Large layer V neocortical pyramidal neurons express elevated levels of oxidative metabolic activity in several carnivore and primate species.

Authors: Yaman Sebai, Clara Guirguis, Sophie Biehler, Ayah Elsamad
Faculty Advisor: Dr. Glenn Kageyama
Department of Biological Sciences

CalPolyPomona College of Science

Kellogg Honors College — A Community of Scholars

Abstract

The mammalian neocortex, is comprised of six layers that differ by neuronal types, level of metabolic activity, and patterns of interconnectivity. We investigated layer V of the neocortex due to the presence of larger pyramidal neurons that have been shown previously to exhibit higher levels of metabolic activity in the cat visual cortex. Cytochrome c oxidase (CO) a large mitochondrial protein complex is the terminal electron acceptor in cellular respiration and is required for the generation of most of a cell's ATP. CO staining is considered the standard for assessing the relative levels of neuronal oxidative metabolic activity. Preliminary findings have shown that there is a distinct pattern of distribution of CO-rich neurons within the neocortex of many different species of mammals, a detail largely overlooked. We conducted a morphometric analysis via enzyme histochemical CO-stained cortex tissue of several carnivore and primate species and measured the diameter of CO reactive and CO unreactive neurons within Layer V. Our results indicate that the darkly CO-reactive pyramidal neurons are significantly larger (+52% diameter) than the neighboring smaller and lighter staining neurons. We theorize these larger, more reactive pyramidal neurons fall under the classification of "Meganeurons," highly metabolic and physiologically active projection neurons, found in specific cortical areas throughout the brain and also tend to be more prominent in higher-level cognitive species. Loss of these neurons has been associated with neurological diseases such as Alzheimer's disease, frontotemporal dementia (von Economo neurons), and ALS (Betz cells), two specific types of mega neurons. We suspect studying these neurons is vital to furthering our understanding of these important neurological diseases.

Introduction

The cerebrum is one of the three main components that compose the human brain. This part of the brain can be further divided into the outer, grey (cellular and synaptic layer) and inner, white (axonal layer) matter [4]. This color difference is largely due to the presence of the neuronal cell bodies (somas) in the outer layers (neocortex) and myelinated axons within the inner (white matter) layers. The expansion of the outer layer, also known as the cerebral cortex is one notable attribute of advanced cognition in mammals [3]. However, this explanation does not suffice to answer the question of human intelligence. One prominent feature to explain the complexity of the mammalian brain is the presence of Thick-Tufted Layer V (TTL5) cells, a subtype of large pyramidal neurons. The TTL5 cells are remarkably larger and more complex than other pyramidal neurons, based on their more extensive dendritic branching patterns. Neocortical pyramidal cells are characterized by a single apical spiny dendrite and multiple basal spiny dendrites that emerge from the soma of a pyramid-shaped cell body. Each spine on the spiny dendrites receives synaptic input from other neurons. These are then integrated across the cell body and converge onto the neuron's axon initial segment, where action potentials are initiated. Once initiated the action potential self-propagates along the axon and all of its many collaterals and terminal branches where they synapse with hundreds to thousands of dendrites or dendritic spines of other neurons. In neurons, the primary source of energy comes from oxidative metabolism within the mitochondria, while glial cells appear to derive most of their energy from glycolysis [5]. A popular view, even amongst neuroscientists, is that most of the oxidative metabolic activity in neurons is due to the electrophysiological activity that neurons are noted for. Based on this view, many neuroscientists expect that axons that conduct action potentials and axon terminals that synthesize, release, and recycle neurochemicals for synaptic transmission should have the highest levels of oxidative metabolic activity. However, based on electron micrographic findings from the Kageyama and Wong-Riley labs, mitochondria have been observed to be concentrated primarily within the dendrites of neurons, rather than the cell body, axon, or axon terminals of neurons. Cytochrome c Oxidase (CO), a key transmembrane mitochondrial protein complex in mitochondria is used to assess oxidative metabolic activity within neurons. TTL5 cells are hypothesized to exhibit the highest amounts of CO staining based on early findings that only two types of neurons exhibit elevated levels of CO in the visual cortex, large layer V pyramids and sparsely distributed large nonpyramidal (stellate) interneurons [2]. The most surprising observations were the relatively low levels of CO observed in most axons and axon terminals, except for some excitatory axospinous terminals located in layer IV, and some axosomatic inhibitory terminals. Even more surprising is the relatively low levels of CO observed in the somata of most pyramidal neurons and smaller interneurons. The lower levels found in astrocytes, on the other hand, were expected [1]. TTL5 cells have been described in many areas of the neocortex and in some areas have been given special names. In the visual cortex, they are known as Meynert cells. In the motor cortex, they are called Betz cells. In the anterior cingulate gyrus and frontal insular cortex, a special type of TTL5-like neurons has been described that takes on a bipolar (von Economo neurons) or triangular (Fork neurons) shape. These specialized neurons appear to be found mostly in the highest cognitive species of mammals such as the pachyderms (elephants), cetaceans (dolphins and whales), and hominids (apes and man). The use of cytochrome oxidase histochemistry as a marker for metabolic neuronal activity allows us to assess the functionality and importance of these large pyramidal neurons. By assessing their distribution among various species of mammals, we hope to observe a pattern shown in previous studies that would assist in further researching these neurons.

Methods

A quantitative analysis of cytochrome oxidase (CO) stained paraffin-embedded tissue of the visual cortex (VC), the motor cortex (MC), and somatosensory cortex (SC) was performed on cat, ferret, and mink species. These slides were obtained from past work that Dr. Glenn Kageyama produced through his academic career. Leica LAS EZ Software was used to obtain 100-400x images of these slides for students within the lab to analyze. Diameter was obtained using ImageJ software via using the freehand tool to outline up to 5 dark, moderate, and light-stained neurons. Measurements were calibrated using a 100 µm scale bar. Yaman Sebai then collected the data and analyzed the data collected into graphs and summaries.

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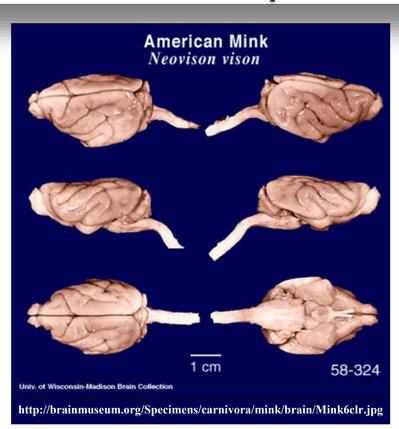

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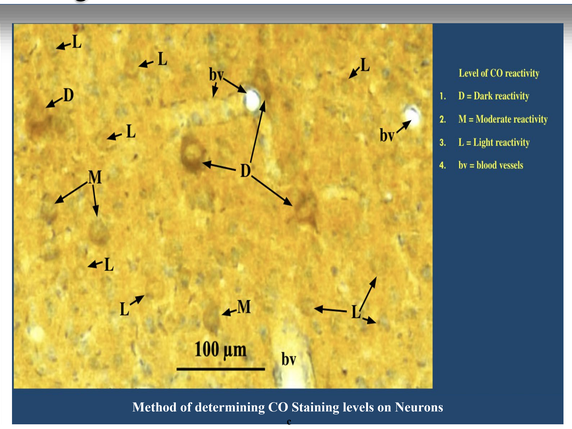
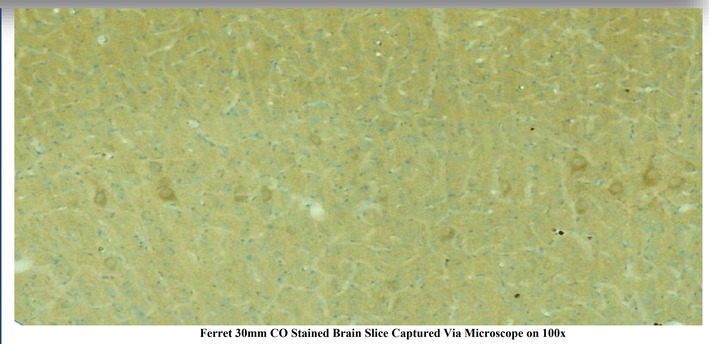
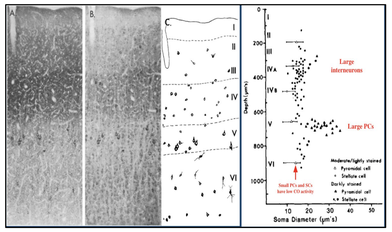


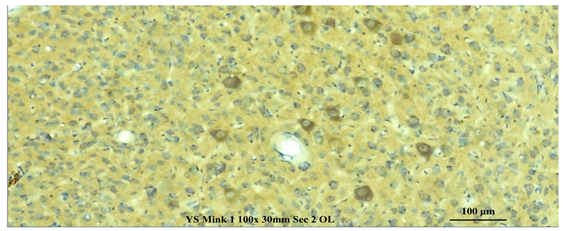
Method of determining CO Staining levels on Neurons



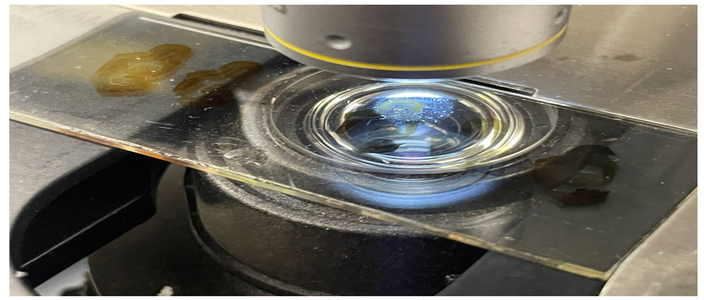
Ferret 30mm CO Stained Brain Slice Captured Via Microscope on 100x



Kageyama GH, Wong-Riley M (1986) Laminar and Cellular Localization of Cytochrome Oxidase in the Cat Striate Cortex, J Comp Neurol 245, 137-159.



Mink 30mm CO Stained Brain Slice Captured Via Microscope on 100x



Mink cytochrome oxidase (CO) stained paraffin-embedded brain tissue

RESULTS

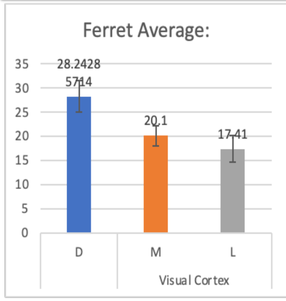


Figure 1. Neuronal Diameter (µm) ± SE of Ferret in Visual Cortex CO Stain

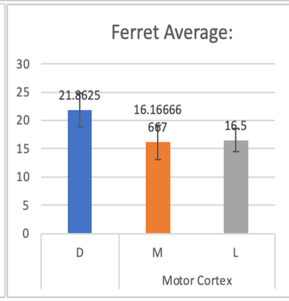


Figure 2. Neuronal Diameter (µm) ± SE of Ferret in Motor Cortex CO Stain

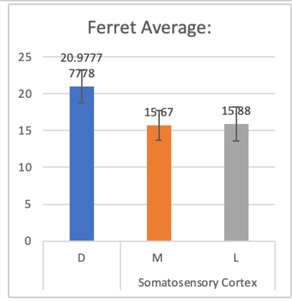


Figure 3. Neuronal Diameter (µm) ± SE of Ferret in Somatosensory Cortex CO Stain

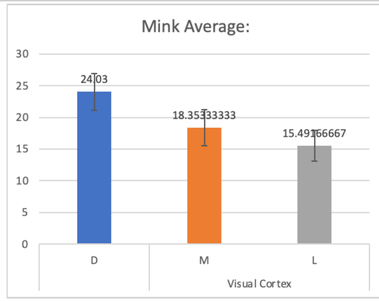


Figure 5. Neuronal Diameter (µm) ± SE of Mink in Visual Cortex CO Stain

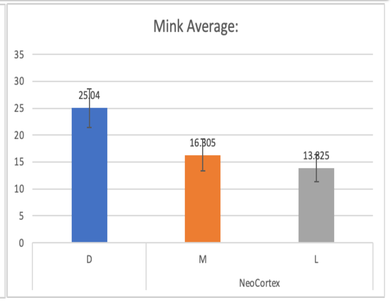


Figure 6. Neuronal Diameter (µm) ± SE of Mink in Visual Cortex CO Stain

Ferret:		Visual Cortex		Ferret:		Motor Cortex		Ferret:		Somatosensory Cortex	
	D	M	L		D	M	L		D	M	L
Mean:	28.2	20.1	17.4	Mean:	21.9	16.2	16.5	Mean:	21.0	15.7	15.9
STDEV:	3.2	2.1	2.7	STDEV:	2.9	3.0	2.1	STDEV:	2.2	2.0	2.3

Table 1. Neuronal Diameter (µm) Mean and STDEV of Dark, Medium, and Light CO Stained Neurons in the Visual, Motor, and Somatosensory Cortices of a Ferret

Mink:		Visual Cortex		Mink:		NeoCortex	
	D	M	L		D	M	L
Mean:	24.0	18.4	15.5	Mean:	25.0	16.3	13.8
STDEV:	2.9	2.9	2.4	STDEV:	3.6	2.9	2.5

Table 2. Neuronal Diameter (µm) Mean and STDEV of Dark, Medium, and Light CO Stained Neurons in the Visual and Neo Cortices of a Mink

Results

Our results showed that the darkly stained neurons were ~52% larger in diameter than the lightly stained neurons in the same layer. This statistic was validated using a t-test where we received a p-value of 0.0003268242, with a significance level of 0.05. Thus, indicating large statistical significance from this data set.

Discussion

Our study shows that pyramidal neurons larger in diameter tend to be more darkly stained compared to their smaller counterparts. This greater intensity of CO staining shows that, as in the cat, these larger neurons exhibit higher functional activity within Minks and Ferrets, a trend that has been shown also to be true in various primate species as well. This increased metabolic activity in TTL5-type neurons of layer V in several species of primates and carnivores (this study) indicates these neurons, may be vital for higher levels of cognitive processing that would be required for species that rely more heavily on these abilities for their survival. The TTL5 "Meganeurons" are also hypothesized to be important for understanding various neurological diseases due to the associated cognitive decline that is associated with their degeneration in such diseases as frontotemporal dementia and Alzheimer's disease (VEN's) and ALS (Betz cells). Studying them more extensively will prove to be important to further our understanding of such conditions.