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Recursive Trace Line Method for Detecting Myelinated Bundles: a Comparison Study with Pyramidal Cell Arrays

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Abstract

Minicolumns are thought to be the smallest cortical modules within the hierarchical organization of the isocortex. Several reports suggest alterations in minicolumnar morphometry may be involved in psychiatric disorders such as autism, dyslexia, and schizophrenia. Thus far anatomical studies of minicolumns have primarily relied on measurements of pyramidal cell arrays. This study expands on a recursive trace line segmentation method used to define morphometric measures for myelinated axon bundles. The results were compared against those of pyramidal cell arrays derived from immediately adjacent serial sections. Width estimates based on cell somas and myelinated axon bundles were highly correlated ($r = 0.9888$). Histograms of signal intensity using the recursive trace line method produced expected features of myeloarchitectonics; that is, bundles of Meynert and intervening interradiary plexus. The close correspondence of derived values for myelinated axon bundles and pyramidal cell arrays suggests their participation and interaction within the same modular arrangement of the isocortex.

Keywords

Computerized image analysis; Minicolumns; Myelin; Neocortex; Pyramidal cells

Introduction

Morphological studies spanning the last 150 years have helped define the remarkable topographical organization of the brain's isocortex. Early studies by Campbell, Brodmann, and von Economo and Koskinas focused on the distribution and arrangement of individual cells (cytoarchitectonics) (Campbell 1905; Brodmann 1909; Von Economo and Koskinas 1925). However, the complexity of cellular arrangements prompted some investigators to question the distinctive cytoarchitectonic nature of previously described homogeneous areas. After extensive parcellation studies in several species Bailey and von Bonin concluded that vast areas

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of isocortex were so closely similar that any attempts at subdividing the same were unprofitable, if not impossible (Bailey and von Bonin 1951). Similarly, independent cytoarchitectural parcellation studies by Lashley and Clark in the brain of two spider monkeys bore little agreement to each other (Lashley and Clark 1946). There was a large amount of individual variability among different brain regions and areas of the brain apparent in one specimen were not evident in the other specimen. Lashley and Clark emphasized the need to corroborate cytoarchitectural fields with other methods, notably those based on fiber connectivity (Lashley and Clark 1946). The subjective approach of these early investigators is now incorporated into multiple parcellation schemes comprising networks of hundreds of different brain regions. According to Daly, "For the moment, (cyto)architectural classification leaves many unresolved problems. Some inevitably result from distortions brought on living cells by fixation and staining. Others result from the subjectivity of the observer's eye in trying to classify cellular patterns, a problem that may yield to the use of computerized techniques of pattern recognition" (Daly 1976).

Recent applications of computer imaging methods has allowed us to analyze a component of isocortical anatomy, more specifically, radially oriented pyramidal cell arrays. These structures, together with bundles of efferent myelinated axons, apical dendrites, and double bouquet cells, are found in a broad range of mammalian species (Douglas and Martin 2004; Buxhoeveden and Casanova 2005). This composite cell and fiber structure, termed the cortical cell minicolumn by Mountcastle has been proposed as the elemental functional microcircuit of neocortex (Mountcastle 1978). Each of its constituent components has been shown to exhibit minicolumnar-scale periodicity in various species and cortical areas (von Bonin and Mehler 1971; Seldon 1981; Seldon 1981; DeFelipe, Hendry et al. 1990; Ong and Garey 1990; Peters and Sethares 1991; Peters and Sethares 1991; del Rio and DeFelipe 1997; Peters, Cifuentes et al. 1997). This suggests that they are part of a general organizing motif of the isocortex in primates.

The present study provides computerized image analysis of myelinated axon bundles which addresses the biases and limitations of cytoarchitectural techniques. Myelinated axon bundles, and the space between them, frame columns of cells and all of the afferent, efferent and interneuronal fiber systems of the minicolumn. Myelin staining techniques label axonal fibers in a consistent and reproducible manner which is not subject to post-agonal and processing artifacts affecting other labeling methods (Chan and Lowe 2002). An additional advantage of the method is that myelinated axon bundles do not bifurcate. All fibers within a given bundle arise from cells in their associated pyramidal cell column thus providing a one-to-one correspondence between these cytoarchitectural features. In contrast, apical dendrites occasionally branch and aggregate into bundles arising from several neighboring cell columns (Rockland 2002).

Materials and methods

We examined 58 pairs of serial sections from several brain regions in a postmortem sample of 16 human patients (Table 1). All patients belonged to a normative aging series and were free from neuropathological changes. Tissue was stored at the Yakovlev-Haleem collection, Armed Forces Institute of Pathology, Washington, D.C. Each brain was celloidin-embedded and cut into 35 μm sections. One section out of every 10 to 25 slices was stained using the Loyez technique, and the section immediately following was stained with cresyl violet (Figure 1).

Photomicrographs of corresponding locations in serial sections were obtained using a Nikon E1000M microscope with 4 $\times$ objective and a DXM1200 digital camera, for an effective sampling frequency of 1.175 pixels per μm . Images were corrected for uneven illumination prior to analysis. In each photomicrograph of Nissl-stained tissue, lamina V was outlined by

hand, and minicolumnar width CW was estimated in this region of interest using established methods (Casanova and Switala 2005). Minicolumnar width was also estimated in the adjacent Loyez-stained section using the methods described below.

An image I of Loyez stained tissue can be segmented into roughly four classes: the white matter W , blood vessels V , myelinated axons A extending through the grey matter, and background G , i.e.

$$I = W \cup V \cup A \cup G.$$

The first two classes have nearly identical grey level histograms and thus can not be distinguished with simple thresholding. The first step is to separate myelinated axons (and background) from the rest of an image using Otsu's (1979) algorithm (Otsu 1979). The resulting set of pixels I_1 includes parts of blood vessels, whose boundaries are smoother than those of axons.

Applying Otsu's method a second time to the set I_1 removes the pixels in V , leaving

$$I_2 = A \cup G,$$

all other pixels being set to zero.

Spacing between bundles of myelinated axons is measured on profiles of I_2 , parallel to the white matter border, roughly perpendicular to the orientation of axon bundles. The white matter W belongs to the complement of I_2 , and can be easily identified as the largest connected subset thereof (Figure 2). The boundary ∂W of W thus identified may be quite irregular. This is dealt with in one of two ways depending on whether the cortex in the region of interest is convex (gyral) or concave (sulcal) (Figure 3). In either case morphological opening with a circular structuring element approximately 20 μm in diameter smooths the boundary somewhat. Then in the gyral case, the convex hull of the smoothed boundary is taken to represent the white matter surface. In the sulcal case, the white matter surface is a cubic curve, least-squares fit to the smoothed boundary.

Profiles of I_2 are then made along level sets of the Euclidean distance map of the computed white matter boundary. These are the point sets $L(r)$ such that

$$\min_{y \in \partial W} d(\vec{x}, \vec{y}) = r \text{ for all } \vec{x} \in L,$$

where $d(a,b)$ is the Euclidean distance. Profiles are smoothed using a kernel function with the bandwidth chosen to satisfy the GCV criterion (Craven and Wahba 1979). The spacing between peaks in the resulting curve provides an estimate of the width of minicolumns as identified by the bundles of myelinated axons through their cores.

Results

Width estimates based on cell somata (CW_S) and myelinated axon bundles (CW_A) are highly correlated with $r = 0.9888$ (Figure 4); this is significant with a lower 95 % confidence limit $r > 0.985$. The linear relationship between the two measurements was estimated as $CW_A = 0.988 CW_S + 0.040 \mu\text{m}$, using orthogonal distance regression. Bootstrap confidence intervals on the slope m and intercept b were $0.9459 < m < 1.0353$ and $0.1410 \mu\text{m} < b < 0.2184 \mu\text{m}$, respectively.

CW_A and CW_S varied with respect to cortical area as revealed by multivariate, repeated measures ANOVA ($F_{10,72} = 2.09$; $P = 0.0363$). Predicted mean widths ranged from $CW_A =$

23.0 μm for area 21 to $CW_A = 28.3 \mu\text{m}$ for area 9. The standard deviation in CW_A pooled across all cortical areas was 3.33 μm . Results for CW_S are essentially the same.

Discussion

Histograms of signal intensity using the recursive trace line method defined expected features of myeloarchitectonics: long streams of vertical radiations denoted as bundles of Meynert (Campbell 1905) and the intervening interradiary plexus described by Edinger (Jones 1984). Furthermore, our results show correspondence for distances between myelinated bundles and those for pyramidal cell arrays. The findings recapitulate previous observations that “the radial arrangement of the myeloarchitecture in many cortical regions often mirrors the suggestion of columnar arrangements better than Nissl preparations” (von Bonin and Mehler 1971).

The recursive line trace algorithm employed in this study is a novel method for quantifying neocortical radial morphometry. Our results are consistent with and complementary to those obtained by previously-employed techniques for imaging pyramidal cell arrays. The close correspondence between measures of myelinated axon bundles and pyramidal cell arrays suggests their presence and interaction within a unifying anatomical structure of vertical cylinders which Lorente de No (1938) designated as an elementary unit of cortical organization (de No 1938). The presence of these closely interacting vertical components lends credence to the supposition of the minicolumn as a canonical architectonic feature of the neocortex.

Pyramidal cell arrays, myelinated axon bundles, dendritic bundles, and double bouquet cells offer complementary information regarding the compartmentalization of the minicolumn (DeFelipe, Hendry et al. 1990; Lohmann and Koppen 1995; Casanova and Switala 2005) and help define the radial organization of the neocortex. Differences in the characteristics of these constituent minicolumnar compartments are exhibited across species (Schmolke and Künzle 1997; Hof, Duan et al. 2002; Yáñez, Muñoz et al. 2005) and cytoarchitectonic areas (Skoglund, Pascher et al. 1996; Peters, Cifuentes et al. 1997; Yáñez, Muñoz et al. 2005; DeFelipe, Ballesteros-Yanez et al. 2006).

Peters and Sethares were the first to characterize the spatial relation of pyramidal cells in layers III and V with the radially oriented translaminal dendritic bundles arising from them (Peters and Sethares 1996). They named these assemblages “pyramidal cell modules.” Lohmann and Koppen further demonstrated in rat visual cortex that apical dendritic and myelinated axon bundles project in register with each other at minicolumnar-scale intervals (52.6 μm and 50.1 μm , respectively) (Lohmann and Koppen 1995). The reported scale dimensions are consistent with other studies (Buxhoeveden and Casanova 2002). Double-bouquet axons in peripheral neuropil similarly were found to align with myelinated axon bundles. This pattern of alignment and of double-bouquet synaptic contacts with pyramidal cells was found to be similar in human temporal and macaque visual cortex (del Rio and DeFelipe 1997). A close association has therefore been demonstrated for all four constituent anatomical elements, the spacing of each providing equivalent information concerning minicolumnar width. However, methodological limitations (see below) have prevented the widespread use of pyramidal cell arrays, apical dendritic bundles and double bouquet cells in studies of cortical modularity.

Among the different features establishing the radial organization of the isocortex, pyramidal cell arrays are probably the most difficult to validate anatomically. Pyramidal cells are intermixed with smaller neurons many of which lack a primary longitudinal axis. Similarly, cell arrays are derived from the ontogenetic minicolumn and are therefore one cell-wide (Casanova, Trippe et al. 2007). Previous column detection routines used this assumption and took the separation between minicolumns as being of the same order of magnitude as the separation between cell soma (Buxhoeveden, Switala et al. 2000). While this assumption may

hold true for columns in laminae II and III, it does not hold for those laminae where granular or stellate neurons abound, i.e., lamina IV. Cellular fields in this lamina tend to be over segmented into fragments that are narrower than true anatomical minicolumns. The difficulty in correcting this artifact lies in the nature of the raw data. In this case, the separation between two cells in adjacent, parallel columns may be indistinguishable from the separation between cells within the same column. Furthermore, in rodent and other non-primate mammalian species, reduction in peripheral neuropil of tangentially-oriented collateral dendritic processes as well as number and complexity of interneurons is associated with increased packing density of pyramidal cell columns. Consequently, visualization and morphometric analysis of these structures is limited in Nissl-stained tissue.

Apical dendritic bundles are well visualized with antibodies to microtubule-associated proteins (MAP2s) which are enriched in dendritic processes. These radially-oriented structures have been well characterized in visual cortex of rodents, cats, rabbits and monkeys (Peters and Kara 1987; Peters and Sethares 1991; Peters and Sethares 1991; White and Peters 1993; Peters and Sethares 1996; Peters, Cifuentes et al. 1997). In human medial prefrontal cortex, apical dendritic bundles were shown to incorporate dendritic fascicles arising from radially aligned chains of pyramidal cells in layer V (Gabbott 2003). While equivalent in density to other minicolumnar elements, these structures may serve to integrate and coordinate input to several neighboring radial cell columns. In fact, the structure of those bundles varies according to cytoarchitectonic area (Viebahn 1990; Peters, Cifuentes et al. 1997; Skoglund, Pascher et al. 2004) and among species (Schmolke and Künzle 1997). Increased branching complexity and spine density of basal dendritic arbors in the peripheral neuropil of human prefrontal cortex in comparison with that of old and new world monkeys suggests that altered morphometry of this compartment in humans results in increased integration of inputs among minicolumns. This difference in the morphometry of the apical dendritic compartment may provide the basis for enhanced human cognition (Elston, Benavides-Piccione et al. 2001). Unfortunately, MAP2 immunocytochemistry does not stain smaller dendritic processes as effectively (Peters and Sethares 1996) and does not provide a high degree of quantitative consistency across samples. This limits its utility as an independent parameter for assessing minicolumnar morphometry.

The double bouquet cell has been identified as a characteristic feature of the minicolumnar peripheral neuropil compartment in monkeys and humans (DeFelipe, Hendry et al. 1990). Antibody labeling for the calcium binding protein calbindin reveals a regular periodic distribution throughout cortex (Yáñez, Muñoz et al. 2005). Quantitative analysis of tissue labeled with this method is therefore subject to the same technical limitations as for antibody-labeled dendritic bundles. Moreover, double bouquet cells have not been identified in large numbers in non-primate species other than in visual cortex of carnivores (Yáñez, Muñoz et al. 2005) and therefore cannot provide a basis for cross-species comparisons of minicolumnar morphometry. In primates, double bouquet cells are not apposed to every pyramidal cell column and therefore cannot be presumed to be an obligate component of a putative minicolumnar microcircuit (Yáñez, Muñoz et al. 2005).

The spacing between myelinated fiber bundles provides an alternative to the scale estimate method employed in measuring distances between pyramidal cell arrays (Buxhoeveden, Switala et al. 2000). While bundles of axons appear to vary in thickness as they traverse laminae, they do not bifurcate. Thus, regardless of the cortical depth at which an observation is made, each bundle of myelinated fibers corresponds to one minicolumn. Furthermore, since collections of myelinated axons extend continuously throughout minicolumns (Peters and Sethares 1996) any fragmentation apparent from a myelin-stained field must result from the plane of cut. Thus, the distribution of myelin bundle lengths yields estimates of the effects of the plane of cut on minicolumns defined by cell somas in serial sections.

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References

- Bailey, P.; von Bonin, G. *The Neocortex of Man*. Urbana, IL: University of Illinois Press; 1951.
- Brodmann, K. *Vergleichende Lokalisationslehre der Großhirnrinde*. Leipzig: 1909.
- Buxhoeveden D, Switala A, et al. Quantitative analysis of cell columns in the cerebral cortex. *J Neurosci Methods* 2000;97(1):7–17. [PubMed: 10771070]
- Buxhoeveden DP, Casanova MF. The minicolumn hypothesis in neuroscience. *Brain* 2002;125(5):935–951. [PubMed: 11960884]
- Buxhoeveden, DP.; Casanova, MF. *The cell column in comparative anatomy*. New York, NY: Nova Science Publishers, Inc; 2005.
- Campbell, AW. *Histological Studies on the Localization of Cerebral Function*. Cambridge: Cambridge University Press; 1905.
- Casanova, M.; Switala, AE. Minicolumnar Morphometry: Computerized Image Analysis. In: Casanova, MF., editor. *Neocortical Modularity and the Cell Minicolumns*. New York, NY: Nova Science Publishers, Inc; 2005. p. 161-180.
- Casanova MF, Trippe J II, et al. A Temporal Continuity to the Vertical Organization of the Human Neocortex. *Cereb Cortex* 2007;17(1):130–137. [PubMed: 16452639]
- Chan, KK.; Lowe, J. Techniques in Neuropathology. In: Bancroft, JD.; Gamble, M., editors. *Theory and Practice of Histological Techniques*. London: Churchill Livingstone; 2002. p. 371-414.
- Craven P, Wahba G. Smoothing noisy data with spline functions: estimated the correct degree of smoothing by method of generalized cross-validation. *Numerische Mathematik* 1979;31:377–403.
- Daly, D. Cerebral Localization. In: Baker, AB.; Baker, LH., editors. *Clinical Neurology*. New York: Harper and Row; 1976.
- de No, L. The cerebral cortex: architecture, intracortical connections, and motor projections. In: Fulton, JF., editor. *Physiology of the nervous system*. London: Oxford University Press; 1938. p. 291-339.
- DeFelipe, J.; Ballesteros-Yanez, I., et al. Progress in Brain Research. 154. Elsevier; 2006. Double-bouquet cells in the monkey and human cerebral cortex with special reference to areas 17 and 18. Part 1:15
- DeFelipe J, Hendry SHC, et al. A microcolumnar structure of monkey cerebral cortex revealed by immunocytochemical studies of double bouquet cell axons. *Neuroscience* 1990;37(3):655. [PubMed: 1701039]
- del Rio MR, DeFelipe J. Double bouquet cell axons in the human temporal neocortex: relationship to bundles of myelinated axons and colocalization of calretinin and calbindin D-28k immunoreactivities. *Journal of Chemical Neuroanatomy* 1997;13(4):243. [PubMed: 9412906]
- Douglas RJ, Martin KAC. NEURONAL CIRCUITS OF THE NEOCORTEX. *Annual Review of Neuroscience* 2004;27(1):419–451.
- Elston GN, Benavides-Piccione R, et al. The Pyramidal Cell in Cognition: A Comparative Study in Human and Monkey. *J Neurosci* 2001;21(17):163RC.
- Gabbott PLA. Radial organisation of neurons and dendrites in human cortical areas 25, 32, and 32'. *Brain Research* 2003;992(2):298. [PubMed: 14625070]
- Hof PR, Duan H, et al. Age-related changes in GluR2 and NMDAR1 glutamate receptor subunit protein immunoreactivity in corticocortically projecting neurons in macaque and patas monkeys. *Brain Research* 2002;928(1–2):175. [PubMed: 11844485]
- Jones, EG. History of Cortical Cytology. In: Peters, A.; Jones, EG., editors. *Cellular Components of the Cerebral Cortex*. 1. New York: Plenum Press; 1984. p. 1-32.
- Lashley KS, Clark G. The cytoarchitecture of the cerebral cortex of Ateles: A critical examination of architectonic studies. *J Comp Neurol* 1946;85:223–305.
- Lohmann H, Koppen HJ. Postnatal development of pyramidal dendritic and axonal bundles in the visual cortex of the rat. *J Hirnforsch* 1995;36(1):101–111. [PubMed: 7751601]

- Mountcastle VB. Brain mechanisms for directed attention. *J R Soc Med* 1978;71(1):14–28. [PubMed: 416210]
- Ong WY, Garey LJ. Neuronal architecture of the human temporal cortex. *Anat Embryol (Berl)* 1990;181(4):351–64. [PubMed: 2346228]
- Otsu N. A threshold selection method from gray-level histograms. *IEEE transactions on systems, man, and cybernetics* 1979;9:377–393.
- Peters A, Cifuentes JM, et al. The organization of pyramidal cells in area 18 of the rhesus monkey. *Cereb Cortex* 1997;7(5):405–421. [PubMed: 9261571]
- Peters A, Kara DA. The neuronal composition of area 17 of rat visual cortex. IV. The organization of pyramidal cells. *J Comp Neurol* 1987;260(4):573–90. [PubMed: 3611411]
- Peters A, Sethares C. Layer IVA of Rhesus Monkey Primary Visual Cortex. *Cereb Cortex* 1991;1(6):445–462. [PubMed: 1726607]
- Peters A, Sethares C. Organization of pyramidal neurons in area 17 of monkey visual cortex. *J Comp Neurol* 1991;306(1):1–23. [PubMed: 1710236]
- Peters A, Sethares C. Myelinated axons and the pyramidal cell modules in monkey primary visual cortex. *J Comp Neurol* 1996;365(2):232–55. [PubMed: 8822167]
- Rockland KS. Visual cortical organization at the single axon level: a beginning. *Neuroscience Research* 2002;42(3):155. [PubMed: 11900825]
- Schmolke C, Künzle H. On the presence of dendrite bundles in the cerebral cortex of the Madagascan lesser hedgehog tenrec and the red-eared pond turtle. *Anatomy and Embryology* 1997;196(3):195. [PubMed: 9310312]
- Seldon HL. Structure of human auditory cortex. I. Cytoarchitectonics and dendritic distributions. *Brain Research* 1981;229(2):277. [PubMed: 7306814]
- Seldon HL. Structure of human auditory cortex. II. Axon distributions and morphological correlates of speech perception. *Brain Research* 1981;229(2):295. [PubMed: 7306815]
- Skoglund TS, Pascher R, et al. Aspects of the quantitative analysis of neurons in the cerebral cortex. *Journal of Neuroscience Methods* 1996;70(2):201. [PubMed: 9007760]
- Skoglund TS, Pascher R, et al. Aspects of the organization of neurons and dendritic bundles in primary somatosensory cortex of the rat. *Neuroscience Research* 2004;50(2):189. [PubMed: 15380326]
- Viebahn C. Correlation between differences in the structure of dendrite bundles and cytoarchitectonic patterns in the cerebral cortex of the rabbit. *J Hirnforsch* 1990;31(5):645–52. [PubMed: 1707078]
- von Bonin G, Mehler W. On columnar arrangement of nerve cells in cerebral cortex. *Brain Res* 1971;27(1):1–9. [PubMed: 4101826]
- Von Economo, CF.; Koskinas, GN. *Die Cytoarchitektonik der Hirnrinde des erwachsenen Menschen*. Wien: Springer; 1925.
- White EL, Peters A. Cortical modules in the posteromedial barrel subfield (Sml) of the mouse. *J Comp Neurol* 1993;334(1):86–96. [PubMed: 8408761]
- Yáñez IB, Muñoz A, et al. Double bouquet cell in the human cerebral cortex and a comparison with other mammals. *The Journal of Comparative Neurology* 2005;486(4):344–360. [PubMed: 15846784]

Figure 1.
Serial 35 µm sections from human cortical area 22, left hemisphere, stained with silver (left) and cresyl violet (right). The scale bar measures 400 µm.

Figure 2.

White matter segmentation. (A) Loyez-stained tissue from cortical area 9 of a 6.5 year old male. (B) Upper third of the image following the thresholding step. Pixels in I_2 are white and pixels in $I - I_2$ are black. (C) The largest connected set of pixels in $I - I_2$ is extracted. (D) The extracted region is filled-in to make it simply connected.

Figure 3.

Convex white matter in a gyrus. Lines outside the white matter are level curves of the Euclidean distance map of the region occupied by white matter. Fiber bundle spacing CW_A is computed using profiles of the image along these level sets.

Figure 4.

Columnar structure appears at essentially the same spatial frequency whether determined by cell somata or bundles of myelinated axons. The line $CW_A = m CW_S + b$ is statistically indistinguishable from the identity function.

Table 1
Summary of postmortem samples. Material was available from all six cortical areas in all cases, but staining quality was such that only the areas marked “✓” were measured.

case	age	sex	Brodmann area							
			4	9	17	21	22	40		
1	3.5	male	✓	✓		✓		✓		✓
2	4	female	✓	✓		✓		✓		✓
3	6.5	male		✓		✓	✓	✓		✓
4	11	female	✓	✓		✓	✓	✓		✓
5	19	male	✓		✓	✓	✓	✓		✓
6	20	male		✓		✓		✓		✓
7	25	male	✓	✓		✓	✓	✓		✓
8	25	male		✓		✓		✓		✓
9	32	female		✓	✓	✓		✓		✓
10	50	female	✓	✓						✓
11	50	male	✓	✓	✓					
12	67	female			✓					
13	71	male		✓		✓				✓
14	86	male		✓						
15	94	female		✓	✓	✓	✓	✓		✓
16	98	male	✓		✓	✓	✓	✓		✓

Table 2

Per-area means and standard deviations for each columnar width measurement, in μm .

Cortical area	CW_A		CW_S	
	mean	s.d.	mean	s.d.
4	24.8	4.9	24.7	4.9
9	28.7	5.6	28.4	5.6
17	23.2	1.9	22.8	2.0
21	22.2	5.0	22.4	5.5
22	24.0	4.1	24.0	4.2
40	24.8	5.6	25.1	5.6