

# THE VON ECONOMO NEURONS: FROM CELLS TO BEHAVIOR

Thesis by

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## **Abstract**

The von Economo neurons are one of the few known specializations to hominoid cortical microcircuitry. The recent emergence of this cell type, as well as its localization to subregions of the frontal cortex, suggest its involvement in sophisticated cognitive behaviors. Studies of this cell may thus provide insights into human uniqueness and origin and may additionally be relevant to the treatment and understanding of mental illness.

The first section of this thesis investigates the anatomical details of these cells, including their structure and surface receptor expression. Using a Golgi preparation of a human postmortem brain, I 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 trees with symmetric apical and basal components. This confirms that the von Economo cells in both ACC and FI share the architectural characteristics of a single population, and that this population is distinct from other layer 5 neurons. I additionally 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 serotonin-2b receptors. Together, these results provide the first detailed anatomical description of a neuron type unique to great apes and humans.

In the second part of this thesis, I explore whether a behavioral stimulus, humor, activates the regions in which this cell occurs. Humor is a hallmark of social discourse and usually depends on the convergence of fast, intuitive assessments with a slow “re-interpretation” of the humor. Because of these characteristics, we thought it likely that humor would activate FI and ACC in addition to other regions in the brain. I used event-related fMRI to differentiate brain activity induced by the hedonic similarities and cognitive differences inherent in cartoons depicting two kinds of humor: visual humor (sight gags) and language-based humor. I found that the brain networks recruited during a humorous experience did indeed include FI and ACC, and that the profile of activation differs according to the type of humor being processed.

Taken together, these projects significantly expand on our knowledge of these unusual cells, and provide a basis that allows us to hypothesize about their function. In the conclusion of this paper, we propose that the role of the von Economo neurons is to facilitate fast decision making in the context of high uncertainty, such as during social interaction.

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## Abbreviations

5-HT—5 hydroxy-tryptamine, also called serotonin

ACC—anterior cingulate cortex

BOLD—Blood oxygenation level dependent

DA—dopamine

FI—fronto-insula cortex

fMRI—functional magnetic resonance imaging

VEN—von Economo neuron

“Each has his own tree of ancestors, but at the top of all sits Probably Arboreal.”

—Robert Louis Stevenson

“The Astonishing Hypothesis is that ‘You,’ your joys and your sorrows, your memories and your ambitions, your sense of personal identity and free will, are in fact no more than the behavior of a vast assembly of nerve cells and their associated molecules.”

— Sir Francis Crick, The Astonishing Hypothesis

# **1 Introduction**

## **1.1 Hominoid brain evolution**

Humanity resides in the human brain, and, as with any other biological organ, the human brain is shaped by evolution. Because the modern human brain only exists by virtue of the adaptations of our primitive ancestors, it shares features with living nonhuman primates. By taking an evolutionary approach towards the study of the human nervous system, we may begin to see what general features we have in common with our closest relatives, as well as how humans are, neurologically speaking, special.

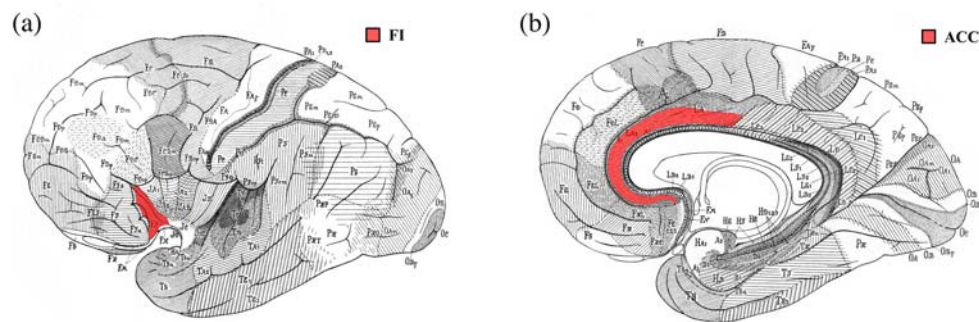
Of the 181 known species of living primates, only five make up the family known as the hominoids: the humans and great apes (Nusbaum et al., 2006). Behaviorally, the great apes are diverse: orangutans are arboreal while gorillas are terrestrial; bonobos are peaceful while chimpanzees are aggressive; gorillas are polygamous while humans are monogamous (or, at least, less promiscuous than bonobos and chimpanzees). There is no

unifying characteristic that defines great apes in terms of social structure, diet, or sexual behavior. As a family, the great apes appear to be more intelligent than the simians, though this has been notoriously difficult to demonstrate in a laboratory setting. It has been fairly well established, however, that great apes use tools, have some form of self-recognition, and transmit culture; and though these traits are by no means unique in the animal kingdom, they have been demonstrated more consistently in the hominoid family than in any other (Biro et al., 2003; Breuer et al., 2005; de Veer et al., 2003; Nusbaum et al., 2006; Sanz et al., 2004; van Schaik et al., 1999; van Schaik et al., 2003; Whiten et al., 1999). In addition, African apes and humans live in dynamic, highly complex social groups, a characteristic that is extremely difficult to quantify but likely to be an important factor in the evolution of general intelligence. It appears that the brains of great apes and humans have evolved to be flexible and adaptive, capable of identifying optimal responses in the context of a multitude of different circumstances and environments. This cognitive feature seems the most prominent in humans, and is responsible for our colonization of every habitable niche, even at the expense of our fellow primate species (Caldecott and Miles, 2005).

What biological structures underlie the flexible, intelligent behavior of the hominoids? Previous speculations that humans are characterized by large frontal lobes have been replaced by empirical evidence that disproportionately large frontal lobes are, in fact, characteristic of the hominoids (Semendeferi and Damasio, 2000; Semendeferi et al., 1997; Semendeferi et al., 2002). Recent exciting studies designed to identify elevated gene expression or mutation rate in humans have not only identified genes that are likely to have contributed to this frontal lobe expansion (Nusbaum et al., 2006), but also genes

associated with metabolism and synaptic plasticity (Caceres et al., 2003). This line of evidence serves as a useful starting point, so that other experimental methods can be employed to explore the functional and anatomical repercussions of these genetic changes, one case of which is explored elsewhere in this thesis (see section 5.2).

In addition to these known differences in gross brain anatomy and genetics are those internal characteristics of the brain that separate the human minds from the apes, and the hominoids from the simians. Perhaps surprisingly, very few differences have been identified on the cellular or molecular scale. In general the microstructure of brains is surprisingly homogeneous across mammalian species.<sup>1</sup> Cajal's pioneering work in the 1800s resulted in the neuronal doctrine, which states that the neuron is the basic anatomical unit in the brain, and that information flow in the brain is in the form of chemical and electrical messages that pass from neuron to neuron. The circuits formed by populations of neurons throughout the brain are the biological substrates that underlie behaviors. Thus, one might expect systematic differences in this circuitry from species to

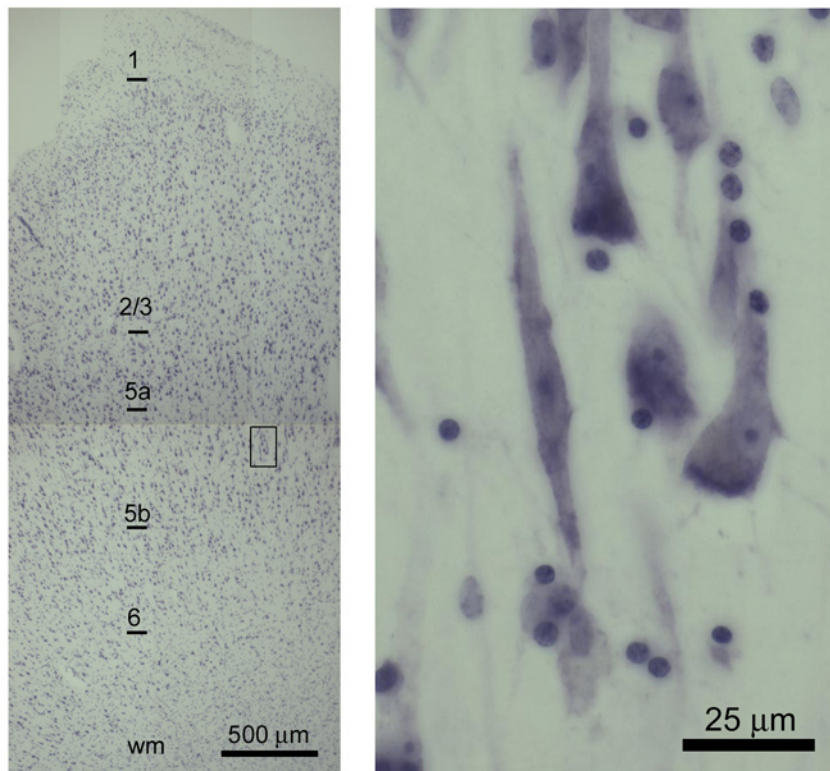


**Figure 1.** Location of the von Economo neurons in the human brain. (a) Lateral view of the brain with left anterior and fronto-insula (FI) demarcated in red. (b) Medial view of the brain with left anterior and anterior cingulate cortex demarcated in red. Illustrations from von Economo and Koskinas (1929) modified by Atiya Hakeem.

<sup>1</sup> This is a disadvantage if one is looking for an obvious “neural correlate” of human brainpower, but an advantage if one wants to use species other than humans to study how the brain works and how to cure diseases in the brain.



species that correlates with differences in species-specific behavior. In some cases these differences are documented, such as in the primary visual region (Preuss et al., 1999; Sherwood et al., 2003), but these cases are the minority. Indeed, in the frontal lobe—still relatively mysterious but known to be crucial in planning, decision making, behavioral inhibition and social interaction—our knowledge of species-specific differences is sparse.



**Figure 2.** Cresyl-violet stained anterior cingulate in a 53-year-old male human. (a) Low-power photomicrograph montage. (b) z-projection of six 1  $\mu\text{m}$  slices collapsed into one depth plane. A single von Economo neuron, center, surrounded by several pyramidal neurons. Photomicrograph taken from boxed area in (a).

## 1.2 The von Economo neurons

In 1999, Nimchinski and colleagues reported a type of cell that they identified as unique to great apes and humans. At the time, they termed this population the “spindle cells,” but to avoid potential confusion with other uses of this name we now refer to them as “von Economo” (VE) cells.

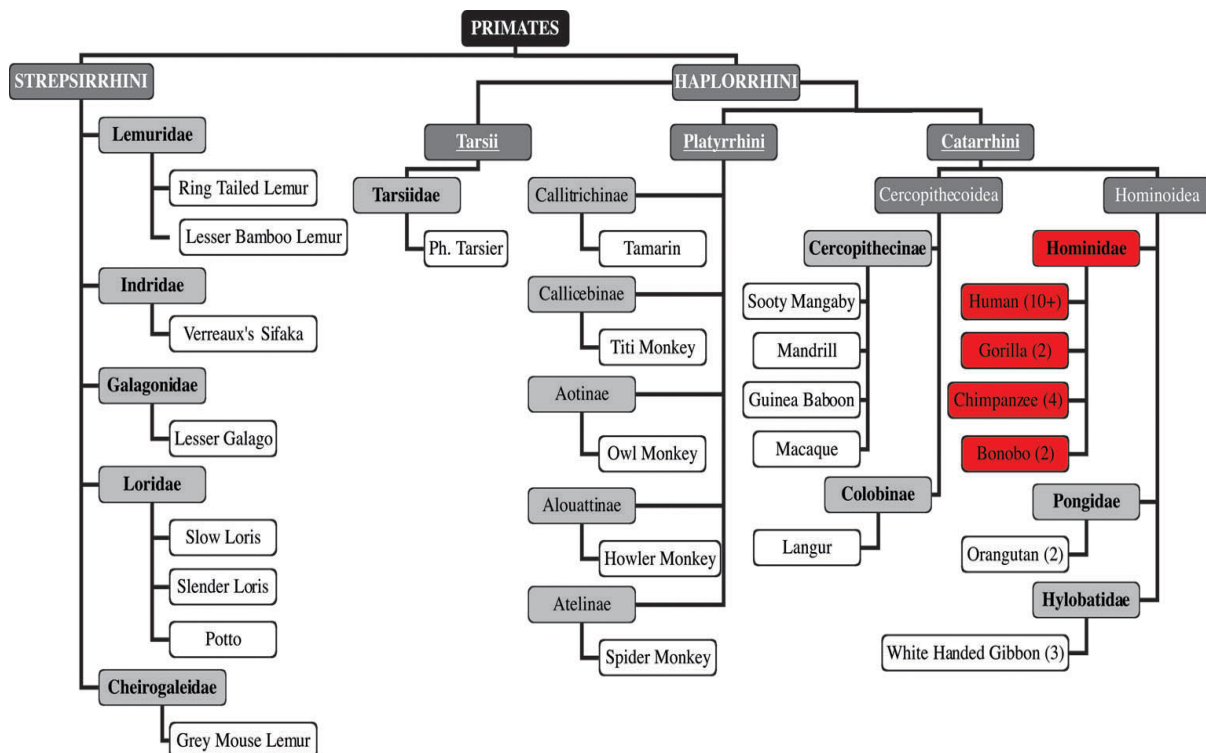
This name is chosen in honor of the neuroanatomist Constantin von Economo, who is the first author of the original 1925 book that contains the classical description of this distinctive class of neurons. Upon inspection of his Golgi preparations of human

cortex, he noted that these large cells were located in layer 5 and restricted to two regions of the human brain: the anterior cingulate cortex (ACC) and in posterior orbitofrontal cortex adjacent to the insula, a region that he termed “fronto-insular” cortex (FI, figure 1). Both of these regions lack a granular layer 4; as in motor cortex, this agranularity may reflect a functional specialization.<sup>2</sup>

In a cresyl violet (Nissl)-stained sample of human or great ape cortex, these cells may be easily distinguished from the neurons around them due to their symmetric, bipolar soma shape and their large size (Figure 2), and it was on the basis of such stains that the phylogenetic uniqueness was determined. The VENs of the anterior cingulate cortex are present in all four living species of great apes and the humans, which implies that they evolved within the last 15 million years (Figure 3). The Von Economo cells in the fronto-insular cortex are present only in the great African apes, and not the orangutan (Allman et al., 2005). This pushes the likely emergence of Von Economo cells in that region to 9 million years ago.

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<sup>2</sup> In general, layer 4 is a granular layer that receives input from the thalamus, and layer 5 contains large neurons that project to other cortical and subcortical regions. It follows that sensory cortex has a very large layer 4, whereas motor cortex lacks layer 4 altogether.



**Figure 3** Primate cladogram. Species with VENs in both ACC and FI are in red. The orangutan, the only living non-African great ape, has VENs in ACC but not FI. Number in parenthesis indicates the number of specimens counted stereologically by N. Teatreault and J.Allman. Figure prepared by Atiya Hakeem.

Relative to other neuronal populations, the VENs develop late in ontogeny as well as phylogeny. They first appear at the 35th week of gestation and only about 15% of the full complement is present at birth (Allman et al., 2005). The adult number is attained by 4 years of age. Whether the VENs emerge by differentiation or migration, there is the possibility that their emergence might be disrupted during postnatal development with dysfunctional consequences.

In all of the great apes and post-natal human brains, the VENs are more numerous in the right FI and ACC than the left (Allman et al., 2005). This hemispheric asymmetry appears to arise after birth, as the VEN are about 6% more numerous in the right hemisphere in the neonate but about 30% more numerous in the adult. This right hemisphere VEN predominance may be related to the right-hemispheric specialization for

the social emotions (Blonder et al., 1993). The fact that this 30% right preference is so tightly regulated and consistent across humans and apes (past the infant period) suggests that this ratio is important for normal functioning and that deviations from it could be dysfunctional.

Little is known about the function of the von Economo cells, despite their unique phylogenetic lineage and their potential importance to human brain pathology. The very features that make these neurons so interesting also make them difficult to study with conventional techniques. Most experimental methods devised to explore single cell function and anatomy are invasive and ultimately require the sacrifice of the animal, which would obviously be inappropriate for the study of the VENs given that they are only present in great apes and humans. However, VENs are a specialization of the circuitry that had been present in a common ancestor to the great apes, and is currently present in other modern anthropoids. Thus, studies of FI and ACC in monkeys can, to some extent, inform our assumptions about the function of the VENs

Monkey studies using anterograde and retrograde tracers indicate the 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).<sup>3</sup> It is more difficult to localize the region analogous (or homologous) to FI in monkeys, simply because of the absence of definitive cortical landmarks.<sup>4</sup> Agranular

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<sup>3</sup> In fact, because these connections are characterized by afferents from multimodal sensory-integration- regions and decision making- regions, and by efferents to motor areas, Francis Crick was moved to speculate that ACC is the site of “free will”! (1994)

<sup>4</sup> Anterior cingulate is always easy to recognize, since by definition – “cingulate” stems from “cingere,” meaning “girdle” in Latin – this region wraps around the corpus callosum, the single most recognizable cortical structure in the brain. FI, on the other hand, is one of many protuberances in the lumpy cortical mantle, and the macaque brain is considerably less lumpy than the great ape or human brain. This rules out

regions of anterior insula that extend into orbitofrontal cortex do exist in macaques, however, and these are extensively connected with medial temporal and cingulate limbic structures. (Carmichael and Price, 1994; Carmichael and Price, 1995; Carmichael and Price, 1996). Thus, the regions in monkeys that are presumably homologous to those containing the VENS in hominoids are coupled to each other anatomically.

### **1.3 Rationale of the approach.**

The spatially localized nature of the VENS, in conjunction with the known modularity of the brain, gives us another advantage in guessing their function. The recent explosion of functional magnetic resonance imaging (fMRI) studies has enabled us to identify those paradigms that activate the von Economo regions. This approach is particularly useful in the case of FI, which seems to be more selective than ACC, the latter of which is active in almost every behavior that involves intense concentration or emotion. In fMRI studies, FI and ACC are coactivated by two broad classes of stimuli: Those that involve decision making in the context of high uncertainty, and those that involve social stimuli.

In order to link the VENS directly to behavior, we can use the fMRI literature in conjunction with pharmacological literature. That is, once we know what sort of behaviors the circuits might be involved in, we can look for molecular agents that might mediate those behaviors. Once we have some likely candidates, we probe for these receptors or molecules using specific antibodies, which subsequently allows us to determine whether these molecules act directly on the VENS.

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a simple 1:1 mapping of cortical regions. Furthermore, the outstanding cytoarchitectonic feature of FI is that it contains von Economo cells, which are not present in monkeys. This makes FI impossible to delineate cytoarchitectonically in the monkey brain!

The von Economo cell regions appear to be strongly activated during periods of high uncertainty. In an fMRI study during which subjects were engaged in a simple gambling task, activation in both FI and ACC got increasingly stronger as the uncertainty in the task increased (Critchley et al., 2001). In a similar vein, both regions were activated during a reversal task, in which a subject attempts to maximize reward during a task that changes contingencies when the subject's behavior stabilizes (O'Doherty et al., 2003a). A series of incorrect answers will prompt the subject to switch strategies, at which point both ACC and FI show increased activity. Recordings from individual dopaminergic neurons in the macaque monkey ventral tegmentum reveals a similar pattern of activation. During trials with high uncertainty of reward, dopamine neurons exhibit a gradual increase of firing rate across the duration of the trial (Fiorillo *et al.*, 2003). Dopamine is known to be involved in reward delivery and expectation, as well as contingency learning. This leads us to probe for the various types of dopamine receptors, especially D3, the high-affinity dopamine receptor, which is known to have a limbic distribution and is implicated in mechanisms of drug addiction (Le Foll et al., 2005). Additionally, because of models that suggest that dopamine and serotonin act in opposition to one another during learning, we probed for several types of serotonin receptors (Daw et al., 2002). Because the serotonin receptor class is so large – there are thirteen different types – and because antibodies were only available for a subset of these receptors, we were not able to perform an exhaustive survey of all serotonin receptors. However, in probing five of the receptor subtypes, we did identify several that labeled the VENS, with interesting implications.

Golgi is an old technique and was used by both Cajal and Golgi for the work that earned them the Nobel prize in 1906. Although the technique is notoriously capricious, we found that the appropriate protocol, when combined with a consistent source of brain tissue, yielded beautiful results, which I describe and quantify in Chapter 2. This work allowed me to describe the dendritic architecture of the von Economo neurons, and to compare it with that of their layer 5 pyramidal counterparts. A computational study by Mainen and Sejnowski in 1996 showed that variations in the morphology of the cell can have a large effect on the firing profile of the neuron. Knowing the dendritic morphology of the VENs may thus allow future computational studies (in progress by Sejnowski's group) to project the likely firing pattern of the VENs

Finally, in chapter 3 I describe my own contribution to the fMRI literature, a study on the neurobiology of humor. Because humor is essentially an error response coupled with emotional arousal, and because it is so frequently used in social circumstances, we hypothesized that humor would activate the von Economo regions. We did indeed find evidence supporting this hypothesis, and, in addition, report a novel result that contrasts cognitive differences and affective similarities during the perception of two different types of humor.