2 Anatomy of the von Economo Neurons

2.1 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 found that, in contrast to layer 5 pyramidal neurons, the von Economo neurons have sparse dendritic tress that have symmetric apical and basal components. We also used immunohistochemistry to probe the receptor expression on these cells, and found that the von Economo neurons strongly express the dopamine D3 and D5 receptors, as well as serotonin-1b and -2b receptors. This receptor profile is consistent with a role in mediating decision making in uncertain contexts. Together, these results provide the first detailed anatomical description of a neuron type unique to great apes and humans.

2.2 Introduction

Von Economo neurons (VENs) are large, bipolar neurons that are located in layer 5 of anterior cingulate cortex and fronto-insula 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 African 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 suggest that these cells have proliferated in the human line of descent (Allman et al., 2005; Nimchinsky et al., 1995). 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. Our understanding of this cell type may thus be relevant to the treatment and understanding of mental illness.

Despite these important characteristics, little is known about the dendritic morphology of the von Economo neurons or about their neurochemical makeup. Cell morphology is crucial to our understanding of these cells, because neuronal shape is directly related to the computation. For example, dendritic structure can establish intrinsic firing patterns (Mainen and Sejnowski, 1996), perform non-linear operations (Koch et al., 1982), or modulate action potential propogation (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.

Another way to gain insight into a cell's function is by cataloguing its receptor expression. Pharmacology studies link certain ligands to behaviors, and therefore the

presence of specific receptors on or in the VENs can indicate a role in those behaviors. In this paper we describe immunohistochemical results that show the presence of the dopamine D3 receptor, and the serotonin-1b and -2b receptors.

2.3 Materials and methods

2.3.1 Golgi

Tissue specimens were obtained via Maryland Brain Bank from a human 23-year-old male (PMI = 18 hours) 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 fronto-insula (FI) cortex and anterior cingulate cortex (ACC) were dissected, photographed, placed immediately in a potassium dichromate fixative solution (FD Neurotechnologies, Ellicott City, MD) 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 to 4 days. They were then Nissl-stained with cresyl violet, processed according to manufacturer's directions (FD Neurotechnologies), and coverslipped in Permount (Fisher Scientific, Fair Lawn, NJ).

Once dry, the specimens were observed using the 4x, 10x, and 40x-oil (N/A = 1.00) objectives of a Recihert Polyvar light microscope equipped with a 10x ocular and a motorized stage. The criteria for classifying a neuron as a VEN was an elongated, large soma in layer five 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 von Economo neuron 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) 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 nonparametric tests (Kruskal-Wallis and Wilcoxon rank sum tests) using Matlab 7.0 (Mathworks Inc, Natick, MA).

2.3.2 Immunohistochemistry

Neurologically normal human postmortem tissue was obtained from Maryland Brain Bank, UCLA Brain Bank, and Dr. Bob Jacobs, and stored in 10% formalin until sectioning. Tissue specimens were ruled out if the known medical history of the donor included neuropharmacological compounds (i.e., oxycotin), if tissue lacked immunohistochemical reactivity, or if Nissl-stained tissue was evaluated as abnormal. The six specimens used in the experiment had a postmortem interval ranging from 8 to 22 hours (mean 15, s.d. 4.3), and were from male donors ranging in age from 17 to 80 years (mean 45, s.d. 21.0). Tissue was sectioned perpendicular to the pial surface in 50 μm slices on a vibratome and stored in a 0.1 M phosphate buffer with 0.01% sodium azide (Sigma Chemical, St. Louis, MO). The presence of von Economo neurons in tissue was determined by Nissl stain prior to immunohistochemistry. The tissue was processed free-floating with an antibody (see Table 1) in a stock solution of 1% normal goat or

Receptor type	Manufacturer/Product number	Antigen	Host	Working dilution or concentration
Dopamine D1	Chemicon AB1784	Nine aa peptide corresponding to the 4th extracellular domain in rat (88% homology with human)	Rabbit	5 µg/mL
Dopamine D2	Chemicon AB1558	Amino-terminal peptide sequence (aa 24–34) near or at the ligand binding domain of rat D2R (91% homology with human)	Rabbit	1:2500
Dopamine D3	Chemicon AB1785p	Human peptide. 19 aa peptide sequence from 3rd cytoplasmic region	Rabbit	20 µg/mL
Dopamine D3	Santa Cruz D3DR (H-50)	Human peptide. aa 1–50 amino terminal extracellular region	Rabbit	15–25 µg/mL
Dopamine D4	Chemicon AB1789p	Human peptide. 25 aa sequence within the 4th cytoplasmic domain	Goat	10 µg/mL
Dopamine D5	Chemicon AB1790	Human peptide. 20 aa sequence within the 4th cytoplasmic domain	Goat	10 µg/mL
Serotonin 2b	BD Biosciences 556334	Human peptide.aa 1–58	Mouse	10–15 µg/mL
Serotonin 2b	Santa Cruz SR-2B	Human peptide. aa 387–481	Rabbit	10 µg/mL
Serotonin 1b	Chemicon AB5651	Human peptide. aa 15–28	Rabbit	4 µg/mL
Serotonin 1b	Chemicon AB5410	Rat peptide. Sequence 100% conserved in human, corresponding to aa 277-291	Guinea pig	1:1000

Table 1 Antibodies and concentrations used for immunohistochemistry experiments aa = amino acids

rabbit serum (Sigma) and 0.2% Triton X-100 (Sigma). The primary antibody was detected by a species-specific biotinylated secondary antibody (Chemicon, Temecula, CA, 1:200) and then a commercial horseradish peroxidase complex (Vector Laboratories Elite ABC Kit, Burlingame, CA). Labeling was revealed using 3,3'-diaminobenzidine (DAB, Vector Laboratories) as a chromagen according to manufacturer's instructions. Specimens were mounted on gelatinized slides and dried overnight at room temperature. In some cases, some slides were subject to a intensification process through the application of a 2% osmium tetroxide solution for 1 minute, followed by rinsing in buffer, water, and graded alcohols. Once dehydrated, all specimens were cleared in Histo-clear (National Diagnostics, Atlanta, GA) or xylene and coverslipped with Permount (Fisher Scientific, Fair Lawn, NJ). Negative controls consisted of performing the same experiment in parallel, omitting the primary antibody. Specificity of localization for the Chemicon dopamine D3 receptor antibody (AB1785p) was confirmed by control experiments in which the primary antibody was preadsorbed with a dopamine receptor peptide (Chemicon AG229), which abolished staining (data not shown). Results for the D3, serotonin 1b, and serotonin 2b receptors were obtained for at least five of the six specimens, for both hemispheres, and from both FI and ACC, and were replicated using two different antibodies for each receptor (Table 1), thus ruling out false positive results.

For some experiments, an Alexa Fluor conjugated secondary antibody (Molecular Probes, Eugene Oregon) was used instead of the biotinylated antibody, in which case the ABC/DAB steps were omitted and the section was mounted, left to dry, and coverslipped

with Vectashield (Vector labs). Slides were visualized with a Zeiss LSM 510 META NLO equipped with a Coherent Chameleon laser.

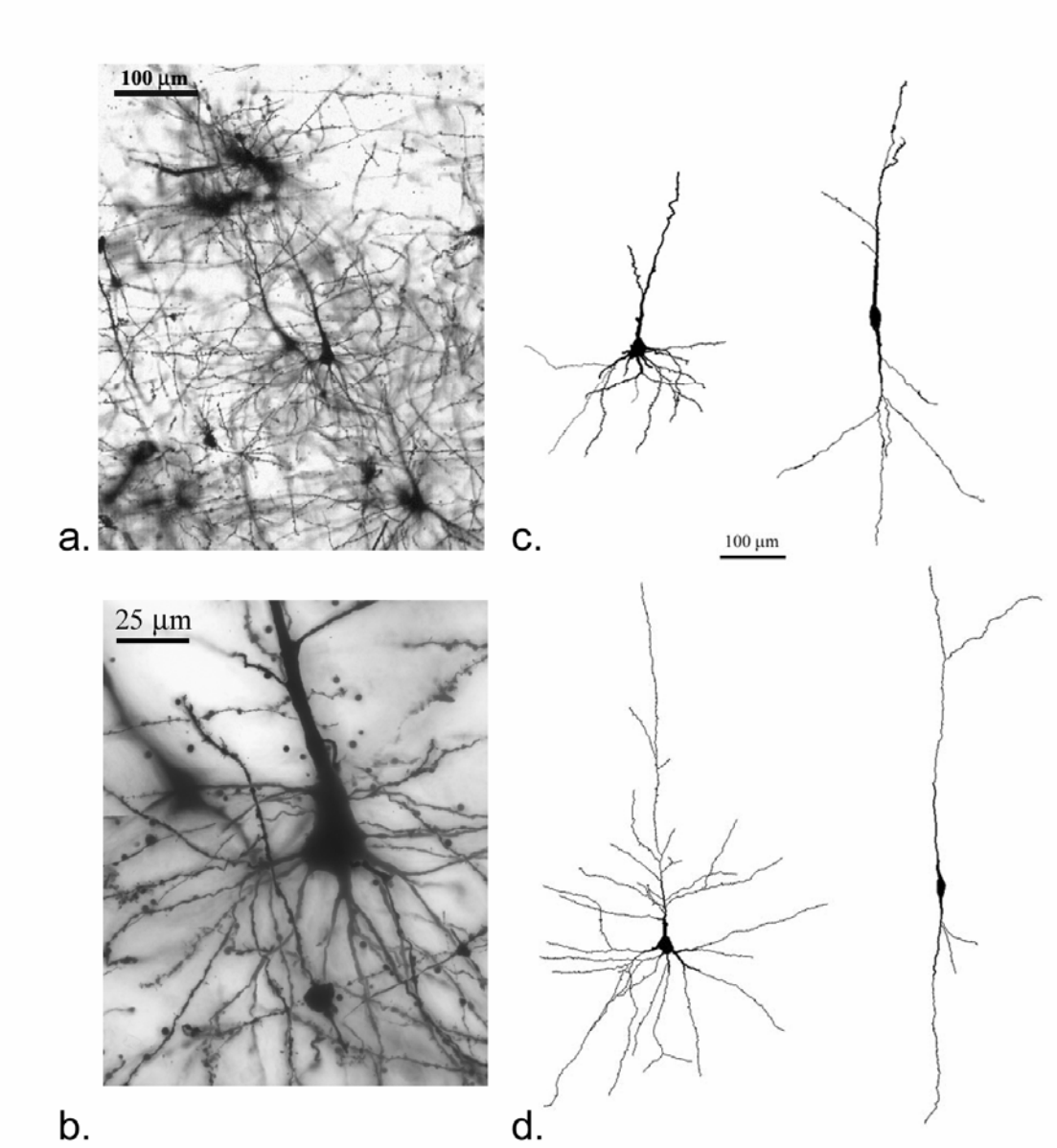

Figure 4. a. Low power photomicrograph of two pyramidal cells in Golgi-stained anterior cingulate cortex, demonstrating the quality of the stain. b High power photomicrograph of pyramidal cell, corresponding to boxed area in (a). Z-projection of 25 slices (taken every 1 µm) projected onto a single plane. c,d Neurolucida tracings of a pyramidal (left) and von Economo (right) neuron from FI (c) and ACC (d). Notice the vertical symmetry and relative sparseness of the VEN dendritic tree. Neurons are oriented so the pial surface is at the top.

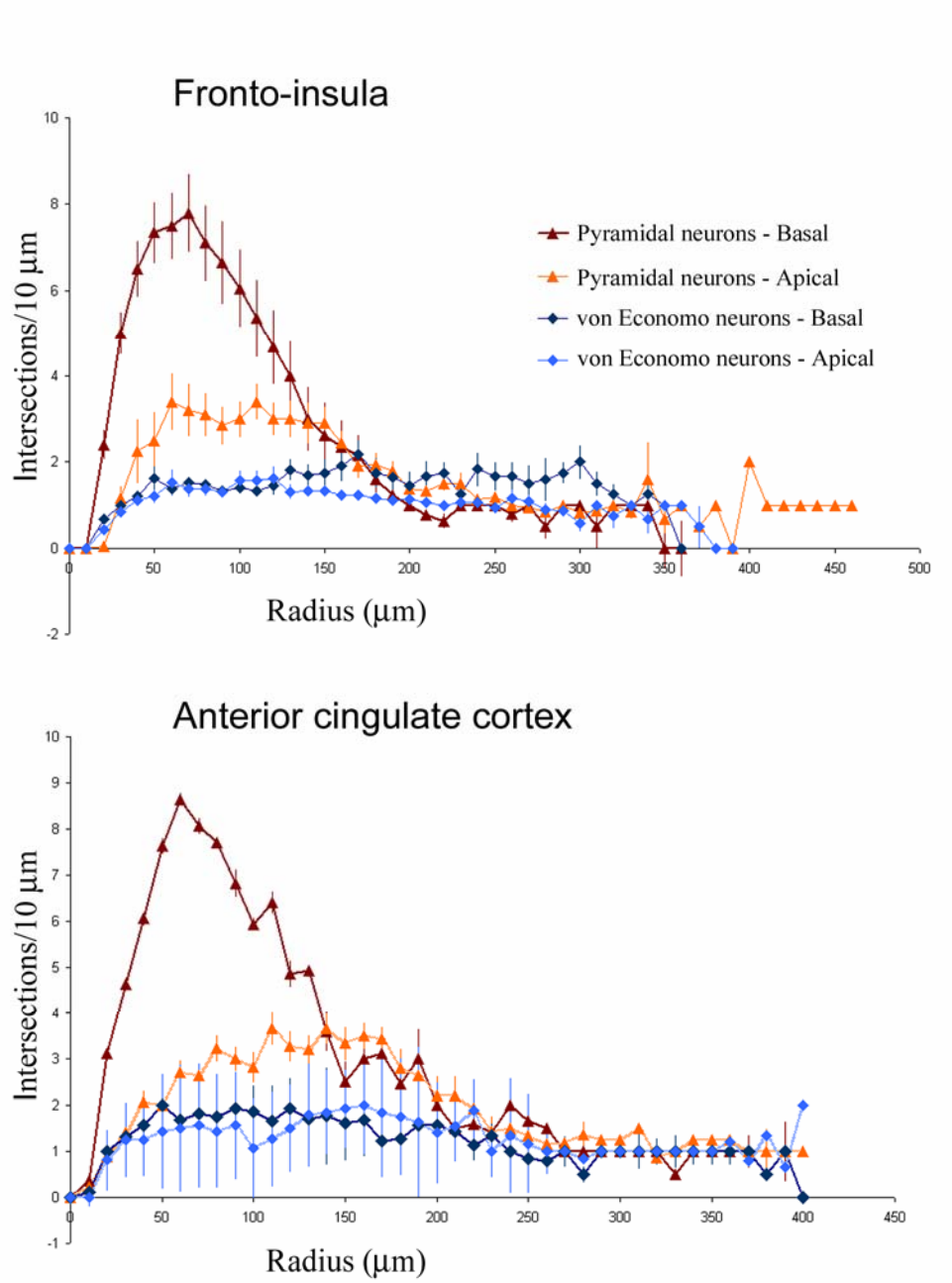

Figure 5 Scholl intersections for FI (top) and ACC (bottom) for pyramidal cells (red triangles, basal tree; orange triangles, apical tree) and von Economo cells (navy diamonds, basal tree; light blue diamonds, apical tree). Note the spike in intersection number that occurs in the pyramidal basal tree 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.

2.4 Results

2.4.1 Golgi

Photomicrographs of the specimen sample demonstrate the overall quality of the stain (Figure 4a). Cortical layers were distinguishable on the basis of the Golgi stain. Neurolucida models were created for 17 pyramidal cells and 15 von Economo neurons in ACC, and for 21 pyramidal cells and 20 von Economo neurons in FI. VENs were noted to be symmetric, with their apical and basal dendrites having similar profiles in terms of "branchiness" and length (Figure 4b). In contrast, pyramidal cells had highly branched basal tufts in comparison to their relatively sparse apical trunks (Figure 4c).

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 (Figure 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 overall 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 the VENs (Figure 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±15.72; pyramidal = 330.52± 17.65) or basal (VEN = 233.43± 14.52, pyramidal = 212.63±10.39) trees (p<0.001, figure 6). Pyramidal cells had a mean total dendritic length 2.5 -fold higher than that of VENs (pyramidal = 2044.3 ±157.1 μm, VENs = 815.8 μm ±66.75).

Spines were distinguishable at 400x 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 were greater than that of the pyramidal

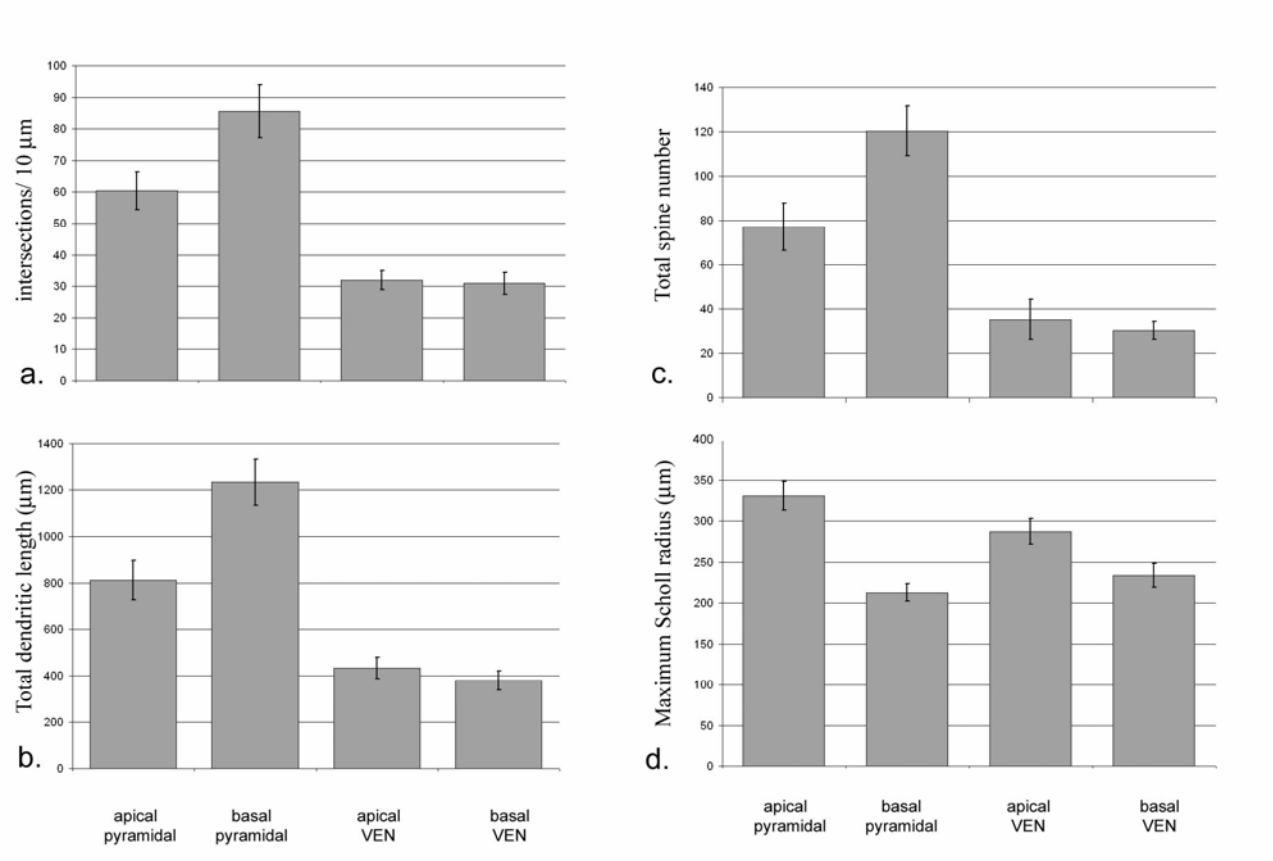

Figure 6. Comparisons of dendritic structure for apical and basal trees of VENs and layer 5 pyramidal cells for (a), total number of Scholl intersections, (b), total dendritic length; (c), spine counts; and (d), maximum scholl radii. Note that, despite significant differences between VENs and pyramidal 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.

for (a), total number of Scholl intersections, (b), total dendritic length; (c), spine counts; and (d), maximum 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 the number of dendritic spines per 10 um 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 to110 μm from the soma, while the maximum number of spines on the apical pyramidal tree occurred at 160 to180 μm distance from the soma. Both the apical and basal trees of the VENs reached the maximum number of spines around 190 to 240 μm from the soma.

age	gender	cause of death	PMI	hemisphere	% of D3 labeled pyramids (number labled/total)	% of D3 labeled spindles (number labled/total)
47	M	Heart attack	15	R	46% (243/522)	78% (47/60)
80	M	Unknown	15	R	35% (98/278)	75% (16/22)
54	M	Coronary artery disease	12	L	56% (60/107)	85% (12/14)
54	M	Coronary artery disease	12	R	56% (85/139)	93% (14/15)

Table 2 Percentage of VENs and pyramidal cells labeled with D3 receptor antibody in four human brain specimens

2.4.2 Immunohistochemistry

2.4.2.1 Dopamine

Dopamine receptors were localized in human brain by immunocytochemistry. The distribution of the D3 receptor in the anterior cingulate cortex and fronto-insula cortex was similar. Labeling was strongest in the deeper layers and was present on the somas and apical dendrites of Von Economo neurons and large pyramidal cells. The apical dendritic shafts of some cells were labeled for 200 μm or greater, long enough to reach the higher layers of cortex (Figure 7a). This profile of labeling was the same for two different antibodies against the D3 receptor, thus ruling out false positives (Table 1). Fluorescent double labeling of the ACC from four hemispheres (three individuals) revealed that a greater proportion of VENs (82.8% ± 8.0) than pyramidal neurons (48.3% ± 10.0) were labeled with the D3 antibody ($p < 0.001$, Table 2, figure 7b). Immunocytochemistry with an antibody recognizing the dopamine D5 receptor revealed a

pattern of labeling similar to the D3 (Figure 7), whereas antibodies to the D1, D2, and D4 receptors did not label von Economo neurons in a recognizable fashion.

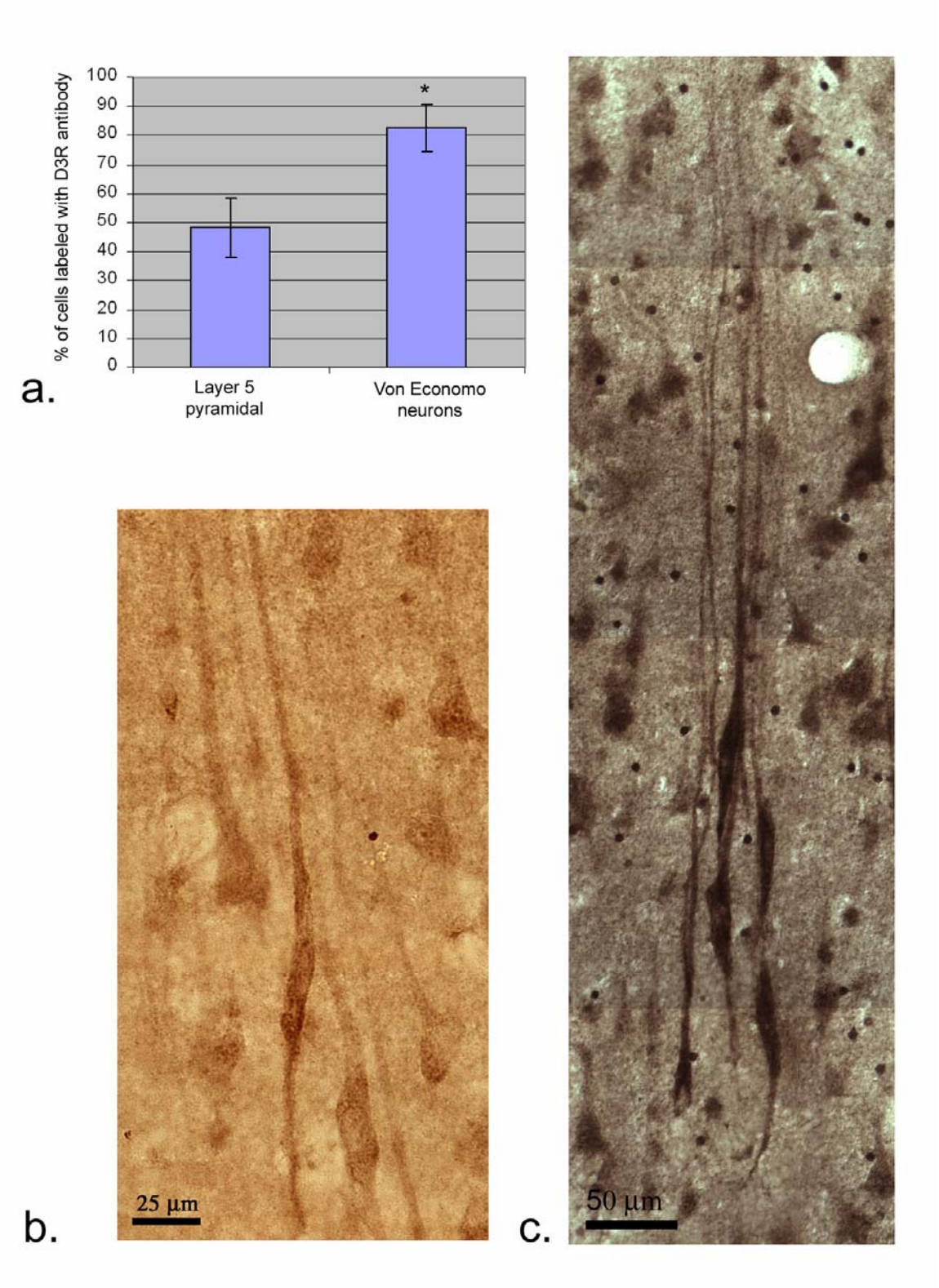

Figure 7. (a.) The percentage of cells labeled with the antibody against the dopamine D3 receptor is significantly lower for layer 5 pyramidal cells compared to von Economo neurons ($p<0.001$). (b) D5 receptor and (c) D3 receptor antibody labeling was evident on the somas and apical dendrites of the von Economo neurons.

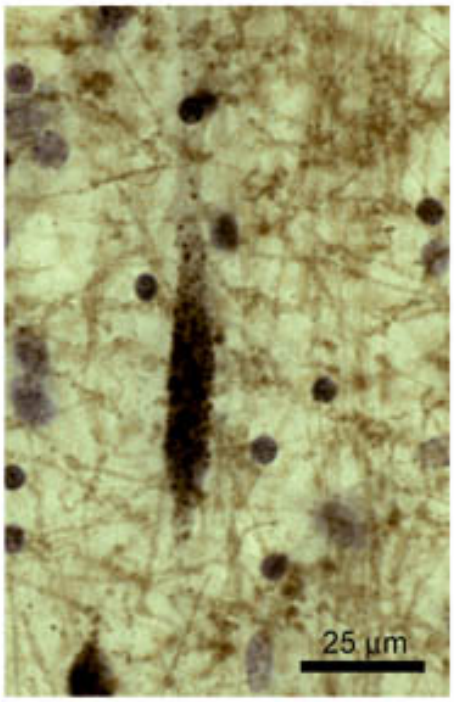

Figure 8. Unlike the D3, D5, and 5-HT 2b receptor antibodies, the 5-HT1b antibody labels the soma but not the apical dendrite of the von Economo neurons

2.4.2.2 5-HT1b

Immunohistochemistry was performed using two different antibodies recognizing different portions of the serotonin 1b receptor (table 1). In the human anterior cingulate cortex, this antibody labeled a network of axons as well as the somas and proximal dendrites of von Economo cells and large pyramidal cells in layer 5 (figure 8). Layers 2 and 3 contained labeled pyramidal somas but few fibers, while layer 6 was nearly confluent with labeled fibers but lacked labeled neurons.

2.4.2.3 5-HT2 receptors

Von Economo cells and other layer 5 pyramidal cells were noted to expess the serotonin 2b receptor, as recognized by two different antibodies. Expression of this receptor was strongly layer 5 specific in human ACC and FI (figure 9a). Expression was strongest on the proximal apical trunk and the soma (figure 9b). von Economo cells were also labeled by an antibody to the 5-HT2a receptor, as were other pyramidal cells in all layers of cortex (data not shown), similar to previous reports. (Jakab and Goldman-Rakic, 1998)

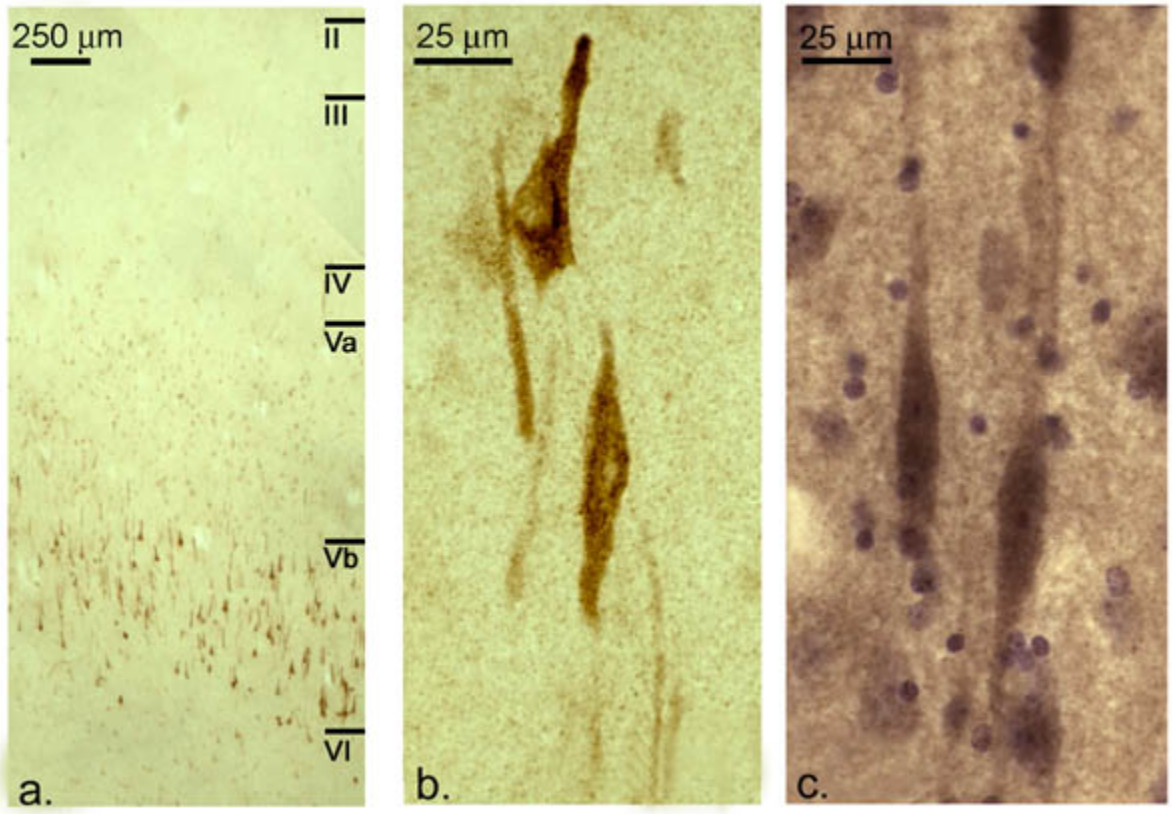

Figure 9. Serotonin 2b antibodies show layer 5 specificity in FI (a) and label the somas and apical trunks of pyramidal and von Economo neurons (b). (a) and (b) are from the same specimen. (c) Two 5-HT2b labeled von Economo neurons from ACC, counterstained with cresyl violet

2.5 Discussion

We used Golgi stains and immunohistochemistry on human brain tissue to characterize the von Economo cells. In doing so, we demonstrated that the von Economo neurons in anterior cingulate and fronto-insula cortex appear to be a single population of cells. Furthermore, we show that the dendritic geometry of the von Economo cells is distinct from that of layer 5 pyramidal cells, and that the VENs express several subtypes of dopamine and serotonin receptors.

2.5.1 Dendritic morphology

The dendritic architecture of neurons reflects the way in which they integrate information (Vetter et al., 2001). Both spines (Nusbaum et al., 2006; Sabatini et al., 2001) and branches (Polsky *et al.*, 2004) can operate as computational compartments, and, compared to 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 to pyramidal neurons.

VENs additionally have only a fraction of the total dendritic length of the average pyramidal cell. The complexity and size of dendritic trees vary with species and brain region. For example, pyramidal dendrites vary according to where they lie in the visual processing stream, with temporal and frontal areas containing neurons of greater complexity than the primary visual area (Elston et al., 2005; Jacobs et al., 2001; Travis et al., 2005). Additionally, Elston found that pyramidal neurons in human prefrontal cortex were more branched and spinous than those in marmoset or macaque monkeys, and that neurons in the cingulate cortex of baboons were similarly more complex than those in vervets and macaques. These results suggest that greater "computational power" comes in the form of more complex and spinous dendrites in the frontal corticies of species that we associate with larger behavioral repertoires. Following this logic, one might hypothesize that a phylogenetically recent neuron type such as the von Economo neuron, found only in great ape and human frontal cortex, would have more extensive dendritic arborizations than the surrounding, presumably more primitive, pyramidal cells.

However, we found that von Economo neurons 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. However, it is also possible that the mode of afferent input is different in VENs, which could also account for these differences in structure. Evidence suggests that a substantial amount of communication to the VENs is extrasynaptic (for more discussion, see below). Extrasynaptic transmission renders dendritic and spinious compartmentation superfluous, and could account for the sparse dendritic architecture of the VENs.

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 (Allman et al, 2002). 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 (Allman et al, 2002; Sherwood et al, 2003). The VENs contain an abundance of non-phosphorylated neurofilaments, which is characteristic of neurons bearing large axons (Hof et al, 1996; Nimchinsky et al, 1995, 1996). 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). Thus 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. However, it is not known where the VENs ultimately project. Studies in monkeys indicate that ACC is connected to prefrontal, orbitofrontal, insular and anterior temporal cortices and to the amygdala,

hypothalamus, various thalamic nuclei, and the periaqueductal gray (Öngür and Price, 2000; Rempel-Clower and Barbas, 1998; Barbas et al, 1999; Cavada et al, 2000).

2.5.2 Immunohistochemistry

While the morphology and connectivity have a large bearing on a neuron's computational properties, neuromodulators such as dopamine and serotonin are crucial as well. In fact, the pattern of input a neuron receives is crucial to the morphology of the dendritic tree. Input patterns guide outgrowth and pruning during development and serve to maintain a stable structure later (Cline, 2001; Wedzony et al., 2005; Wong and Ghosh, 2002). Thus, morphology and receptor expression are synergistic systems that endow a neuron with its particular functional role. Pharmacological and electrophysiological studies provide links between the various receptors and animal behavior. Using immunohistochemistry, we were able to probe some of the receptor types that are strongly expressed in the von Economo cells, which in turn provides evidence with respect to the likely function of this population.

2.5.2.1 Dopamine D3 receptor

Our results indicate that the von Economo neurons, as well as a subpopulation of layer 5 pyramidal neurons, express the D3 dopamine receptor on their somas and apical shafts. We found a similar pattern of labeling for the dopamine D5 receptor. In contrast, antibodies against the dopamine receptors D2, D4, and D1 labeled the von Economo neurons weakly or not at all.

The dopaminergic system projects from the midbrain to the basal ganglia and the frontal cortex, and is the fundamental component of the reward systems that motivate action and signal error and uncertainty. In mammals, there exist two classes of dopamine receptors. The D1 type activates adenylyl cyclase and includes the D1 and D5 receptors, while the D2 type inhibits adenylyl cyclase and includes D2, D3, and D4 and (Missale *et al.*, 1998). Although some studies show that the two classes of dopamine receptors can act synergistically (Hopf *et al.*, 2003), most indicate that they are opposed on the molecular (Aizman *et al.*, 2000; Chase *et al.*, 2004) and behavioral (Chase *et al.*, 2004) levels. For example, D1-class and D2-class agonists can inhibit or facilitate, respectively, pair bonding behavior in voles (Aragona *et al.*, 2006). The von Economo neurons appear to express a receptor from each class (the D5 receptor is D1-like, and the D3 receptor is D2-like), which suggests a dose-dependent effect of dopamine on the cell's output.

The pattern of labeling we observed for both the D3 and D5 receptor is consistent with extrasynaptic transmission of dopamine. Extrasynaptic dopamine action at D3 receptors, originally hypothesized because of the receptor's high affinity (Levesque et al., 1992; Sokoloff et al., 1990) and the disparity between dopaminergic innervation and dopamine receptor expression (Levesque *et al.*, 1992), has been further substantiated by studies confirming that dopamine escapes the synaptic cleft during transmission (Garris *et al.*, 1994; Venton *et al.*, 2003), and that behavior can endogenously elicit changes in extrasynaptic dopamine (Wightman and Robinson, 2002). Our results are similar to the perisomatic immunolabeling of the D5 receptor reported by Paspalas and Goldman-Rakic (2004), who used electron microscopy to demonstrate that the receptors were localized to microdomains specialized for dopamine volume transmission.

The D3 receptor's high (25 nm) affinity for dopamine (Sokoloff *et al.*, 1990) may endow it with a special role in monitoring the expectation of reward under uncertainty (Sokoloff and Schwartz, 2002). When reward is uncertain, the dopamine neurons in the ventral tegmental area exhibit a steady ramp-like increase in activity associated with excited expectancy culminating in the receipt or non-receipt of the reward (Fiorillo et al, 2003). Receptors of varying affinity, such as the D3 and D5 receptors on the von Economo neurons, may serve to encode different aspects (i.e., uncertainty, error, and expected value) of the reward signal. This is also consistent with fMRI activations in ACC and FI during decision making under uncertainty (Critchley et al, 2001), as well as with recent pharmacological, genetic, and behavioral studies that implicate the D3 receptor in motivation when response requirements are high and in self-administration of rewarding drug stimuli in response to environmental cues (Le Foll *et al.*, 2005). These paradigms – high response requirements, and learning based on contextual cues – increase the level of complexity in the task, and, consequently, the uncertainty that reward will be obtained. Interestingly, in humans, performance on an intuitive probabilistic learning task varies depending on a polymorphism in the D3 receptor, a result consistent with the rodent studies (Keri *et al.*, 2005).

2.5.2.2 Serotonin 1b and 2b receptors

As a neurotransmitter in the CNS, serotonin is involved in a plethora of cognitive processes, including mood, anxiety, pain, and aggression. The breadth of these various processes may parallel the breadth of experience that occurs in the periphery, where it mediates smooth muscle growth, blood vessel constriction, and platelet aggregation, among other things (Fozard and Saxena, 1991). Indeed, it has been estimated that 90% of

the body's serotonin is manufactured not in the CNS, but in the chromaffin cells of the gastrointestinal system (Bueno, 2005). Behaviorally, serotonin is known to be involved in aversive responses, which has led to development of models in which serotonin mediates the punishment component to learning in a manner complimentary to the encoding of reward by dopamine (Daw et al, 2002). This model supports the hypothesis that the VENs participate in a circuit involved in adaptive decision making, because they, as well as a subpopulation of the surrounding neurons, strongly express at least two types of serotonin receptor.

The VENs express the 5HT1b serotonin receptor on their somas and proximal dendrites. Although we did not explore whether this type of receptor was coexpressed with dopamine D3 receptors on the same cell, it seems likely that this is the case, given its widespread presence in layer 5 neurons and that activation of this receptor is known to inhibit dopamine release in striatal synapses (Sarhan *et al.*, 1999; Sarhan *et al.*, 2000). This particular receptor subtype appears to mediate behavioral inhibition. Application of a 5HT1b antagonist or knocking out the receptor increases aggressive behavior (Bouwknecht *et al.*, 2001) and sex drive (Rodriguez-Manzo *et al.*, 2002) in rodents, while applying an agonist to the receptor has inhibits both types of behavior (de Almeida et al., 2001; Fernandez-Guasti and Rodriguez-Manzo, 1992).

Less is known about the 5HT2b receptor, the other serotonin receptor that we found to be strongly expressed in VENs. This may be because this receptor is relatively rare in the central nervous system (Baumgarten and Göthert, 1997), although it is strongly expressed in the human stomach and intestines. The gastrointestinal serotonin 2b receptor promotes contractions of the smooth muscles responsible for peristalsis, a

role which appears to be restricted to humans (Borman et al., 2002). Serotonin may serve as an antagonistic signal to dopamine, with serotonin signaling punishment and dopamine signaling reward (Daw et al, 2002). The activation of the serotonin 2b receptor on Von Economo neurons might be related to the capacity of the activity in the stomach and intestines to signal impending danger or punishment (literally "gut feelings") and thus might be an opponent to the dopamine D3 signal of reward expectation. The outcome of these opponent processes could be an evaluation by VENs of the relative likelihood of punishment versus reward and contribute to "gut level" or intuitive decision making in a given behavioral context. ACC and FI are known to have an important role in interoception or the conscious awareness of visceral activity (Craig, 2004). Indeed, evidence shows that an insult to the periphery, such as a challenge to the immune system, can result in a change in serotonin level in the frontal cortex (Gardier *et al.*, 1994). Conversely, the duration of social interaction, which requires rapid, context-dependent behavioral adaptation, is altered via manipulations of the 5HT2b receptor in rats (Duxon *et al.*, 1997). In his theory of "somatic states," Damasio (1995) proposed that this monitoring of sensations arising from the gut is crucial to adaptive decision making. The presence of a serotonergic receptor type on the von Economo cells that is otherwise rare in the brain but strongly expressed in the intestinal tract suggests an interesting extension of the concept that these areas are monitoring activity in the gut. Perhaps the expression of the serotonin 2b receptor on the von Economo cells represents a transposition of this monitoring function from the gut into the brain, which would enable the organism to react much more rapidly to potentially threatening circumstances than if that individual depended solely on sensations arising from the gut. In other words, the strong serotonin

2b expression in the von Economo cells could be a model of the gut reaction in the brain that could enhance rapid decision making.

This immunohistochemical profile dovetails nicely with the fMRI literature, which, due to the highly localized distribution of the VENs, also provides information regarding the types of behaviors that involve the VEN regions. For example, ACC and FI are active when subjects make decisions under a high degree of uncertainty (Critchley et al, 2001). These areas are involved in the subjective experience of pain (Singer et al, 2004a), which is powerfully magnified by uncertainty. These areas are also active when subjects experience guilt, embarrassment and engage in deception (Shin et al, 2000; Berthoz et al, 2002; Spence et al, 2001). ACC and FI are also active in humor (Allman et al., 2005), trust, empathy, and the discrimination of the mental states of others (Singer et al, 2004a; Singer et al, 2004b, Baron-Cohen et al, 1999). All of these social emotions are influenced by the degree of uncertainty involved.

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. They can thus be seen as an adaptation supporting the increased complexity of hominoid and especially human social networks. This is reflected in evidence that the capacity for empathy is better developed in chimpanzees than in monkeys (Preston and DeWaal, 2002). We hypothesize that the VENs and associated circuitry enable us to reduce complex social and cultural

dimensions of decision making into a single dimension that facilitates the rapid execution of decisions. Other animals are not encumbered by such elaborate social and cultural contingencies to their decision making and thus do not require such a system for rapid intuitive choice. We are not suggesting that animals lacking VENs lack intuition, but rather that the VENs are a specialization that facilitates rapid intuitive decisions in complex, often social situations.

2.6 Acknowledgments

We thank Sarah Teegarden for her help with the histology and immunohistochemistry, Patrick Codd for help with the Neurolucida tracing, and Dr. Bill Yong for providing us with the specimens for the pilot studies. This work was funded by the Gordon and Betty Moore Foundation, and the Swartz Foundation.