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DENDRITIC ARCHITECTURE OF THE VON ECONOMO NEURONS

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Abstract—The von Economo neurons are one of the few known specializations to hominoid cortical microcircuitry. Here, using a Golgi preparation of a human postmortem brain, we describe the dendritic architecture of this unique population of neurons. We have found that, in contrast to layer 5 pyramidal neurons, the von Economo neurons have sparse dendritic trees and symmetric apical and basal components. This result provides the first detailed anatomical description of a neuron type unique to great apes and humans. © 2006 Published by Elsevier Ltd on behalf of IBRO.

Key words: spindle cell, anterior cingulate, fronto-insula, Golgi, human brain, ape.

von Economo neurons (VENs) are large, bipolar neurons that are located in layer 5 of anterior cingulate cortex (ACC) and fronto-insula (FI) cortex (von Economo and Koskinas, 1929). Elsewhere we have referred to them as the "spindle" neurons, but because of potential confusion with other uses of this term, we now refer to them by the first author of the best classical description of these cells. Unlike most neuron types, the VENs are present in the great apes but are absent in the lesser apes, Old and New World monkeys, and prosimians (Nimchinsky et al., 1999). This suggests that they arose in the hominoid clade within the last 15 million years. The volume of the soma is much larger in humans than in apes, and stereological counts indicate that these cells have proliferated in the human line of descent (Nimchinsky et al., 1995; Watson et al., 2006). The recent emergence of this cell type, as well as its localization to subregions of the prefrontal cortex, suggests its involvement in sophisticated cognitive behaviors. This suggests that studies of this cell may provide insights into human uniqueness and origin. Furthermore, because the force of natural selection has had only a relatively short time to shape their functioning and integration with other cell populations, the VENs may be particularly vulnerable to dysfunction. Thus knowledge of the morphology of the VENs may be useful in evaluating possible pathological variants in neurogenetic and neuropsychiatric disorders.

Despite these important characteristics, little is known about the dendritic morphology of the VENs. Cell morphology is crucial to our understanding of these cells, because neuronal shape is directly related to the computations performed by the cell. For example, dendrites can establish intrinsic firing pattern (Mainen and Sejnowski, 1996), perform non-linear operations (Koch et al., 1982), or modulate action potential propagation (Vetter et al., 2001). In the current study, we used a modified Golgi technique that enabled us to quantitatively describe the dendritic architecture of the von Economo cells from a young adult human male. Comparisons of the extended dendritic trees allowed us to determine whether the populations of VENs were consistent across regions, and if and how the dendritic trees of VENs differed from those belonging to layer 5 pyramidal cells.

EXPERIMENTAL PROCEDURES

Tissue specimens were obtained via Maryland Brain Bank from a human 23 year old male (postmortem interval=18 h) who suffered sudden cardiac arrest. Toxicology reports indicate that there were no drugs or alcohol present in the body at time of death. The right hemisphere FI cortex and ACC were dissected, photographed, placed immediately in a potassium dichromate fixative solution (FD Neurotechnologies, Ellicott City, MD, USA) and mailed overnight to the authors. The specimens were kept in this fixative for 17 days, and then placed in FD Neurotechnologies Solution C for 9 days.

Specimens were sectioned at 200 μm intervals on a freezing microtome, mounted on gelatinized slides, and allowed to dry for 2–4 days. They were then Nissl stained with Cresyl Violet (Sigma-Aldrich, St. Louis, MO, USA), processed according to manufacturer's directions (FD Neurotechnologies), and coverslipped in Permount (Fisher Scientific, Fair Lawn, NJ, USA).

Once dry, the specimens were observed using the 4×, 10×, and 40×-oil (N/A=1.00) objectives of a Reichert Polyvar light microscope (Leica Microsystems Inc., Bannockburn, IL, USA) equipped with a 10× ocular and a motorized-stage. The criteria for classifying a neuron as a VEN was an elongated, large soma in layer 5 of the FI or ACC, a prominent basal dendrite, and symmetrical morphology along the horizontal and vertical axes of the cell (Nimchinsky et al., 1999). We further constrained the category to include only those neurons that had no additional dendrites or branching for a half-soma's distance along the length of the proximal dendrites. For every VEN traced, we also traced the nearest complete pyramidal cell that had two or more prominent basal dendrites. Using Neurolucida 6.0 (MicroBrightField Inc., Williston, VT, USA) we created three-dimensional reconstructions of the spines, soma, and dendrites of VENs and pyramidal cells in FI and ACC, and used NeuroExplorer (MicroBrightField) for visualization and to perform Scholl analysis (Scholl, 1953). Statistical comparisons were made with non parametric tests (Kruskal-Wallis and Wilcoxon rank sum tests) using Matlab 7.0 (Mathworks Inc., Natick, MA, USA).

RESULTS

Fig. 1 illustrates soma and proximal dendrites of a pyramidal and a VEN. The dendritic arborization is much richer in

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Abbreviations: ACC, anterior cingulate cortex; FI, fronto-insula cortex; VEN, von Economo neuron.

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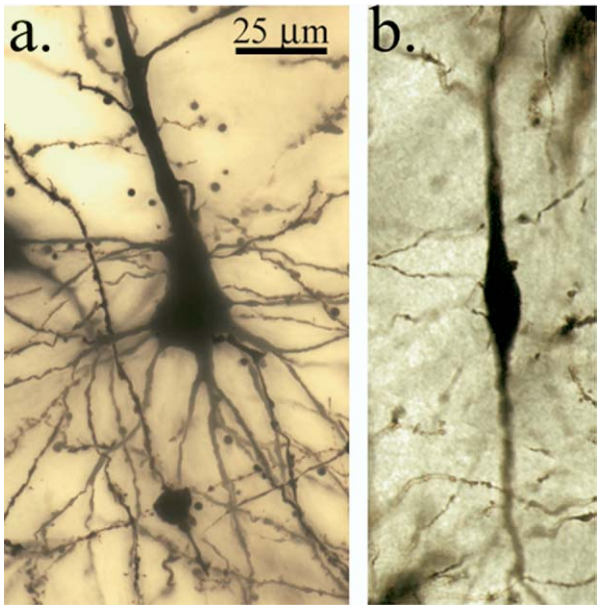

Fig. 1. Photomicrographs of soma and proximal dendrites of (a) a pyramidal and (b) the VENs stained with the Golgi method. Photomicrogaphs are montages taken of several planes and/or fields of view. Scale bar applies to both images.

the pyramidal neuron; a finding which is confirmed quantitatively in this study. Fig. 2 illustrates the long narrow radial arborization of a VEN, and Fig. 3 shows at higher magnification of the distribution of the spines on basal dendrites of a pyramidal and a VEN. Neurolucida models were created for 17 pyramidal cells and 15 VENs in ACC, and for 21 pyramidal cells and 20 VENs in FI. VENs were noted to be symmetric, with their apical and basal dendrites having similar profiles in terms of "branchiness" and length (Fig. 4, right). In contrast, pyramidal cells had highly branched basal tufts in comparison to their relatively sparse apical trunks (Fig. 4, left).

We used Scholl analysis to measure dendritic length and the number of branch points ("intersection number") as a function of distance from the soma. Similar to previous findings in macaque temporal lobe (Elston and Rosa, 2000), we found that the peak dendritic complexity of layer V pyramidal cells occurred in the basal tree 50–75 μm from the soma. This spike in dendritic complexity was not present in apical tree of the pyramidal neurons, nor in the apical and basal trees of the VENs (Fig. 5).

Between regions (ACC and FI), there were no significant differences in mean total branch length or intersection number for either the pyramidal or von Economo populations ($P>0.25$). Therefore, data from both regions were pooled into a single von Economo group and a single pyramidal group for statistical analyses. When summed over all Scholl radii, neither the total length nor intersection number of the apical and basal dendritic trees of the Von Economo cells differed significantly from one another. In contrast, the basal dendritic trees of the pyramidal neurons contained significantly greater total dendritic length and more Scholl intersections than the apical dendrites of the pyramidal neurons as well as the apical and basal trees of

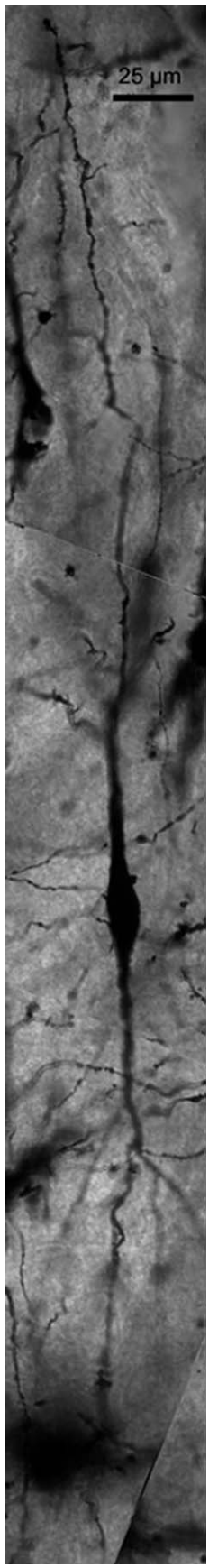

Fig. 2. Dendritic arbor of the VEN depicted in Fig. 1.

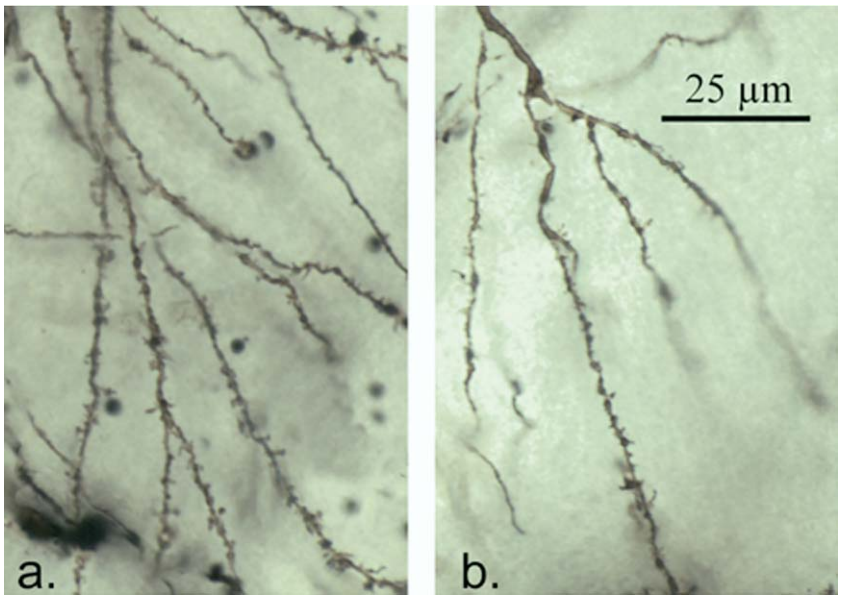

Fig. 3. Higher magnification photomicrograph of basal dendrites of (a) a pyramidal neuron and (b) a von Economo neuron. Scale bar applies to both images.

the VENs (Fig. 6, $P<0.001$). The maximum Scholl radii for the von Economo and pyramidal neurons were not significantly different for either the apical (VEN=287.14 μm$\pm$15.72; pyramidal=330.52 μm$\pm$17.65) or basal (VEN=233.43 μm$\pm$14.52, pyramidal=212.63 μm$\pm$10.39) trees ($P<0.001$, Fig. 6). Pyramidal cells had a mean total dendritic length 2.5-fold higher than that of VENs (pyramidal=2044.3 μm$\pm$157.1 μm, VENs=815.8 μm$\pm$66.75).

Spines were distinguishable at 400$\times$ magnification. Because the mean total number of spines did not vary by region, data were pooled across ACC and FI. Kruskal-Wallis non parametric ANOVA tests indicated a significant difference in total spine counts between cell and tree types ($P<0.001$). Post hoc rank sum tests indicated that the mean sum of spines on the basal pyramidal trees was greater than that of the pyramidal apical, VEN apical, or VEN basal trees ($P<0.005$). The sum of spines on the pyramidal apical tree was greater than that of the VEN apical or basal trees ($P<0.001$). The VEN apical and VEN basal trees had the same mean total numbers of spines ($P=0.98$). We counted

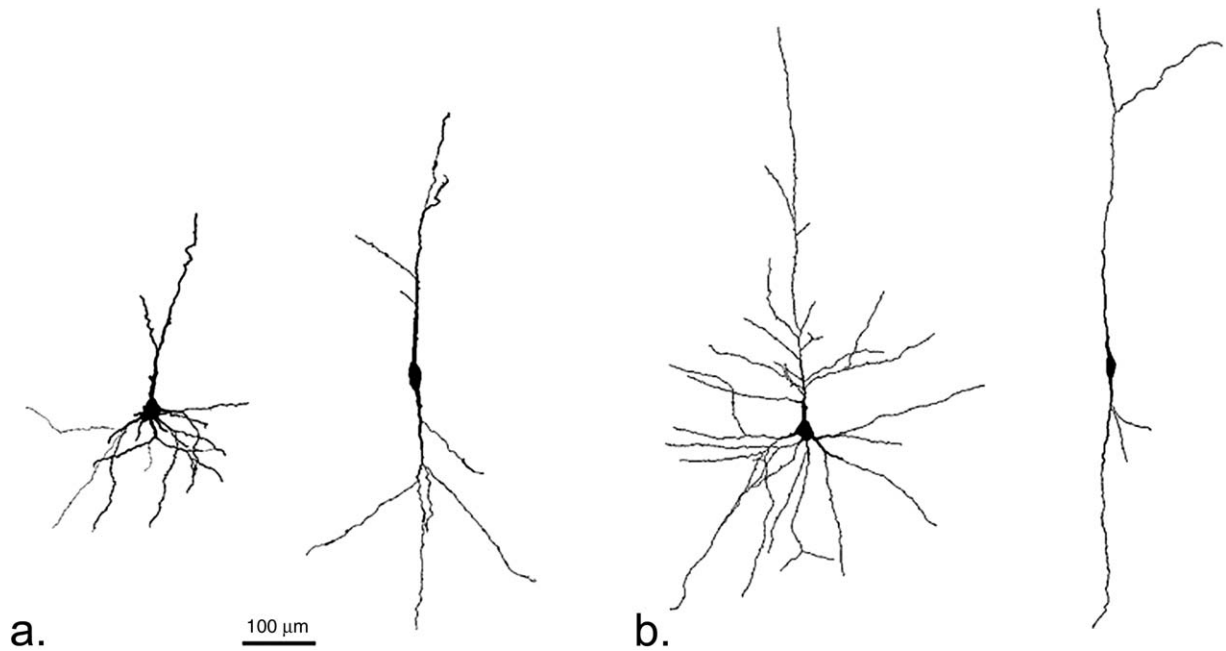

Fig. 4. Neurolucida tracings of pyramidal (left) and von Economo (right) neurons from FI (a) and ACC (b). Notice the vertical symmetry and relative sparseness of the VEN dendritic tree. Neurons are oriented so the pial surface is at the top.

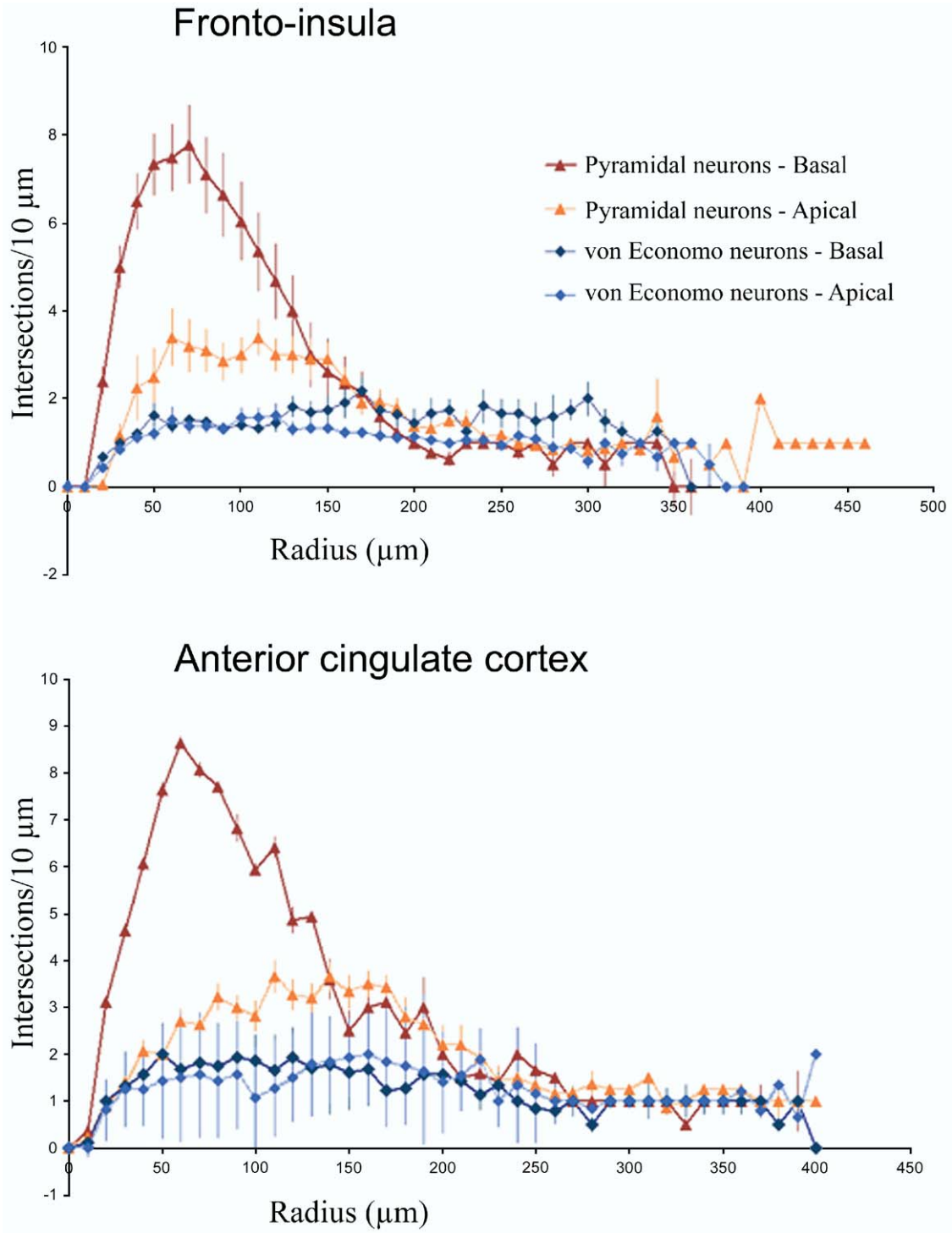

Fig. 5. Scholl intersections for FI (top) and ACC (bottom) for pyramidal cells (basal tree, red triangles; apical tree orange triangles) and von Economo cells (basal tree navy diamonds; apical tree light blue diamonds). Note the spike in intersection number that occurs in the pyramidal basal tree that occurs at a radius of 50–100 μm from the soma, and the symmetric intersection number in apical and basal dendritic tress of the VENs in both regions. Error bars represent S.E.M.

the number of dendritic spines per 10 μm along the extent of all dendrites on a tree and found that the number of spines on the basal tree of the pyramidal neuron was maximal from 70 to 110 μm from the soma, while the maximum number of spines on the apical pyramidal tree occurred at 160–180 μm distance from the soma. Both the apical and basal trees of the VENs reached the maximum number of spines around 190–240 μm from the soma.

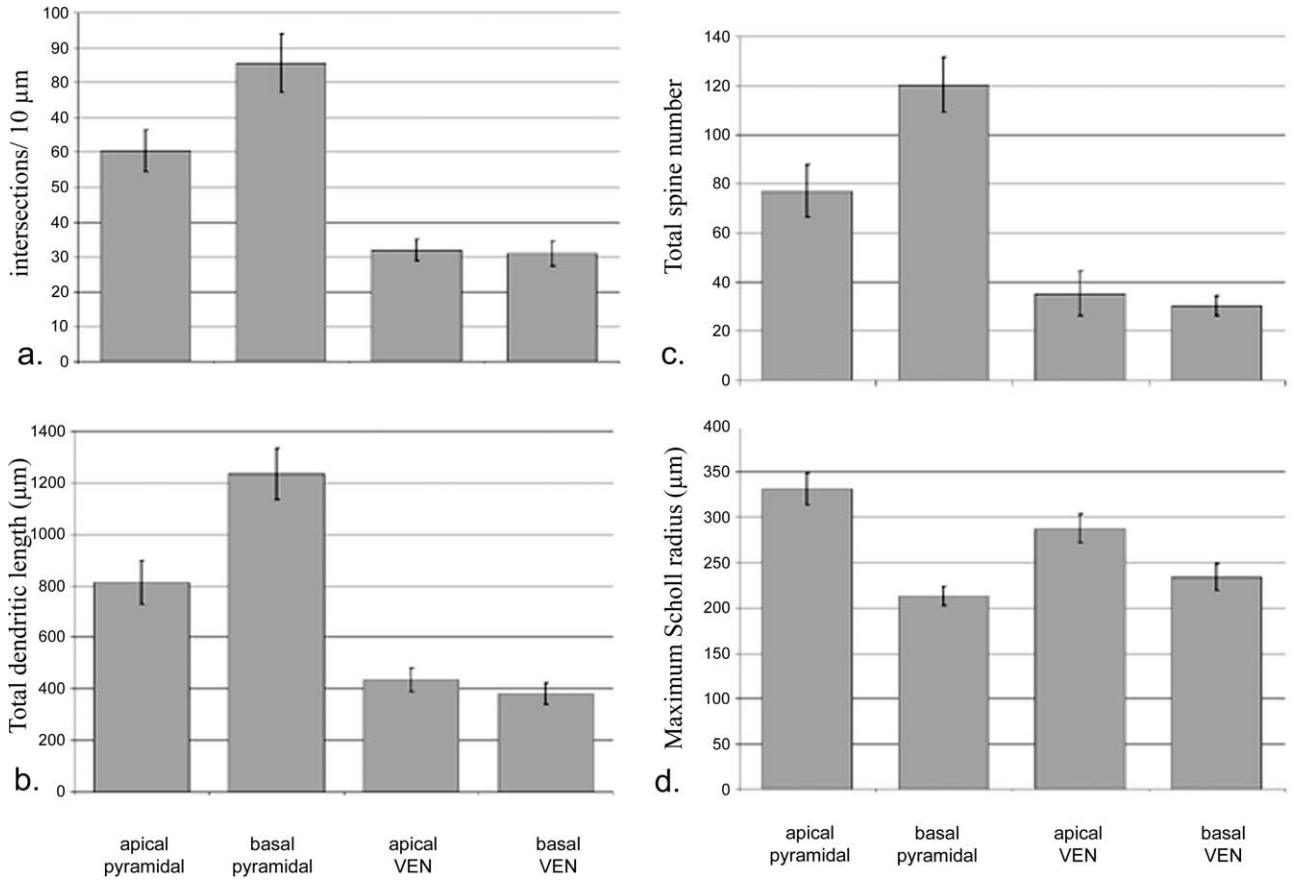

Fig. 6. Comparisons of dendritic structure for apical and basal trees of VENs and layer 5 pyramidal cells for (a) total number of Scholl intersections (b) total dendritic length (c) spine counts and (d) maximum Scholl radii. Note that, despite significant differences between VENs and pyramidal cells for the first intersections, length, and spine count, there are no significant differences in maximum Scholl radii, suggesting that the observed differences are not due to variations in the degree of Golgi staining. Error bars denote S.E.M.

DISCUSSION

We used Golgi-stained human brain tissue to characterize the von Economo cells. In doing so, we demonstrated that the VENs in anterior cingulate and FI cortex appear to be a single population of cells. We also found that VENs have fewer spines, fewer intersections, and overall, less dendritic length than their layer 5 pyramidal counterparts, which suggests that the von Economo cells receive, and therefore integrate, fewer inputs than pyramidal neurons. The dendritic architecture of neurons reflects the way in which they integrate information (Vetter et al., 2001). Both spines (Sabatini et al., 2001), and branches (Polsky et al., 2004) can operate as computational compartments, and, compared with their layer 5 pyramidal counterparts, VENs have fewer of both. Studies of rat sensorimotor layer 5 pyramidal cells reveal a relationship between depolarization and output frequency that is linear near the soma and proximal dendrites but non-linear in higher order dendritic branches (Oakley et al., 2001). This suggests that VENs are computationally simple compared with pyramidal neurons.

The radially polarized structure of the VENs is reminiscent of the theme of radial organization that occurs throughout cortex. A vertical orientation bias is present in pyramidal and non-pyramidal cortical cells (Jin et al., 2001), and axonal afferents project within the bounds of narrow columns. These and other features form the basis of the minicolumn, a collection of 80–100 vertically aligned cells which, through virtue of their common input, share functional properties (Mountcastle, 1997). The narrow dendritic tree of the VENs suggests that it samples only a subspace of a minicolumn, which is reported to be 35–60 μm wide in humans (Buxhoeveden and Casanova, 2002). In the case of either synaptic or extrasynaptic input, the VENs probably receive neurotransmission only within their individual minicolumns. Latency measurements by Hubel and Wiesel (1977) show that input into a column gets relayed rapidly in a vertical direction, but not on a horizontal dimension. The VENs could be a specialization that facilitates this rapid radial signal transmission, providing an output response that reflects the minicolumnar input within one or two synapses.

Although we show that the dendritic tree of the average VEN is sparser than that of the average pyramidal cell, previous research shows that the cell bodies of VENs in ACC are, on average, 4.6 times larger than that of layer 5 pyramidal cells in this area (Nimchinsky et al., 1999). The VENs' large size suggests that they bear large, rapidly conducting axons, which is a characteristic feature of big

neurons in layer 5 elsewhere in the cortex (Sherwood et al., 2003). The VENs contain an abundance of non-phosphorylated neurofilaments, which is characteristic of neurons bearing large axons (Nimchinsky et al., 1995). Lipophilic dye injected into the anterior part of the cingulum bundle backfills VENs in ACC, thus indicating that they project axons into the white matter (Nimchinsky et al., 1995). Together this evidence suggests that the function of the VENs may be to provide a rapid relay to other parts of the brain of a simple signal derived from information processed within FI and ACC.

Functional magnetic resonance imaging studies indicate that FI and ACC are coactivated when subjects experience social emotions such as empathy (Singer et al., 2004), guilt (Shin et al., 2000), violation of social norms (Berthoz et al., 2002), deception (Spence et al., 2004), and humor (Watson et al., 2006) As of yet, we do not know the mechanisms responsible for the differentiation of the complex social emotions that activate FI and ACC, but we do know that the VENs are a recently evolved population that probably serves to relay output of the processing within FI and ACC to other brain structures. Their large size suggests that the VENs may relay a fast intuitive assessment of complex social situations to allow the rapid adjustment of behavior in quickly changing social situations (Allman et al., 2005). They can thus be seen as an adaptation supporting the increased complexity of hominoid and especially human social networks.

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