Running Head: PYRAMIDAL NEURONS IN BROCA

Quantitative Analysis of Specific Structures of Magnopyramidal Cognitive Neurons in Broca's Region

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Abstract

Magnopyramidal neurons of a deep IIIC human cortical layer have a major role in important cognitive functions such as memory, language and thinking. This study looks at quantitative measurements of twenty post-mortem neurons impregnated with GolgiCox solution from IIIC layer of left and right Broca's region in male and female brains. After a 3D reconstruction of neurons, software extracting the data from the digital images was applied, and subsequently Minitab conducting paired t-tests was used to statistically analyze the hemispherical differences. The hypothesis for nine variables was predicting no statistical differences however, four variables significantly differed in the left from the right hemisphere. Limited implications can be given due to the small sample size and further research ideas are offered.

Introduction

Loss of cognitive pyramidal neurons in deep layer IIIC of the human cortex results in declination of cognitive functions such as memory, language and thinking, depending on the neuronal loss locality (Rajkowska, Selemon, & Goldman-Rakic, 1998). Many researchers have been concentrating on the qualitative characteristics of pyramidal neurons, but not on the quantitative factors (Kostovic, Judaš, Petanjek, & Šimic, 1995). In this study we will focus on quantitative parameters of neuron morphology in layer III in Broca's motor speech area (Brodmann area 45) of the left and right hemispheres of the human male and female brain. Results obtained from this study will lead towards a better understanding of neurobiology of human cognitive functions such as verbal memory and its pathology. For instance, Rajkowska, et al. (1998) found significant size declination of large pyramidal neurons only in layer III in schizophrenic patients.

In the next several paragraphs pyramidal neurons, Broca's region and development of layer III in the human brain will be defined. All mentioned factors are basic in understanding the nature of the current study. Review of related research will then follow.

Pyramidal Neurons in the Neocortex. There are three main parts of a neuron: its body – the soma, an axon and the dendrites. In neocortex (here also referred to as cerebral cortex or cortex), there are two types of neurons: spiny neurons (pyramidal and spiny non-pyramidal) and aspiny non-pyramidal neurons (DeFelipe & Fariñas, 1992). Here, spiny pyramidal neurons will be discussed.

Pyramidal neurons usually have long axons and are found in all six layers of cortex except the first one. They comprise up to 85% of all neurons in the cortex. These neurons are projection neurons of the cortex and are subdivided accordingly. Not all pyramidal cells look the

same, each displaying different morphology. This includes size, shape, dendritic branching, density of spines, pattern of axon's collaterals and site of their projection, as already mentioned (DeFelipe & Fariñas, 1992).

DeFelipe & Fariñas (1992) divide pyramidal neurons into two more categories: typical pyramical cells and atypical. Morphology of a typical pyramidal cell includes three factors. First, the cell's soma is shaped as a pyramid. It has an apical dendrite stretching from the upper pole toward the pia mater and is branched. From the other side of the soma, there are other dendritic branches which stretch in the opposite direction from the apical dendrite. Next, the axon also stretches from basal part of the soma, in a direction opposite from the apical dendrite and can give a few collaterals: near the soma, above or below the layer the soma is in, or horozontally in the same lamina but with some distance. The third factor includes spines which are found on dendrites and dendritic branches but are not found on the soma and immediate segment of the dendrite that arises from the soma.

Atypical pyramidal neurons, according to DeFelipe & Fariñas (1992), show four distinguishing factors: pattern of dendritic branches, shape of the soma, spine density and the axon. DeFelipe & Fariñas give six examples of atypical cells. First, they describe cells in layer II which have a short apical dendrite (if they have one at all) and usually a larger soma. Second, there are cells in layer V which have more than one apical dendrite. Usually, other apical dendrites in this cell arise from one dendrite and are described as radial branches. The next example are cells located in layer VI which show different shapes of somas (ovoid, fusiform). The apical dendrite, which is mostly spiny, does not go beyond the fourth layer. The fourth example the authors give for atypical pyramidal cells are those which have little or no spines. They can be found again in layer V. Interestingly, these cells, when compared to spiny pyramidal

cells, have greater velocity of axon conduction. Furthermore, there are cells whose axons do not have collaterals. As the authors (DeFelipe & Fariñas, 1992) indicated, axon's collaterals appear around the first and second week after brith. These axons leave the grey matter and enter the white matter. The last example of atypical cells is of intrinsic cortical pyramidal neurons. Their axons do not leave the grey matter and can be found mostly in layers II, III and V in the cat's visual cortices.

In the current study I will observe the arborizations, tree-like branching arrangement of either a dendrite or an axon (Merriam-Webster, 2009), primarily on the dendrites. According to Uylings & van Pelt (2002), roles of the branches are reception and flow-conductance. Authors say the dendritic tree is usually easily distinguished from the soma. Each tree has a root, a branch point, a terminal tip and connecting segments (terminal and intermediate segments). Counting the number of segments in a tree is often indicative of the type of neuron as well as looking at the pattern of connectivity. Degrees are used to measure the number of sub-trees relative to the root tree. Usually, we will see a bifurcation of trees at the branch point which means that from one branch point we will have two sub-trees stretching out. To label the sub-trees, some researchers use the centrifugal order which is defined as a topological distance from the root, according to Uylings & van Pelt (2002). Figure 1 in the Appendix has been adopted from Uylings & van Pelt (2002) and presents centrifugal order of the segments. Figure 2, indicates segments in a centrifugal order on a pyramidal neuron.

Broca's area. Broca's area is commonly known as a primary cortical area for language production in human brains. It can be observed from two perspectives: gross anatomic and cytoarchitectonic. Paul Broca found importance of the third frontal convolution in language expression by observing patients with language disorders (Keller, Crow, Foundas, Amunts, &

Roberts, 2009). Two of his historical cases include brains of Leborgne and Lelong (Dronkers, Plaisant, Iba-Zizen, & Cabanis, 2007). Today, more common name for this part of cerebral cortex is the inferior frontal gyrus or Broca's area. Furthermore, it can be divided into 3 smaller regions based on the Sylvian fissure: posterior or Par opercularis, anterior or Pars triangularis and ventral or Pars orbitalis (Keller, et al., 2009). Broca's area is evident in both hemispheres of a human brain, but left hemisphere is dominant in 95% of population (Branche, Milner & Rasmussen, 1964, cited from: Amunts, Schleicher, & Zilles, 2004). Some researchers noted a certain morphological hemispherical asymmetry for this region however, a great variability exists among individuals and thus quantifications are impacted (Keller, et al., 2009).

Cytoarchitectonic observations look at laminae, and neuron density patterns at different locations (Grozdinsky & Santi, 2008). This implies that Broca's area is a part of a six layered neocortex, areas 44 and 45 according to Brodmann's cytoarchitectonic map. Some researchers (Amunts, Schleicher, Ditterich, & Zilles, 2003; Uylings, Jacobsen, Zilles & Amunts, 2006) searched for a difference in number of neurons in each of the Brodmann's areas (44 and 45) and have found different results.

Uylings, Malofeeva, Bogolepova, Jacobsen, Amunts and Zilles (2005) did a study on examining the number of neurons in BA 44 and 45. Previous studies that they took into consideration showed opposite results. Some research showed doubling of neurons in these areas after birth while other studies found opposing results. In their current study (Uylings et al., 2005), results support latter research that stated no doubling of neurons occurs in post natal years. In other words, some researchers did not find a significant asymmetry between two human hemispheres (Uylings, et al., 2006; Keller, et al. 2009) and others have found asymmetry in both areas BA 44 and BA 45 (Amunts, et al., 2003). Both BA 44 and BA 45 are, however, six-

layered. Layers III and V are known for their magnopyramidal (large pyramidal) neurons whereas layer IV is described as granular as it largely contains granular cells. In relation to the earlier statement, García, Montiel, Villalón, Gatica, and Aboitiz (2004) have shown the left-right asymmetry of magnopyramidal neurons in layer III, rich with acethycolinesterase only in BA 45.

Development of layer III. The human cortex has six horizontal layers which are different in their morphology, neurons that comprise them, function and finally, density. Layer III is the third such layer and is also called the pyramidal layer as it mostly consists of pyramidal neurons (Petanjek, et al, 2008). In the 32nd week of gestation layer III is being formed, according to Kostovic, et al. (1995). During the first postnatal month, the first dendritic growth occurs. Subsequently, there is a silent period which is then followed by a growth period in 2-year-olds (Petanjek, Judas, Kostović, & Uylings, 2008). Kostovic, et al. (1995) add that in the first postnatal year, chemical characteristics are changing in this layer. Cholinergic pyramidal neurons are developing and receiving a great role in commissural and associative projections. Thus, these neurons have a role in cognitive functions. Researchers also say that described development continues until an individual is a young adult and mention that this finding has only been seen in humans (see Kostovic, et al., 1995; Petanjek, et al., 2008)

The first study counting the number of neurons in BA 44 and 45 (Broca's area) was done by Uylings, et al. (2006). Researchers used Nissl-staining to look for differences in number of neurons in BA 44 and 45. Results yielded statistical significance in the BA 44 of the left hemisphere but only for male subjects. However, BA 45 turned out to be significantly larger in women's left hemispheres. Generally, the total number of neurons is greater in the left hemisphere but this asymmetry was not statistically significant (Uylings, et al., 2005).

Chronologically next study was done by Zeba, Jovanov-Milosevic and Petanjek (2008). Authors looked at the basal dendritic morphology of rapid Golgi and Golgi Cox impregnated cells in primary motor (BA4), associative mangnopyramidal (BA9) in the left hemisphere and BA 45 (Broca's area) of both hemispheres. For purposes of this review, the attention will be given to the results from Broca's area. Researchers looked at the soma cell surface, total dendritic length, number of basal dendrites, number of segments and spine density. They have found no statistical difference between the left and right Broca's area.

A more recent quantitative study of dendritic trees in human prefrontal cortex, distinguishing the cortical layers, already mentioned, was done by Petanjek, et al. (2008). Authors concentrated specifically on layers III and V. Researchers studied 25 human brains ranging from 1 week of age to 91 years of age, which had no medical record of psychopathology or neuropathology. Samples from superior and middle frontal gyri were used and only large pyramidal neurons of deep layer IIIC and layer V were used. For purposes of this analysis, we will consider author's results from layer III. Qualitative results showed that at birth, large pyramidal cells were still immature and without spines, but progressing to the third postnatal month, significant changes were reported. At the third month, cells appear to be the same as in adults concerning the branching, spine morphology and dendritic orientation (vertical, horizontal). As mentioned earlier, quantitative results show two periods of growth: around 3rd month and at the second year of age (biphasic pattern of dendritic growth). In the first phase, dendritic trees triple in size. But, there was not a great difference between a one-year old and an adult brain as most of the branches were already grown. The second phase involved increase in radial distance of terminal segments. So, there was a significant difference found between two-

year olds and 2.5-year olds. Researchers also report an increase in soma of a pyramidal neuron in the third layer. These changes occur in preschool children and afterwards in their adolescence.

In addition, Petanjek, et al. (2008) mention a study on nonhuman primates which has shown that pyramidal neurons in layer III are involved in working memory and cognitive functions of the prefrontal cortex. Disorders of those higher cognitive functions show loss in the layer III of prefrontal cortex. Furthermore, biological bases of individuality are particularly associated with density and morphology of large pyramidal areas, mostly located in layer III. Due to the amount of acetylcholine in early developmental years, we can see the two phases of dendritic growth, but after the second year, it is considered that growth occurs due to the social and environmental factors. Final stages of maturation can last until the person is even thirty years old

Amunts, et al. (2004) have conducted a study comparing an extraordinary language competent individual (E.K.) to 11 controls. Even though in the past, researchers used weight and obvious macroscopic patterns to infer about mental capacity, today new, cytoarchitectonic techniques are used. E.K. was interesting due to his ability to learn fast and speak fluently over 60 languages. His brain was post-mortem compared to 11 human male brains that comprised the control group. Cytoarchitectonic measures indicated significant difference in both left and right BA 44 and right BA 45 when compared to controls. Difference was also found in left BA 45, but was not statistically significant. This pattern of asymmetry was concluded to be unique for E.K. this research suggests that hemispherical asymmetry may be responsible for better or even worse cognitive functions.

Based on the research done in the field of Broca's region (Uylings, et al., 2006; Zeba, et al., 2008), the overall goal of this study was to question the differences in Broca's deep

layer IIIC between hemispheres of the human male and female brain. Research suggests that some mental illnesses such as schizophrenia show a statistical decrease in somal size in a deep layer III of prefrontal cortex (Pierri, Volk, Auh, Sampson, and Lewis, 2001). To test these findings, I have digitally reconstructed 20 neurons of deep layer III of Broca's region: ten neurons belonged to a normal male brain (five of the right hemisphere and five of the left hemisphere) and ten from a normal female brain (five of the right hemisphere and five of the left hemisphere). 'Normal' implies that there were no psychological disorders associated with the brain. Thus, these variables were testsed: (1) cell soma area, (2) dendritic length, (3) quantity of dendrites, (4) length of branched structures, (5) base diameter of branched structures, (6) average diameter of branched structures, (7) spine density of branched structures, (8) quantity of markers and (9) the number of segments.

I hypothesized no significant differences in all nine variables indicated above between hemispheres in both male and female brain.

Method

Histology. Slides from one female (40 years old) and one male (37 years old) adult, healthy brains were provided by the Croatian Institute for Brain Research in Zagreb, Croatia. The sample consists of twenty (20) magnopyramidal cognitive neurons taken from the third layer of Broca's motor speech area (BA44) of the left and right hemispheres of adult human male and female post-mortem brains. Five neurons were selected from each hemisphere (male: right, left; female: right, left).

Staining. Slides were previously impregnated by the Golgi-Cox solution consisting of potassium chromate, potassium dichromate and mercuric chloride (Zeba, et al., 2008).

Variables. The variables included in this study are based on topological and metrical measures reviewed by Uylings and van Pelt (2002).

1. Cell soma area represents the body of a cell that spreads until the origin of the dendrites and an axon. Due to the shadows and the depth of a slide, this number is usually an estimate and expressed in squared micrometers (μm^2).
2. Dendritic length is the total length of all dendrites of a neuron. It includes the number of normal segments (those that are terminal, not further branched), intermediate segments (those that are further branched) and the incomplete segments (those which are not possible to follow through completely due to: the staining methods, another neuron cutting the dendrite, insufficient depth of a slide). Dendritic length is expressed in micrometers (μm).
3. Quantity of dendrites is the number of dendrites coming out of the neuron's soma.
4. Length of branched structures is the length of specific segments (or branched structures). For the purposes of this study I have incorporated only "normal" and "branched" segments and did not include the "incomplete" segments. Again, the unit of measurement for this length is the micrometer.
5. Base diameter of branched structures is the diameter measured at the beginning of a dendrite; at the location where the dendrite originated from the cell soma. It is measured in micrometers.
6. Average diameter of branched structures is the average thickness of a dendrite from its beginning to the terminal point.

7. Spine density of branched structures is the overall number of spines across all the segment area in that particular neuron. This number does not include the incomplete segments.
8. Quantity of markers is the number of spines per micrometer of dendrite surface which includes the incomplete segments, as well.
9. Number of segments is the number of normal, branched and incomplete segments of a particular neuron.

Procedure

3D Reconstruction. To reconstruct the neurons in three dimensions, I used a computerized system Neurolucida (Figure 3), software for reconstruction and mapping of the neurons, connected to a microscope (MicroBrightField Inc., n.d.). First, the slides were prescreened to find most utilizable neurons. Upon decision on which neurons to incorporate into the study, a contour of the slide was made on the lowest zoom on the microscope. Using a specific option in the software, the neuron was precisely located within the contour (Figure 4). The slide was then zoomed and cells were reconstructed under a 60x zoom. After setting the baseline depth for the soma's reconstruction, dendrites were added in a specific format. Each degree of a dendritic branch was marked using 'bifurcation nodes' and using a different color. For each degree of the dendritic branch, spines were added separately. For purposes of this study, apical dendrites were not included in the reconstructions and axons were not included in the statistical analysis. Figures 5 and 6 are examples of such reconstructions.

Quantitative observations. NeuroExplorer, software for neurophysiological data analysis (MicroBrightField Inc., n.d.), was used in gathering the data from Neurolucida 3D reconstructions. The program generated three tables of data used in statistical analysis: Neuron

Summary (for the cell soma area, dendritic length and the quantity of dendrites), Branched structure analysis (for the length of branched structures, base and average diameters of branched structures and the spine density) and Markers (for the number of spines analysis). Minitab package for Windows was applied to statistically analyze the data. The nine variables were tested separately using paired t-tests to determine statistical differences between hemispheres separately in the male brain (B1) and the female brain (B2) . A p-value lower than 0.05 was considered significant.

In more detail, 26 paired t-tests have been done to test for the hemispherical differences in the third layer of Broca's region:

1. *Cell soma area*. No statistical differences between two hemispheres in both normal male and normal female brains were hypothesized for cell soma surface. The first paired t-test examined the means of the right and the left hemispheres in the male brain (B1R vs. B1L).The second paired t-test looked at the five neurons of the female right (B2R) versus the five neurons in the female left (B2L) hemisphere.
2. *Dendritic length*. The hypothesis stated no significant difference in the total dendritic length between hemispheres in either a male or a female brain. Values for the total dendritic length, generated with NeuroExplorer, were used for two paired t-tests. The tests looked at the hemispherical differences separately for the male and the female brain.
3. *Quantity of dendrites*. The Neuron summary table contained the number of dendrites coming out of the cell soma per each neuron. These numbers were used in the next two paired t-tests to confirm or reject the hypothesis stating no statistical difference between hemispheres in either the male or the female brain.

4. *Length of branched structures*. Four paired t-test were analyzing hemispherical differences in the segment length. Normal segments were tested separately (B1R normal vs B1L normal; B2R normal vs B2L normal) from branched segments (B1R branched vs B1L branched; B2R branched vs B2L branched) and incomplete segments were not used in the analysis. The values of normal segment length per neuron were added and divided by the number of normal segments to get an average. That average number was used in the t-test. The same procedure was used for branched segments. No statistical differences were predicted in either of male normal and branched or female normal and branched segment length.
5. *Base diameter of branched structures*. Another four paired t-tests were done for base diameter analysis. This variable was also tested for hemispherical differences between normal and branched. The values of normal segment base diameters per neuron were added and divided by the number of normal segments to get an average. That average number was used in the t-test. The same procedure was used for branched segment base diameter. It was predicted that no such differences existed in either male or female brain.
6. *Average diameter of branched structures*. It is predicted that neither normal nor branched average diameters are statistically different in right versus left hemisphere in both brains. The values of normal segment average diameters per neuron were added and divided by the number of normal segments to get an average. The average numbers of the right hemisphere were compared to the means of the left hemisphere in a paired t-test. The same procedure was used for branched segments.
7. *Spine density of branched structures*. This variable was again divided into the normal and branched segment analysis. The values of normal segment spine density per neuron were

added and divided by the number of normal segments to get an average. The right hemisphere was then compared to the left hemisphere in a paired t-test. The same procedure was used for branched segments. Similarly, the hypothesis assumed no statistical differences neither in normal segments nor branched segments in either brains.

8. *Quantity of markers*. The third table generated by NeuroExplorer was indicating the number of markers on a whole neuron (all three terminal types of dendrites: normal, branched and incomplete). This number was divided by the total dendritic length (from the Neuron summary table) which included the incomplete segments. Given quantities were then categorized according to the hemisphere and compared using the paired t-test. It was hypothesized that the quantity of markers will not be significantly different in either hemisphere in the male and female brain.
9. *Number of segments*. The number of all three types of segments was simply taken from the Branched structure analysis table and used for a paired t-test. It was hypothesized that there will be no statistical difference in the number of segments in any hemispherical analyses (both for the male and the female brain).

Qualitative observations. Qualitative observations were limited to the deep layer IIIC of Broca's region and magnopyramidal cognitive neurons. All of the properly stained neurons included one axon, one apical dendrite and several basal, branched dendrites. Axons of these neurons spread towards the white matter whereas the apical dendrite spreads towards the cortical surface. Basal dendrites are faced toward the lower cortical layers meaning, towards the white matter.

Results

Statistical analysis included 26 paired t-tests (t-statistic) examining hemispherical differences for nine variables in one male and one female normal, adult brain. Five neurons were taken from each hemisphere. A p-value lower than 0.05 was considered significant and would indicate a significant difference between the right and left hemispheres.

Cell soma area. A paired t-test for the male brain (B1) indicated no statistical significance in cell soma surfaces ($t(4)=0.69$, $p=0.528$) between the right hemisphere ($M=284.6$, $SD=53.3$) and the left hemisphere ($M=258.3$, $SD=57.4$). A t-test done for the female brain (B2) also reported no statistical differences ($t(4)=0.46$, $p=0.528$).

Dendritic length. Differences found between the male right and the male left hemisphere for the dendritic length were not significant ($t(4)=-0.20$, $p=0.851$). The female brain yielded similar results ($t(4)=0.98$, $p=0.381$).

Quantity of dendrites. Neither the male nor the female brain showed any hemispherical differences in the dendritic quantity (respectively, $t(4)=0.14$, $p=0.893$; $t(4)=-0.53$, $p=0.621$).

Length of branched structures. Both the male and the female brains showed a statistically longer normal terminal typed segments in their right hemispheres ($t(4)=3.04$, $p=0.038$). The normal segment length in the right male hemisphere averaged 104.86µm ($SD=20.75$) while the corresponding left hemisphere averaged 74.14µm ($SD=21.27$). The paired t-tests for length of branched (intermediate) segments again showed no difference between the male hemispheres ($t(4)=0.79$, $p=0.472$) and a statistically longer branched segments in the left female hemisphere ($M=24.05$, $SD=3.05$) as opposed to the right ($M=19.25$, $SD=2.77$, $t(4)=-5.46$, $p=0.005$).

Base diameter of branched structures. No significant differences were found in either male and female brains for the normal segment base diameters ($t(4)=2.22$, $p=0.090$; $t(4)=-1.80$,

p=0.145, respectively). An average base diameter for the right male hemisphere is 0.868 µm (SD=0.23) and the left male hemisphere is 0.646 µm (SD=0.08). Values for the female brain were somewhat lower: right hemisphere averaged to 0.5195 µm (SD=0.07) and the left to 0.6030 µm (SD=0.08). Similarly, a paired t-test for branched segment base diameters did not result in hemispherical differences in either male (t(4)=-0.91, p=0.415) or female brain (t(4)=1.31, p=0.260).

Average diameter of branched structures. Paired t-tests for normal segment average diameter showed no statistical differences in neither male (t(4)=-.278, p= 0.05) nor female brain (t(4)=-2.39, p=0.075) which is confirming the set hypothesis. T-tests done for the male and female brain branched segment average diameter did not show statistical significance either (t(4)= -1.81, p=0.144; t(4)=0.62, p=0.568).

Spine density of branched structures. Paired t-tests did not yield any statistical differences between hemispheres in a male (t(4)=-2.65, p=0.057) and female brain (t(4)=-0.27, p=0.802) for the normal segment spine density. When tested for differences in branched segment spine density, enough evidence is found to reject the hypothesis for the male brain (t(4)=-3.99, p=0.016). The difference between the male right and male left hemisphere is significant. The opposite is concluded for the female brain (t(4)=-2.38, p=0.076).

Quantity of markers. Quantity of markers did not differ in the male right (M= 4.77, SD=1.29) versus male left (M=3.215, SD= 1.23) hemisphere (t(4)=1.52, p=0.203). Similar results are reported for the female brain. Right hemisphere (M=4.183, SD=0.63) did not statistically differ from the left (M=3.99, SD=0.44) hemisphere (t(4)=0.76, p=0.489).

Number of segments. Number of segments was not statistically different between hemispheres in either male ($t(4)= 1.4238$, $p=0.2276$) or female brain ($t(4)=0.3835$, $p=0.7209$).

Discussion

Based on the studies researchers previously conducted (see Uylings, et al., 2006; Zeba, et al, 2008), I hypothesized no statistical differences for all nine variables, when p-value lower than 0.05. Because paired t-tests did not indicate statistical differences for cell soma surface in neither sexes, the hypotheses were confirmed. Same conclusion was made for the dendritic length. Neither in the male nor female brain was the hemispherical difference statistically significant. This would mean that dendrites are overall similar in length when compared between hemispheres, both in an adult male and an adult female brain. Figure 6 illustrates 10 neurons from both the left and right male Broca's regions. Also, the number of dendrites coming out of the soma is statistically similar between hemispheres in the two brains. Hypothesis stating no difference is confirmed. Same conclusion is made for the number of markers. Due to the lack of statistical difference, it is appropriate to conclude that the number of markers is similar in both hemispheres of both brains. When comparing normal segment length across hemispheres, we find statistical significance in both male and female brain. This finding rejects the hypotheses which indicated no statistical differences in dendritic length between hemispheres in both sexes. However, if we look at the findings of branched segment length, we will confirm the hypothesis set for the male brain, but the prediction of having no differences in length of branched structures for the female brain will be rejected. The predetermined no-difference hypothesis is confirmed when calculating hemispherical differences of both sexes for normal segment base diameter and branched segment base diameter. This suggests that no matter if the segment is terminal of intermediate, there is no statistical difference in its base diameter, the branching point from the

soma. Similarly, the hypothesis is confirmed when testing for hemispherical differences in normal segment average diameter and branched segment average diameter. Without performing a statistical analysis, difference between the average diameter of normal segments and branched segments in both sexes is evident. Using the male brain as an example, normal segment average diameter for the right hemisphere is 0.3520 μm (SD=0.06) while its branched counterpart is 0.960 μm (SD=0.08). The normal segment average diameter in the left hemisphere is 0.4232 μm (SD=0.06) whereas the branched segment average diameter for the left hemisphere is 1.250 μm (SD=0.327). Assuming statistical significance, I would conclude that intermediate segments are thicker (larger in their average diameter) than the terminal segments. When looking at the normal segment spine density, we confirm the hypothesis that finds no differences between the hemispheres in both brains. This means that the number of spines per segment on the left side is similar to the right side of the both male and female brains. However, as already indicated in the quantitative measurement section, we reject the hypothesis for branched segment spine density due to the presence of significant hemispherical differences in the male brain. This is not, however, true for the female brain and we do confirm the hypothesis. Lastly, no statistical difference was found in the number of segments between hemispheres of the male and the female brain and thus the hypothesis is confirmed. This implies that the number of segments in the five neurons of the right male hemisphere is approximately the same as the number of segments in the five neurons of the left male hemisphere, and the same is true for the female brain.

To summarize, across nine variables, statistically significant hemispherical differences were found in male and female normal segment length, female branched segment length and male branched segment spine density. These results were supported with enough evidence to

reject predetermined hypothesis which predicted no statistical difference in any of the nine variables.

Results of this study are somewhat in accordance to other studies in the field. One hypothesis that was applied to all nine variables was determined in relation to the research conducted by Uylings, et al. (2006) and Zeba, et al. (2008). In comparison to Zeba, et al. (2008), the age of cell donors were similar (about 40 years), the cells were taken from the same areas (BA45) and cells were impregnated with the same solution (Golgi cox). However, Zeba, et al. (2007) showed no differences in any of their variables measured for Broca's region (soma cell surface, dendritic length, number of basal dendrites, etc.). The variables in both studies were mostly the same but the most probable cause to this discrepancy in our results is due to the limited sample in the current study. Uylings, et al. (2006) reported results in accordance with Zeba, et al.(2008) meaning, no statistical asymmetry in hemispheres in BA 45. These results again do not correspond to findings in this study.

It is to be noted that statistical significance in e.g. somal size is reported in schizophrenic patients. Rajkowska, et al. (1998) found a declination in somal size of magnopyramidal neurons of layer IIIC. Because the study did not look specifically at Broca's region, and did not do a hemispherical analysis, comparative conclusions to this study would be incorrect, but it is nonetheless important to consider in future studies.

If one would to replicate the study using the same hypothesis, it is primarily recommended to take a sample size of more than five neurons per hemisphere. The results of this study are limited in their implications due to the small sample size. More than two brains are necessary to test for sex differences, as well. Not only would one look at hemispherical

differences, but how are the values for the nine variables spread across the male brains versus female brains. When choosing which neurons to reconstruct from those available in the slides, I chose the ones stained the best, visible the best across various microscopic depths, with similar numbers of basal dendrites originating from the soma and those that seemed rich in branches while prescreening. Another suggestion for further studies is to compare the post-mortem schizophrenic neurons to the healthy controls on the variables used in this study. Looking at healthy cells only can give us an insight into Broca's hemispheric dominance, or perhaps dominance expressed in only one sex and not in the other. But implications about cognitive functions are limited if we look at only one type of cell donors. If the results of healthy controls were compared to the cells of schizophrenic donors (as previously mentioned research suggests), we could learn more about the cytoarchitectonic nature of this serious mental illness and cognitive abilities in the adult human frontal cortex.

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Figure Captions

Figure 1. Diagram of centrifugal order of segments (Uylings & van Pelt, 2002)

Figure 2. Diagram of centrifugal order of segments on a pyramidal neuron (Uylings & van Pelt, 2002)

Figure 3. Neurolucida software system connected to computer and microscope (MicroBrightField. Inc, n.d.)

Figure 4. A neuron within its contour reconstructed with Neurolucida.

Figure.5 3D Reconstruction of a neuron using Neurolucida software

Figure 6. Comparison of neurons of the left and right Broca in the male brain

Figure 1.

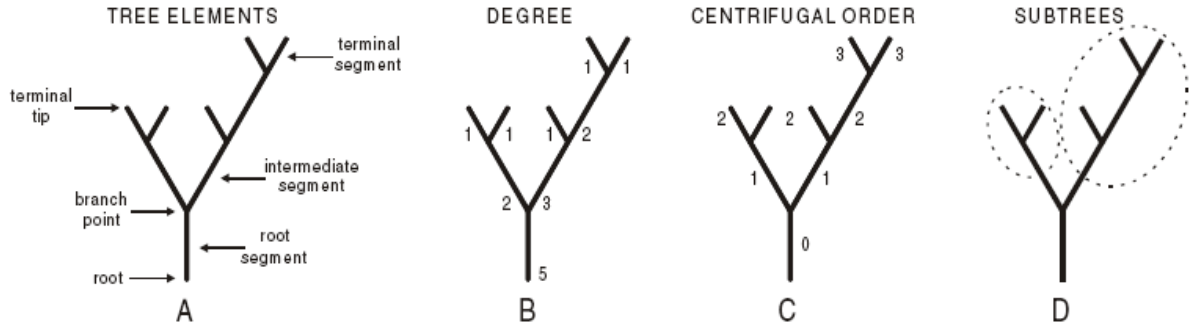

Figure 1. (A) Elements of a topological tree: points (root, branch point, terminal tip) and connecting segments (terminal and intermediate segments); (B) classification of segments according to the number of terminal tips peripheral to the pertinent segment, i.e. degree; (C) classification of segments according to their topological distance from the root, i.e. centrifugal order; (D) a bifurcation into two subtrees.

Figure 2.

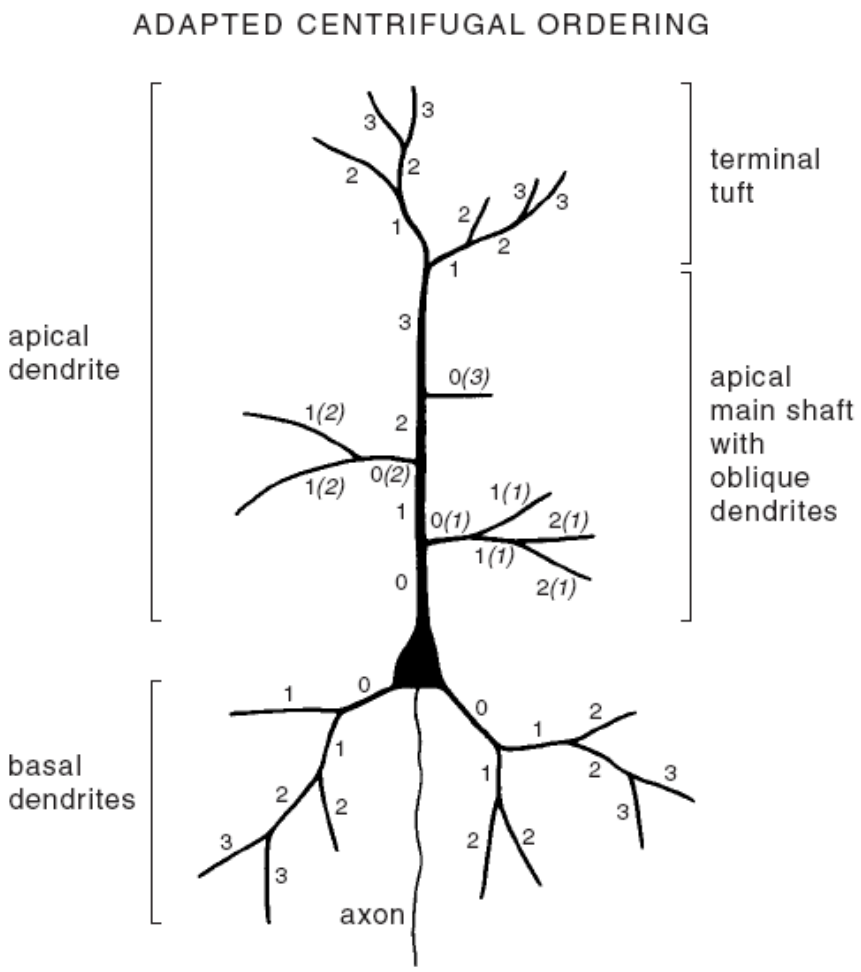

Figure 4. Centrifugal ordering for basal dendrites of pyramidal neurons. The centrifugal ordering has been adapted for apical dendrites. To avoid assigning equal orders to different kinds of dendritic segments, the apical dendrite is divided into a main shaft with a terminal tuft and oblique dendrites (from Uylings *et al* (1986b)).

Figure 3.

Figure 4.

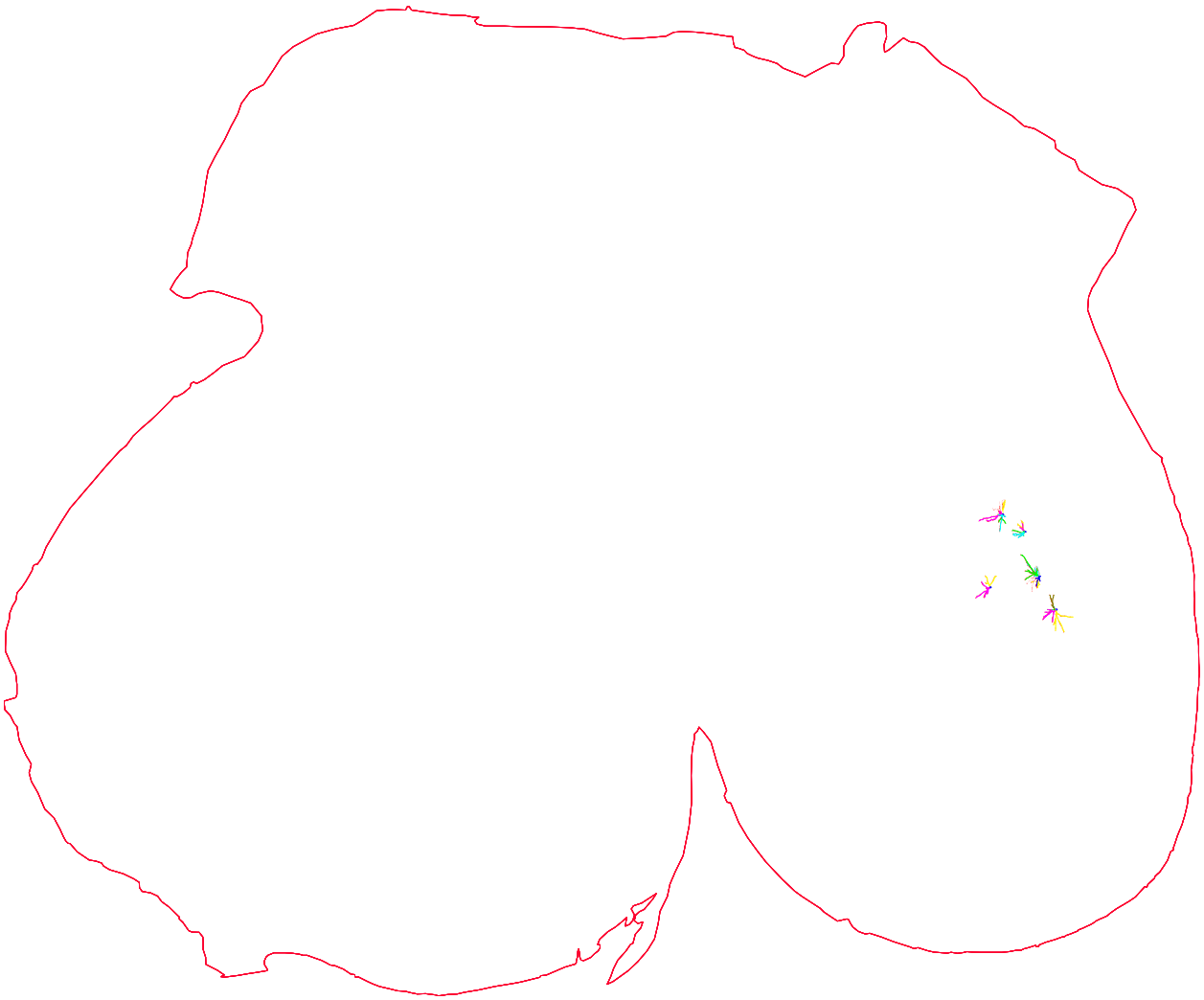

Figure 5.

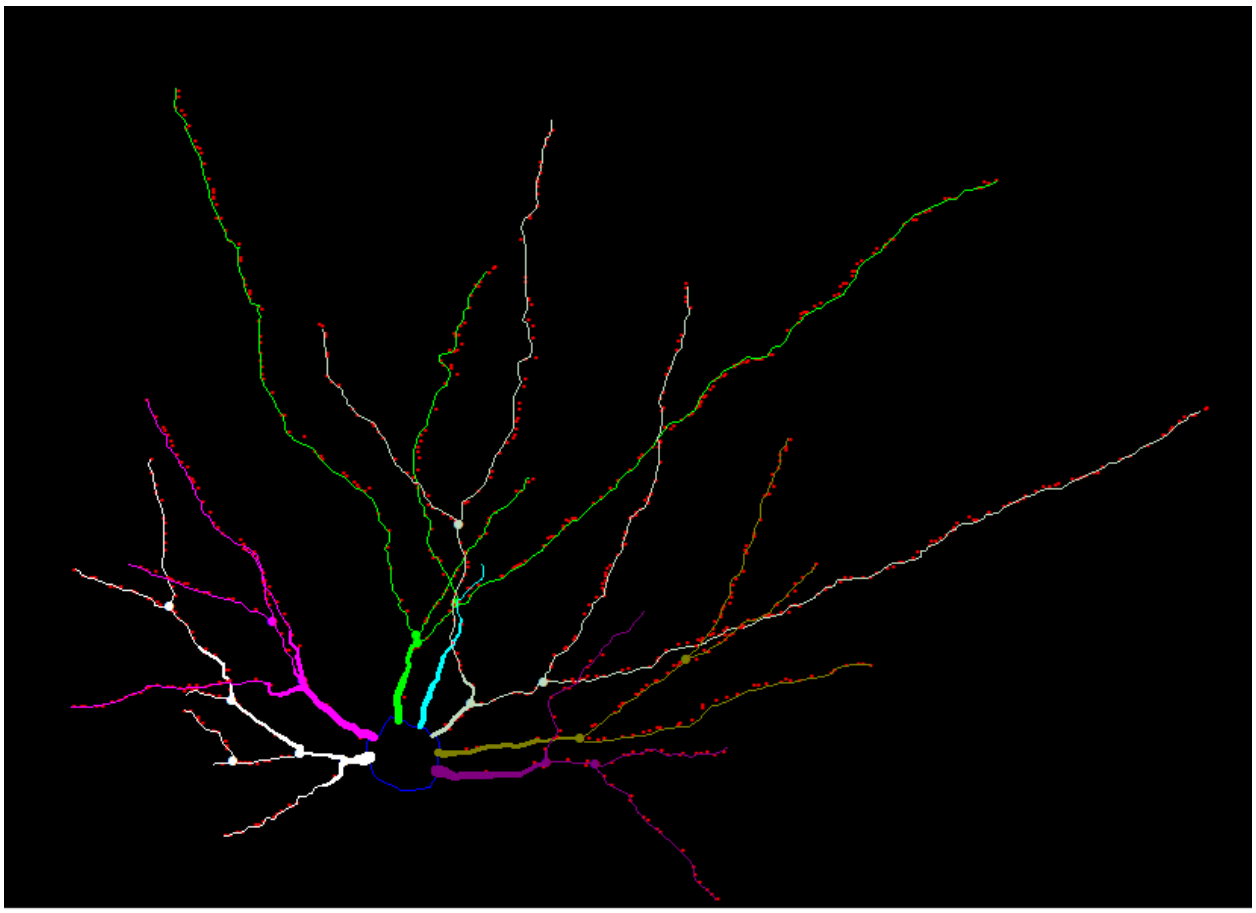

Figure 6.