apical bundles, which is consistent with the description of small-medium pyramidal cells in cows and horses (Barasa 1960), but differs substantially from the canonical vertical apical bundle-type architecture that typifies primate and rodent neocortex (Fleischhauer et al. 1972). The resulting apical dendritic bundling, variations of which have been observed in other species (cat: Fleischhauer 1974; rat: Wyss et al. 1990; tenrec, red-eared pond turtle: Schmolke and Künzle 1997; echidna: Hassiotis et al. 2003, 2005), raises interesting questions regarding neocortical computation in cognitively sophisticated species such as the elephant. Although dendritic bundles appear to help synchronize signals involved in cortical processing (Scheibel and Scheibel 1975; Roney et al. 1979; Geisler et al. 2000), their exact functions remain elusive. It is possible that this V-shaped arrangement represents a variation of the vertical column or serves to facilitate communication across wider domains of cortex, either independent of columns or between columns that are more widely spaced than in other species, as has been noted in whales (Hof and Van der Gucht 2007).

The regional differences observed in elephant superficial pyramidal neurons, with frontal neurons exhibiting more complex dendritic systems than occipital neurons, are generally consistent with findings in human supragranular pyramidal neurons, where basilar dendrites in area 10 were found to be more complex than those in area 18 (Jacobs et al. 1997), and where greater dendritic/spine complexity has been documented in heteromodal (areas 6β, 39) and supramodal (areas 10, 11) cortices than in primary (areas 3-1-2, 4) and unimodal (areas 22, 44) cortices (Jacobs et al. 2001). Nevertheless, further research is required in the elephant to determine if there are progressive increases in basilar dendritic complexity along hierarchically arranged pathways, as has been demonstrated in non-human primates (Elston et al. 1996; Elston and Rosa 1997, 1998a, b), or if regional differences in basilar dendritic extent are due to intrinsic properties within each cortical region, as appears to be the case in the mouse (Benavides-Piccione et al. 2006).

Finally, although TDL was similar between elephants and humans, there was a difference in how this overall length was distributed within the neuron. Human supragranular pyramidal neurons appeared smaller and denser, with more complex branching patterns, whereas elephant superficial pyramidal neurons appeared larger and more expansive in terms of dendritic spread. Thus, although the elephant brain is $\sim$3 times larger than the human brain, elephant cortical neurons are not simply "scaled up" versions of a general mammalian type. As such, simple models of neuron scaling and computations across phylogeny may be problematic (Wittenberg and Wang 2007). Each species has its own phylogenetic heritage and functional design principles (Harrison et al. 2002). Elephant neuromorphology may reflect a particular phylogenetic heritage and/or may be associated with the lower cell packing density in the cortex. Indeed, the elephant cortex appears to have a lower neuronal density than dolphins and primates (Haug 1987; Lyck et al. 2009). These data, combined with calculations of total volume of cortex dedicated to somatic processing (Hofman 1982), suggest that elephants have approximately 10 billion non-somatic neurons spread across a large area of neocortex, compared to 6.5 billion tightly packed neurons for chimpanzees and 20 billion tightly packed neurons for humans (Hart et al. 2008). As such, it has been suggested that elephant neocortex may favor long-distance connections over the more compartmentalized primate model, a possibility tentatively supported by the longer basilar dendritic systems documented here.

Evolutionary and cognitive considerations

The present results confirm that the African elephant cerebral cortex contains a rich variety of large neurons with expansive, spine-rich dendritic systems and, presumably, long axonal projections to interconnect these relatively sparsely packed neurons (Wang et al. 2008). These findings reinforce the high metabolic expense of large brains (Grande 1980), and appear consistent with evidence in the elephant neocortex for high glia-neuron ratios (Haug 1987), high neocortical white–gray matter ratios (Zhang and Sejnowski 2000), and evolutionary selection on aerobic energy metabolism genes (Goodman et al. 2009). Reasons for the extensive dendritic spread of elephant neurons, as opposed to the more compact, branched primate model, remain to be explained.

Perhaps, the most distinguishing characteristic of the elephant cortex is the presence of widely bifurcating apical dendrites that branch close to the soma. This seems consistent with the closest living relatives of elephants, including other afrotherians and especially xenarthrans (e.g., the giant anteater), but contrasts with findings in the rock hyrax, where pyramidal neurons have single apical dendrites resembling those in rodents and primates (Sherwood et al. 2009; Bianchi et al, in review). Variations of bifurcating apical dendrites also appear in cetaceans (Ferrer and Perera 1988; Hof et al. 1992). More generally, bifurcating apical branches appear in caniforms and perissodactyls (Barasa 1960), which is consistent with the close association of odd-toed ungulates and carnivores, and the possible assemblage of carnivores and cetartiodactyls together into superclade Ferungulata (Hou et al. 2009). Thus, it is possible that bifurcating apical dendrites may be a feature of animals with large bodies/brains, but to the general exclusion of primates due to their position within the euarchontoglire lineage. This raises the interesting possibility that geometric effects on neocortical neuron scaling are constrained by phylogenetic history.

Although the arrangement and morphology of cortical neurons crucially interact with an elephant's cognitive and sociobehavioral repertoire, the relationship between cortical architecture and cognition remains unclear. The presence of V-shaped apical bundles, high synaptic density, and neurons with a wide, horizontal dendritic spread collectively illustrate that neocortical neurons in elephants are designed to synthesize a large number of diverse inputs. Nevertheless, much more research on elephant neocortex is required before any functional conclusions can be supported. Certainly, the elephant's integrative cortical circuitry seems consistent with its complex spatial and social abilities, as well as its behaviors indicating empathy and theory-of-mind. These cortical characteristics may also contribute to the elephant's ability to synthesize a variety of information over an extended period of time. In conclusion, although the present study provides an initial window into the complex neuromorphology of the elephant cortex, it also highlights the need for more comparative research to elucidate the multiplicity of evolutionary options for constructing large, elaborate brains.

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