A comparative cross sectional study of the morphological relationship between the superficial and deep gray matter structures in a random sample of cadaveric adult human brains in the Discipline of Clinical Anatomy at University of KwaZulu-Natal

By

Eman Y Haghegh
211559003

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School of Laboratory Medicine and Medical Sciences,
College of Health Sciences
University of KwaZulu-Natal
Durban -South Africa

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Master of Medical Science in Anatomy

Supervisor: **Dr. O. O. Azu**
Co-Supervisor: **Dr. E. C. S. Naidu**

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Preface

The study described in this dissertation was carried out in the Discipline of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa from May 2014 to November 2015, under the supervision of Dr. O.O Azu and Dr. E.C.S Naidu.

Declaration

I, Mrs. Eman Haghegh declare as follows:

1. That the work described in this thesis has not been submitted to UKZN or other tertiary institution for purposes of obtaining an academic qualification, whether by myself or any other party.

2. This thesis does not contain other person's writing, data, pictures, or other information, unless specifically acknowledged as being sourced from other persons or researchers. Where other written sources have been quoted then:
    - Their words have been re-written, but the general information attributed to them has been referenced.
    - Where their exact words have been used, then it has been properly referenced in the reference section.

3. Signed…EMAN HAGHEGH…… Date…16 March 2016……..

Dedication

To the memory of my

Father

Who instilled in me the value of hard work

Who was always emphasizing on the value of education

From whom I learned honesty and generosity

The day I said goodbye to him will always be in my mind and heart.

You will be forever loved and remembered.

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First of all it's my great pleasure to express my gratitude, gratefulness and devotion to ALLAH (S W T), for giving me the courage, strength and power to accomplish this work despite all the obstacles and difficulties.

I would like to thank all the people who in one way or another helped me to put all this work together;

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My thankfulness and appreciation to **Dr. O.O Azu**, my supervisor for his guidance, invaluable advices and constructive criticism throughout the production of this dissertation.

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My unreserved thanks to the staffs and Colleagues in the Discipline of Clinical Anatomy, Nelson R Mandela Medical School, University of KwaZulu-Natal, South Africa for their teaming and technical support during my clinical work, as well as their continuous encouragement.

Last but not least my great debt of appreciation and thankfulness is to my Family, my Mother, for her love, care and continuous support, to whom I shall remain indebted forever, my Brothers and Sisters and their families for their support since I have left home.

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Abbreviations and Symbols

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
BREC	Biomedical Research Ethics Committee
fMRI	functional Magnetic Resonance Image
HD	Huntington's disease
MRI	Magnetic Resonance Image
PET	Positive Emission Tomography
ROI	Region of interest
SD	Standard Deviation

Abstract

Background: While various neurodegenerative diseases affect the cortical mass and mass of deep gray matter differently, finding an optimal and accurate method for measuring thickness and surface area of the cerebral cortex remains a challenging problem due to the highly convoluted surface of the cortex. We therefore investigated the superficial and deep gray matter thickness and surface area in a sample of cadaveric specimens at the Discipline of Clinical Anatomy, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, South Africa to provide some clue as to possible variations in these parameters.

Materials and Method: With ethical approval, 60 brain samples were uniformly sectioned at 5mm thickness and eight slices containing the deep nuclei were taken from each brain and stained by Mulligan's technique. Thickness was measured at selected angles 0º, 45º, 90º, 135º and 180º for both right and left cerebral hemispheres. The cortical thickness and surface area of selected slices for both the superficial cortex and the corresponding deep nuclei were measured.

Results: Mulligan's stain produced good gray mater differentiation and clear images that enabled manual delineation of structures. There was rightward asymmetry of cortical thickness of the selected slices at the suggested angles which corresponded to structurally and functionally important brain regions. There was a positive correlation between the mean surface area of superficial cortex and deep nuclei across the regions of interest (ROI).

Discussion and Conclusion: Baseline data from 55 brain samples provided a range of means and 95% confidence intervals for the three parameters of cortical thickness, cortical surface area and surface area of deep nuclei to be made for a reference table comprising eight coronal slices taken at five angles. This allows an objective assessment of thinning of the cortex or loss of deep gray matter to be made from measurements of the same parameters for the equivalent slices from a postmortem brain slice or an appropriate radiographic image.

Keywords: Brain, Morphometry, Stains, Gray matter.

CHAPTER ONE: INTRODUCTION

1.1 Background

The cerebrum constitutes the largest part of the human brain and composed of the outer thin gray matter called the cerebral cortex and an inner layer of white matter encompassing the deep gray nuclei (basal ganglia, thalamus, hippocampus, and amygdala). The cerebral cortex is largely responsible for higher brain functions, including sensory and motor activity, memory, reasoning, and thought. The human cerebral cortex is 2 to 4 millimetres and makes up to 75% of human brain (Kandel et al., 2000). In order to increase the surface area of the cortex, it shows multiple convolutions consisting of elevated regions called (gyri) which are separated by grooves called (sulci). These are of different extensions and depth and vary from one individual to another (Brodal, 2010, Kandel et al., 2000). The gyri and sulci are the basic functional units of the cerebral cortex and the sulco-gyral folding pattern is the key to understanding the complexity of the cerebral cortex and its connections (Regis et al., 2005).

1.2 Structural Neuroanatomy of the cerebral cortex

The precise reason for the highly convoluted cortical surface is not yet fully known. It serves mostly to accommodate a large number of neurons. As it will be shortly described, the cerebral cortex is organized in functional layers and information is processed across these cortical microcircuits in an interconnected set of neurons called columns, or minicolumns and the number of neurons in the cerebral cortex is considered to be the crucial determinants of the cortex's capacity for information processing. The surface area is considered to be relatively larger in the human brain than other species and this increase will permit a greater number of columns and interconnections thus providing greater capacity for information processing (Kandel et al., 2000, Mountcastle, 1997).

1.2.1 Neuronal layers of cerebral cortex

In the early 20th century, different histological cross-section staining of the cortex applied to study a detailed description of the laminar cortical structure and to reveal the arrangement of the neurons with intracortical axons. The cortex parcellated into six main layers, from the pial surface to the white matter as shown in figure 1.1, (Brodmann, 1909).

Layer I is called the molecular layer. While it consists mainly of extensions of apical dendritic tufts of pyramidal neurons and horizontally oriented axons, there are also a few scattered neurons and some glial cells as well as Cajal-Retzius and spiny satellite cells (Shipp, 2007).This layer receives inputs from the M-type thalamus cells as received from the cortex itself (Rubio-Garrido et al., 2009).These inputs are thought to be essential for the interactions in the cerebral cortex involved in associative learning and attention(Gilbert and Sigman, 2007).

Layer II is the external granular layer. It contains small-size pyramidal neurons and many satellite neurons (Kandle et al., 2000).

Layer III is the external pyramidal layer. It contains mainly small and medium-size pyramidal neurons, as well as non-pyramidal neurons with vertically arranged intracortical axons; layers I through III are forming the main origin of corticocortical afferents and layer III is the main supplier of corticocortical efferent (Kandle et al., 2000).

Layer IV is similar to layer II "the internal granular layer" which contains special types of pyramidal and stellate neurons. This layer is the main origin of corticocortical and thalamocortical afferents, the latter arising from type C neurons in the thalamus (Jones, 1998).

Layer V is the internal pyramidal layer, which contains large-size pyramidal neurons that send afferents to subcortical structures (such as the basal ganglia). It also contains Betz cells in the primary motor cortex. The axons of these cells travel through the internal capsule, the brain stem and the spinal cord to form the corticospinal tract, which is responsible for voluntary motor activity (Kandle et al., 2000).

Layer VI is the polymorphic or multiform layer, which contains mainly multiform neurons with many small spindle-like pyramidal cells and few large pyramidal neurons; this layer sends efferent axons to the thalamus and forming a reciprocal cortico-thalamic interconnection (Creutzfeldt, 1995, Lam and Sherman, 2010).

The cortical layers are not just arranged over each other with clear demarcations between each layer, there is a characteristic connection which extends through all the thickness of the cortex. Therefore, it forms cortical microcircuits that are gathered into cortical columns and minicolumns which are the basic functional units of the cortex (Mountcastle, 1997).

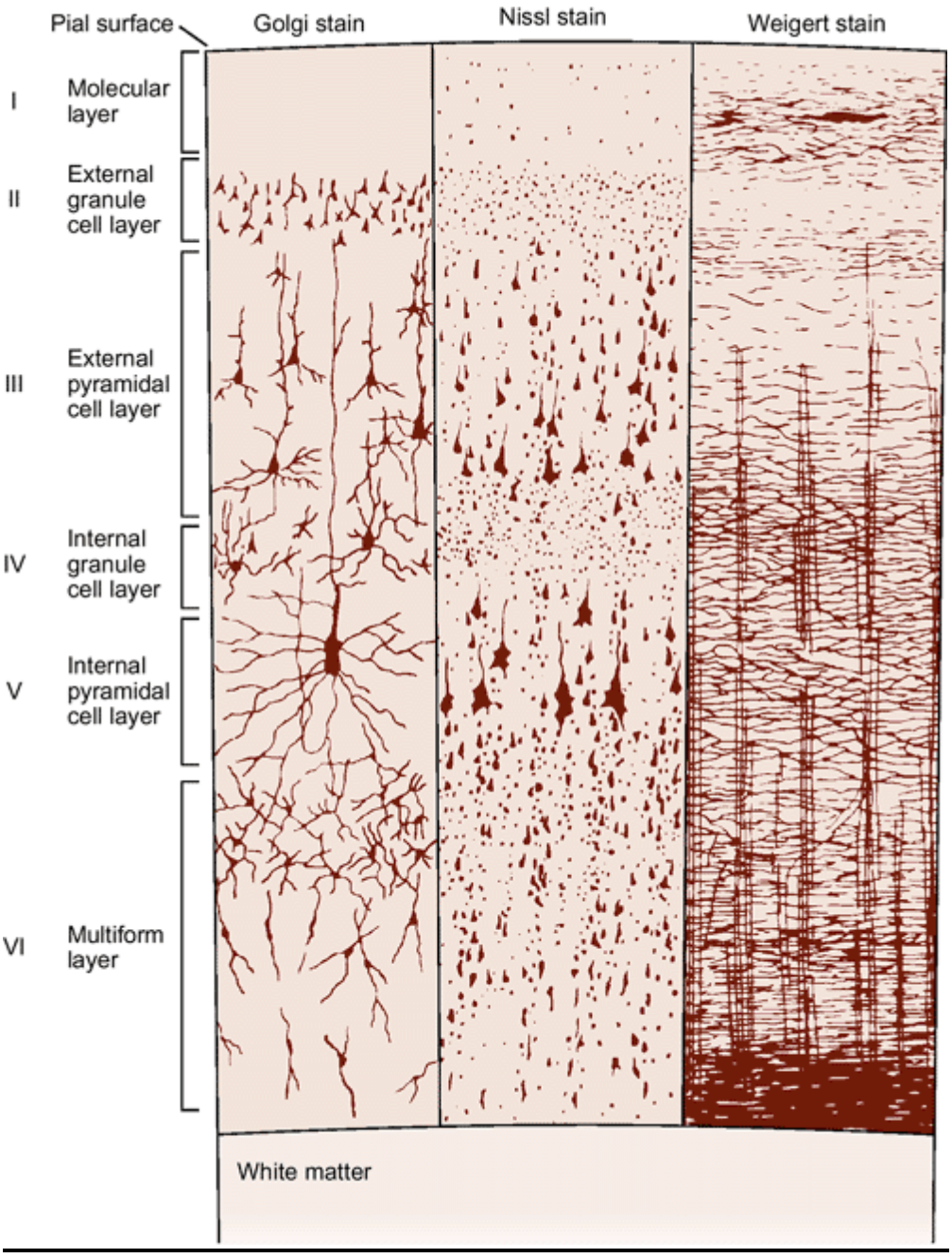

Figure 1.1: The arrangement of the cerebral cortical neurons into characteristic layers. The Golgi method shows neuronal cell bodies and dendritic trees and the Nissl stain reveals the cell bodies and proximal dendrites, while a Weigert stain shows the pattern of myelinated axonal distribution (Heimer, 1995).

1.2.2 Cortical Areas

As shown in Figure 1.2, the cerebral cortex is organized into regions consisting between 3-6 layers. The number of layers and the details of their functional organization vary throughout the cortex. It is classified depending on the differences in the lamination of the cerebral cortex into a large area called the neocortex and a much smaller area of allocortex. It may also be classified based on the gross topographical conventions into four lobes (Kandle et al., 2000).

1.2.2.1 Differences in lamination of the cerebral cortex

Referring to Figure 1.2, the following is noted

1. The neocortex (isocortex or neopallium), shows a mature cerebral cortex with six distinct layers, which found in the primary motor cortex (Brodmann's area 4) and the primary visual cortex (Brodmann's area 17). The neocortex is further subdivided into two types of cortices, the true isocortex and the proisocortex. The latter is found in Brodmann's areas 24, 25, and 32.
2. The allocortex, it is less than six layers and has three areas.
    i. The archicortex, which consists of three cortical layers and it is mostly present in the olfactory cortex and hippocampus.
    ii. The paleocortex, which consists of four to five layers and it is present in parahippocampal gyrus, piriform cortex, periamygdalar area and prepyriform area
    iii. The periallocortex, which is a transitional area adjacent to the allocortex.

The paralimbic cortex is a transitional area where layers 2, 3 and 4 are merged. The proisocortex of the neocortex and the periallocortex of the allocortex are combined in this area.

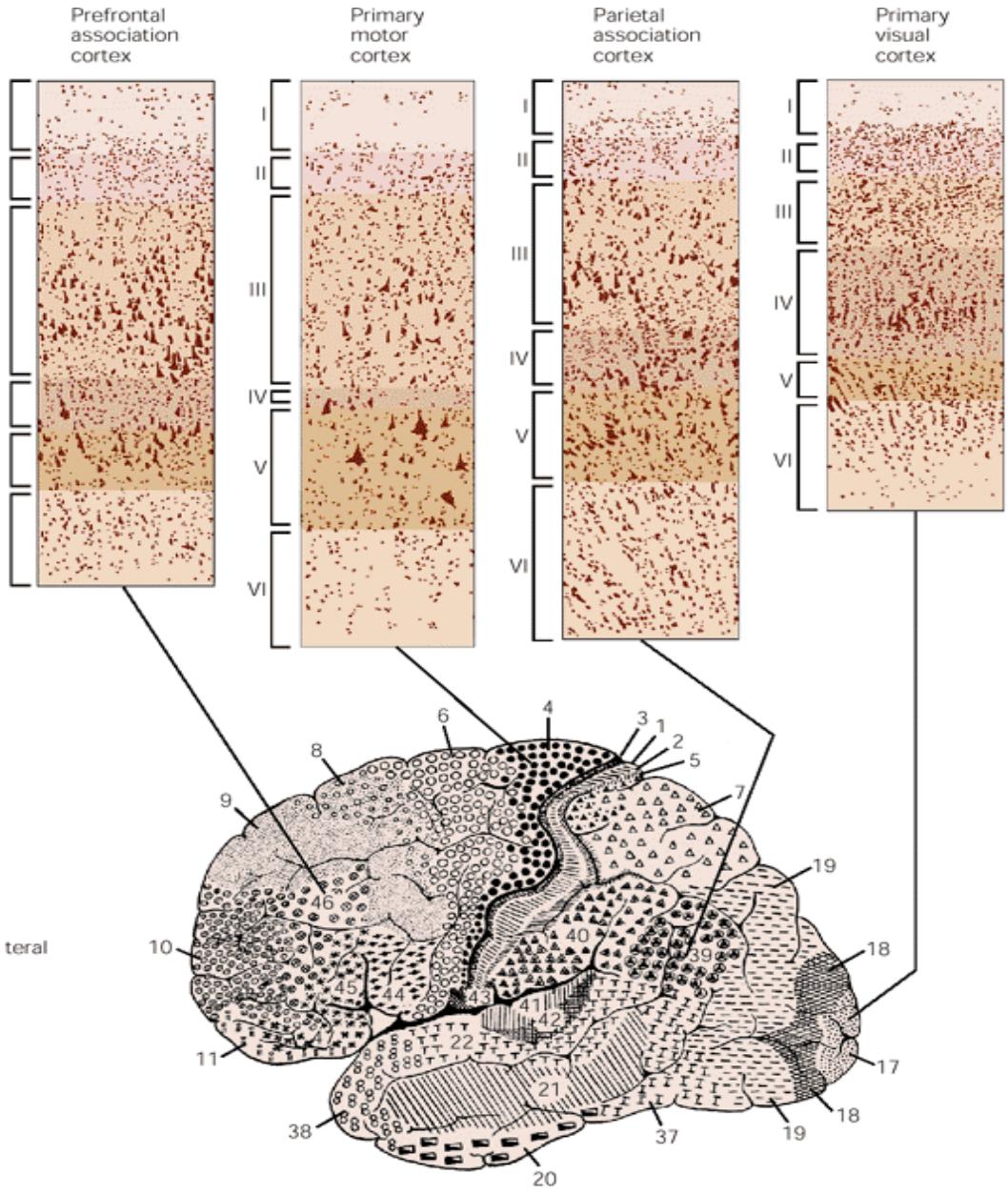

Figure 1.2: The prominence of the cortical cell layers varies throughout the cortex for example, the primary visual cortex has very prominent internal granular layer. The primary motor cortex has a very small layer IV while layer V shows prominent output. This variation made Brodmann and others to turn the century by dividing the brain into different cytoarchitectonic regions. The subdivision by Brodmann (1909) seen in the **bottom half** of this figure, which based on a single human brain analysis (Martin, 1996).

1.2.2.2 The gross topography of the cerebrum

The cerebral cortex has been parcellated into four lobes according to the presence of certain sulci as a landmark. These lobes identified according to the overlying bones into the frontal, parietal, temporal and occipital lobes. The central sulcus separates between the frontal lobe anteriorly and the parietal lobe posteriorly. The Sylvian (lateral) fissure distinguishes between the temporal lobe inferiorly and the frontal and parietal lobes superiorly. While the parieto-occipital fissure demarcates between the parietal lobe anteriorly and the occipital lobe posteriorly, as shown in Figure 1.3 (Miller, 2011).

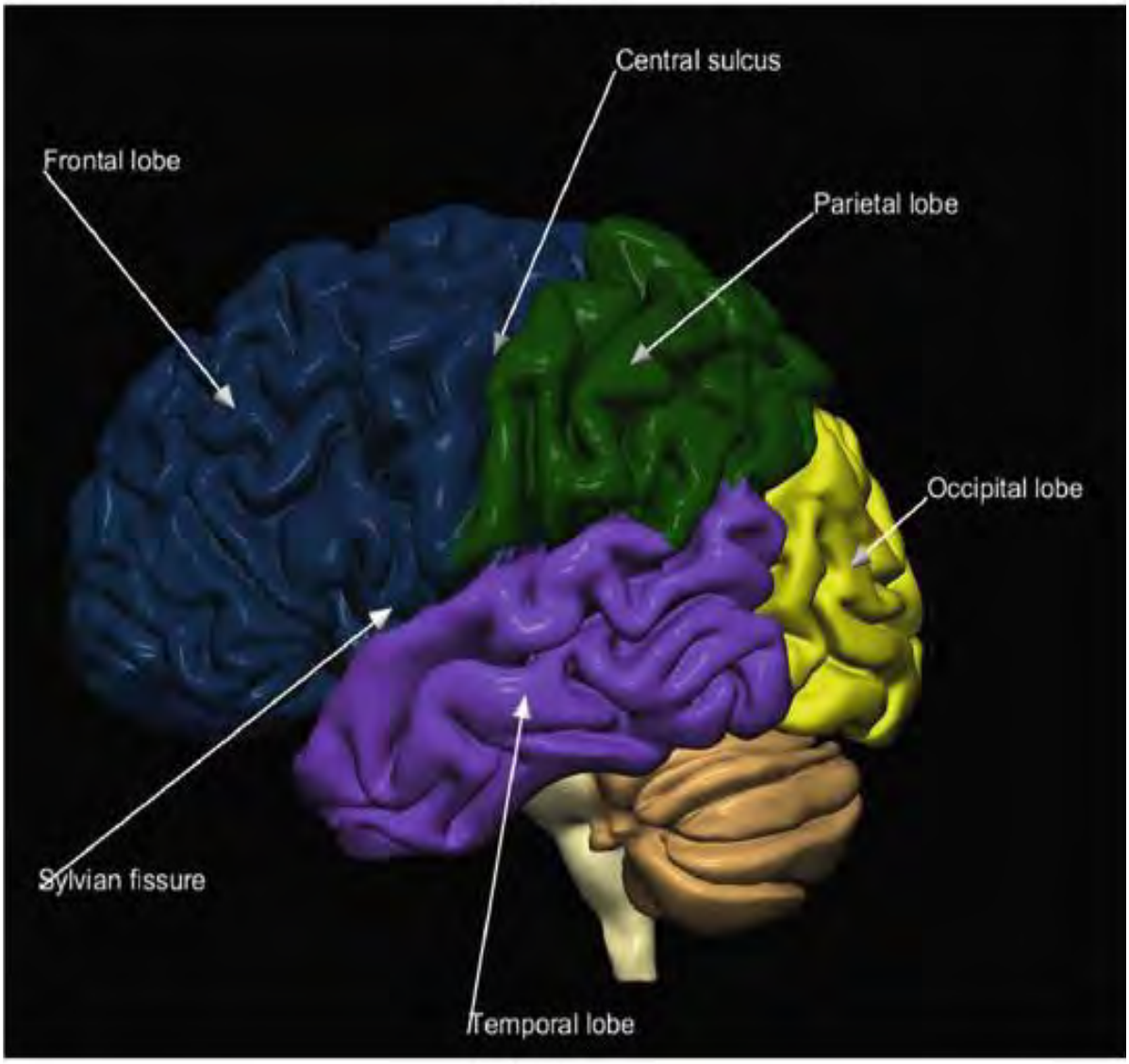

Figure 1.3: Gross topography of the left cerebral hemisphere: Lateral view (Miller, 2011).

1.3 Functional Neuroanatomy of the Cerebral cortex

In most brain functional areas, the process of information is arranged hierarchically. For example, each neuron in the lateral geniculate nucleus (within the thalamus) is responsive to a spot of light in a particular region of the visual field. The fibers of thalamic neurons send output of information to the primary visual

cortex, where each cell will discharge only when a presynaptic cell is active. From the primary visual cortex, information will be sent to the visual association cortex. The visual information processing in the cortex is very delicate, where each single neuron is responsive for a highly complex of information. One of the most remarkable features of this hierarchical configuration of sensory systems is the peripheral receptive layer found in the retina, the cochlea and the receptors in the skin, which is represented topographically throughout successive phases of processing. However, these neural maps do not only reflect the position, but also the density of the receptors and similarly in the motor system, the neurons that regulate each body part are gathered to form a motor map which represent every part of the body differently, as in the primary motor cortex. Each part of the body represented in the cortex in proportion to its degree of function (Kandle et al., 2000).

1.3.1 Cortical Areas

The cortex is divided into three parts functionally: motor, sensory and association areas (Kandle et al., 2000).

i. Motor areas

The motor areas are found in both cerebral hemispheres. They control the motor activity of voluntary movements and each hemisphere control the opposite (contralateral) side of the body (Kandle et al., 2000). There are two motor areas of the cortex, the primary motor cortex, which control the voluntary movements, the supplementary motor areas and premotor cortex where the voluntary movements are selected. In addition, motor functions have been controlled by the posterior parietal cortex for voluntary movements and the prefrontal cortex for higher-order functions, rules and self-generated thoughts (Kandel et al., 2000).

ii. Sensory areas

The sensory areas receive and process information from the sensory endings and the thalamus sends sensory inputs to the primary sensory area. The vision, audition and touch are operated by the primary visual cortex, the primary auditory cortex and the primary somatosensory cortex, respectively. In general, each cerebral hemisphere receives information and inputs from the opposite (contralateral) side of the body (Kandle et al., 2000).

iii. Association areas

Association areas make a significant perceptual experience of the surroundings, which provide an effective interaction in thinking and language. Generally, the association areas arranged as networks which scattered across different regions of the cortex (Yeo et al., 2011). This specific distribution of the association areas reflected the interactions and functional relationships (Srivastava et al., 2014). Previously, it was thought that area 44 and 45 (Broca's area) is responsible for language expression and area 22 (Wernicke's area) for language reception, which are localized in the left hemisphere. However, more recent research implies that the language processes of expression and reception arise in areas other than just those areas including the frontal lobe, basal ganglia, cerebellum, pons and the caudate nucleus (Price, 2000).

1.3.2 Cortical and Subcortical Connections

The cerebral cortex is connected to numerous subcortical regions through efferent and afferent connections. These connections are up to 99% higher at the level of cortical connections (Braitenberg and Schüz, 1991). However, the superficial cerebral cortex is connected with subcortical areas of gray matter called deep gray nuclei which include the basal ganglia, thalamus, hippocampus and amygdala. These nuclei receive inputs from the motor areas of the cerebral cortex and substantia nigra of the midbrain and then resend the signals back to both of these locations. They are involved in controlling the motor activity. The basal ganglia are composed of the caudate nucleus, the putamen, the globus pallidus, the substantia nigra, the nucleus accumbens and the subthalamic nucleus. The putamen and globus pallidus are also collectively called the lentiform nucleus, because they appear as lens-shaped body. While the putamen and caudate nucleus are called the corpus striatum due to their striped appearance (Saladin, 2010), as shown in Figure 1.4.

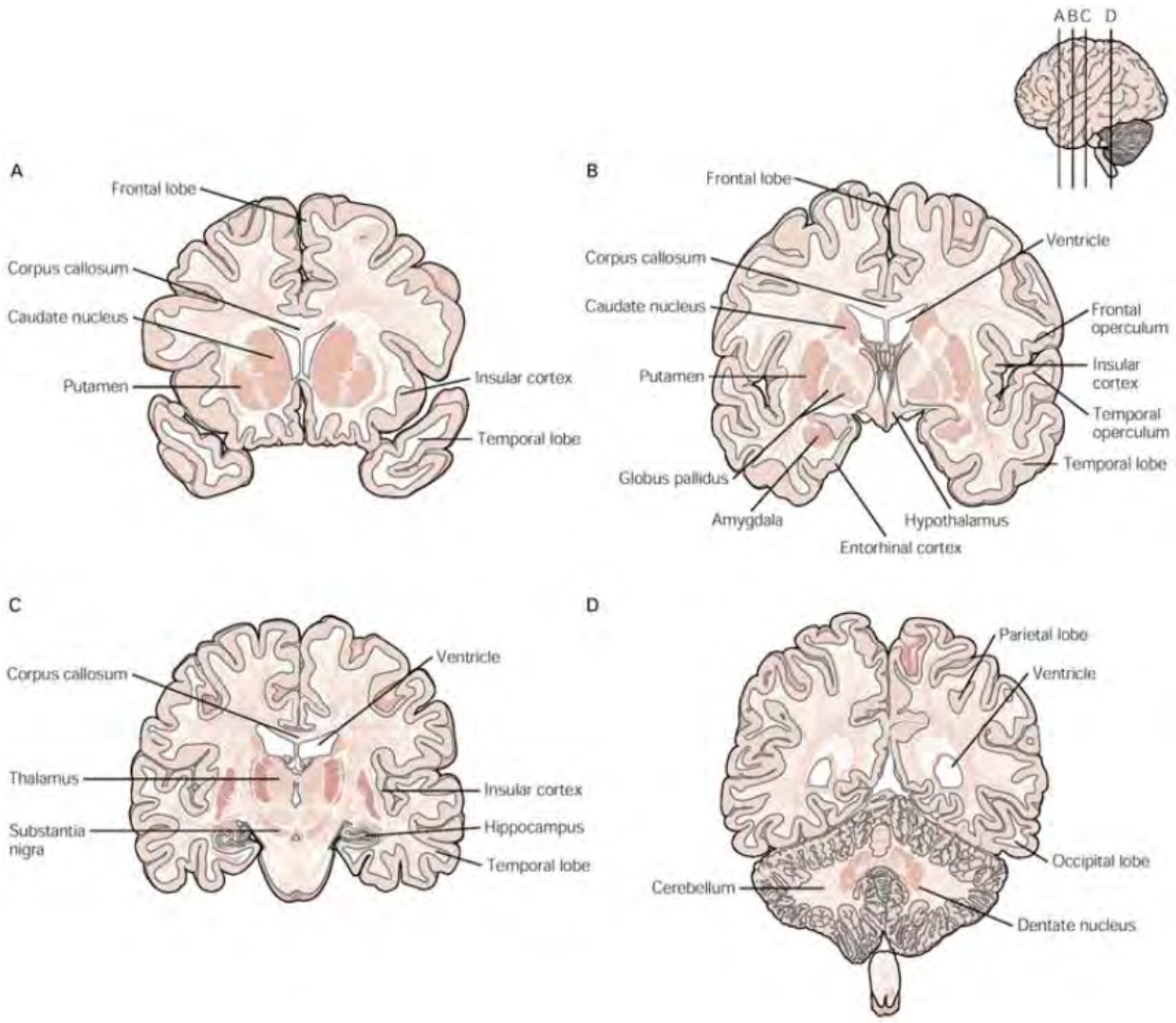

Figure 1.4: Brain regions show deep gray nuclei at different coronal sections. The sections are arranged from frontal (A) to occipital (D) regions (Nieuwenhuys et al., 2007).

For a better understanding of brain structure, it is important to have comprehensive data on the cortical structure in order to compare and relate structural and functional activity across the cortex. It is therefore necessary to establish a detailed mapping of the cortical surface. More impediments have been added to studies of functional specialization because of the complexity and irregularity of cortical geography (Liu, 2011, Passingham et al., 2002, Rettmann et al., 2002, Zilles and Amunts, 2010). This folding pattern raises more obstacles to the investigation of the cortical surface which stems from the fact that gyri are buried in the depth of sulci.

One of these approaches relies on the 3D normalization as described by Talairach (Talairach et al., 1967, Talairach and Tournoux, 1988). Although this type of approach has certain advantages being easy to use and appropriate for subcortical structures, it has been shown to have significant disadvantages because the amount of buried cortex range from as much as 60–70% (Van Essen et al., 1998, Zilles et al., 1988). This distance measured in 3-D space between two points on the cortical surface will substantially underestimate the true distance along the cortical sheet, particularly in cases where the points lie on different banks of a sulcus. For example, the lateral tip of the central sulcus frequently lies within a centimeter of the superior temporal gyrus when the distance is measured in the Cartesian coordinate space. The distance between the same two points as measured along the actual cortical surface is 10 cm due to the depth of the Sylvian fissure (Fischl et al., 1999). This results in poor anatomical accuracy. Other studies have explained the variation between individuals in the anatomical landmark location as being in the order of a few centimeters (Hunton et al., 1996, Thompson and Toga, 1996). So this approach results in loss of accuracy in distinguishing between two neighboring fine cortical features.

Other studies tried to achieve more accurate alignments and measurement through different methods such as using ‘high-dimensional warping’ by recording two volumes (Christensen et al., 1995, Evans et al., 1994, Joshi et al., 1997, Miller et al., 1993). Although such warping methods produce a better match between 3D intensity values of different brains, it is due to its non-rigid alignments and using tens of millions of degrees of freedom to transform one entire 3D volume to another. However, all of this manipulation does not guarantee the accurate alignment of sulci and gyri which are the landmarks for the location of functional areas. Volume-based deformation methods described by Talairach which is depending on 3D normalization of cortical surface provide poor anatomical precision and the metric properties do not reflect the true distances along the cortical surfaces (Fischl et al., 1999).

The third approach has depended on the spherical coordinate system where the cortical surface is first mapped and then reconstructed onto a sphere. The main advantage of this technique is that it respects the intrinsic topological structure of the cortical surface and the metric distortions are thereby minimized where every point on the cortical surface has unique coordinates. Although the above mentioned

techniques try to measure the cortical surface by different approaches, they rely on transformation and reconstruction of the cortical surface to measure the distance between only two structural or functional points. This technique of transformation and reconstruction therefore has poor accuracy due to distortion of the anatomical structure of the cortical surface (Fischl et al., 1999, Miller et al., 1993, Talairach et al., 1967).

There are many other studies that depend on various high-tech imaging systems. For example, automated 3D segmentation by Magnetic Resonance Imaging (MRI) which rely on the tissue homogeneity difference of the brain tissue as explained by (Zeng et al., 1998). The technique has been done by segmentation and measurement of cortical surfaces driven by 3D imaging techniques. It depends on modelling the cortex as a volumetric layer enclosed by two boundaries with different homogeneities thereby segmenting out the cortical gray matter from the white matter and the surrounding brain tissue. Cortical and subcortical gray matter volume, surface area and volume can be measured after this isolation.

Other studies use the functional Magnetic Resonance Image (fMRI) to reveal structure-function relationships and connectivity (Deng et al., 2014). Although this system is a highly sophisticated technique which provide precise information of the interrelationship between the regions of interest (ROI), its disadvantage arises from the high devices expense with its complexity and difficulty of use. Moreover, these studies rely on *in-vivo* human as well as animal studies which are less accurate and therefore less informative when compared to data obtained from *in-vitro* anatomically dissected brain samples. The latter provides better structural resolution (Deng et al., 2014, Fischl and Dale, 2000).

In contrast, this study will attempt to lay the foundation for the compilation of just such a relational morphometric database. Detailed measurements cortical thickness and surface area of cortical and deep gray matter will begin to establish morphological relationships taken over different populations with the key variables of age, gender and population groups. Including deep gray matter to elucidate morphometric variations will further broaden understanding of this important area. To date, research on developmental and pathological changes in brain size had focused dominantly on volumetric measures of brain structure, beside a more recent concentration on cortical thickness. In spite of limited studies, outcomes have provided to propose that the cortical surface area is as important as cortical thickness and volume, as the individual variation in cortical volume is largely contributed to the variability in cortical surface area instead of thickness (Im et al., 2008). In addition, the volume is representing the surface area and thickness; therefore, combination of surface area and thickness structural properties could present the cortical volume measurements. The cortical thickness is influenced by the number of cells, whereas cortical surface area is influenced by the number of columns (Rakic, 1988); in this way, cortical thickness and surface area are strongly related to normal brain development and brain aging, as well as structural

effects of neurodegenerative disorders. For example, findings to date suggest a decrease of the cortical surface area but not necessary the thickness with aging (Østby et al., 2009). (Dickerson et al., 2009b) Showed a link between cortical surface area, cortical thickness, and volume of medial temporal regions in patients with Alzheimer's disease and healthy young and old adults. It has been noticed that age-related differences between healthy young and old adults were most prominent for volume and surface area measures, whereas disease-related differences between patients with Alzheimer's disease and healthy old adults were most prominent for volume and thickness measures. Therefore, this study will investigate the differences in cortical thickness of both right and left cerebral hemispheres in a random sample of adult human brains and to correlate the cortical surface area with certain deep nuclei using specific staining methods to contrast gray and white matter architecture. The deep gray matter as defined by regions of interest (ROI), will include the thalamus, caudate nucleus, globus pallidus (corpus striatum).

Several studies have investigated the cortical thickness asymmetry of both cerebral hemispheres (Luders et al., 2006, Paus et al., 1996, Watkins et al., 2001). These studies use different approaches to measure cortical thickness and provided evidence of thickness asymmetry between the two hemispheres. This asymmetry is mainly regional. For example, there is a rightward asymmetry in the frontal cortex, cingulate sulcus, caudate nucleus and the anterior insular cortex (Watkins et al., 2001). There are also a leftward thickness asymmetry in the precentral gyrus, middle frontal, anterior temporal and superior parietal lobes (Luders et al., 2006).

The asymmetry in hemispheric cortical thickness can be linked to specific functional specialization found in each hemisphere. This hemispheric asymmetry can be used to investigate the structure- function relationships across regions of interest. The importance of structural asymmetry analysis is that it helps to identify the cortical changes which are associated with specific developmental disorders.

There have been many studies which have tried to achieve variant techniques of cortical mapping and measurement to reveal more information about the diseases that affect the cortex. Some studies have devoted more effort to studying how diseases affect the cerebral cortex. For example, one study shows that psychosis in adolescence is associated with a volumetric decrease of cortical structure. Therefore, this study focuses on the differences in gyral/ sulcal cortical thickness, surface area, folding and volume by using magnetic resonance imaging brain scans. The results show that the cortical thinning of gyri and sulci seem to underlie many cortical volume deficits in adolescent patients with first episode early-onset psychosis (Janssen et al., 2009).

Moreover, a new longitudinal mapping technique used to record the transit structural deficits across the cortex in Alzheimer’s disease (AD) (Thompson et al., 2003). There was a loss of neurons in cerebral

cortex and several other subcortical regions. This loss led to generalized atrophy of the affected regions, including degeneration in the frontal, parietal, temporal cortex, as well as in the cingulate gyrus (Desikan et al., 2009, Moan, 2009, Wenk, 2003).

Several studies investigated the effect of diseases on deep nuclei. For example, Huntington's disease (HD), which is a neurodegenerative disorder that affects behavior and muscle coordination. It affects the brain in different areas with some areas more susceptible than others. The early effects are in basal ganglia (the neostriatum which consists of caudate nucleus and putamen), layers 3, 5 and 6 of the cerebral cortex and several areas of the thalamus. In the early stages of the disease, the atrophic changes first appear in the caudate nuclei, while cortical cerebral atrophy can be seen in advanced stages of the disease using magnetic resonance imaging (MRI). In contrast, these advanced changes can be spotted earlier by functional neuroimaging techniques which are not routinely used clinically(Vonsattel and DiFiglia, 1998, Walker, 2007).

Furthermore, neurodevelopmental disorders such as schizophrenia, affect the brain as well. This mental disorder is associated with changes in brain chemistry and structure in 40-50% of cases (Van Os, 2009). However, many studies used imaging techniques such as fMRI and Positive Emission Tomography (PET) scans to investigate the effect of schizophrenia on the cerebral cortex. Volume reduction has been reported in the frontal and temporal cortex (Kircher and Thienel, 2005), however, it is still uncertain whether these volumetric changes are progressive or precede the onset of the disease (Parnas and Jorgensen, 1989).

Hence a comprehensive comparative database will facilitate the analysis and interpretation of morphometric patterns, providing more information that would help in the understanding of the structural and functional relationship that exists in normal as well as diseased conditions. Most of previous studies mentioned above and many others have shown that a lot of diseases affect the cortical surface differently. Moreover, many studies showed the importance of cortical thickness measurement in both normal development as well as in diseases. These changes can be seen in normal aging (De Leon et al., 1997, Jack et al., 1997), dementia (Kaye et al., 1997) and amyotrophic lateral sclerosis (ALS) (Kiernan and Hudson, 1994). In contrast, measurements of cortical thinning are of great benefit in assessing the efficacy of a wide range of treatment for such diseases. This study will provide the basis for developing a database of information supporting such studies by facilitating the analysis of the morphological patterns associated with some of these diseases.

1.4 Problem statement

The cortical thickness is of great importance in both normal and disease conditions such as neurodegenerative and psychiatric disorders. This is because many diseases affect the cortex and deep gray matter differently. Gray matter manifests a variety of changes seen in normal aging (De Leon et al., 1997, Jack et al., 1997), schizophrenia (Kwon et al., 1999), amyotrophic lateral sclerosis (ALS) (Kiernan and Hudson, 1994), Huntington's disease (HD) (Vonsattel and DiFiglia, 1998), as well as dementia (Kaye et al., 1997). In addition, cortical thinning can appear in specific regions and provide information about the causative factors of disease. Moreover, the study of cortical atrophy is useful in the assessment of the efficacy of different treatments. Therefore, establishing the range of cortical thickness throughout the entire surface of the brain is a useful first step to objectively quantify the changes associated with the specific diseases mentioned above. Often these changes are usually subjectively determined clinically by visual inspection of radiographs such as MRI. However, for reliable and repeatable comparisons, measures of cortical thinning or loss of deep gray matter still need to be objectively quantified. The purpose of this study is to establish a basis for setting up a reference database where these comparisons can be made.

1.5 Study justification

There is insufficient morphometric data available on sulco-gyral folding pattern which continues to attract the interest of scientists to find accurate and efficient methods to measure the thickness of the cerebral cortex. The dearth of studies is due to the complexity of the cortical surface which makes the evaluation of cortical structure and function difficult to interpret. This study will provide the basis for developing a database of morphometric information.

Although, automated and highly sophisticated methods of measurements such as a high-resolution T1-weighted MRI scans can provide information of the interrelationship between the regions of interest (ROI), this involves great expense and requires well trained technicians (Fischl and Dale, 2000). This differentiation is preferably performed with *in-vitro* samples as morphometric data obtained from postmortem cadaveric brains provide significantly more detailed structural information from direct observations of the regions of interest (ROI). There is less structural resolution in the regions where the gyri and sulci are buried (Van Essen et al., 1998, Zilles et al., 1988; Deng et al., 2014, Fischl and Dale, 2000) which manual measurements can significantly improve.

Although manual methods are labour-intensive and typically focus on a few *a priori* defined regions (Dickerson et al., 2009) with resultant extensive number of slices and numerous regions that needs to be studied. Staining by Mulligan's technique allows clear differentiation between the gray and white matter which overcomes the measurement errors, especially where the cortical surfaces are not perpendicular to the cardinal axes.

1.6 Research questions

i. Is there any difference between right and left cortical thickness at the regions of interest (ROI)?
ii. Is there any correlation between the cortical surface area and certain deep nuclei at equivalent levels?

1.7 Aim of study

The aim of this study is to investigate the difference between the cortical thickness of both cerebral hemispheres at the regions of interest (ROI), as well as studying the correlation of superficial gray matter versus the deep gray matter in a random sample of adult human brains.

1.8 Objectives of study

The objectives of this study are focused on:

i. Coronal cross sections obtained from cadaveric brain samples.

ii. Specific staining to contrast gray and white matter architecture.

iii. To obtain the thickness of the cerebral cortex at slices which contain deep gray matter at angles 0º, 45º, 90º, 135º and 180º, which define significantly important structural and functional regions.

iv. To obtain the surface area of the superficial cortex versus deep nuclei at equivalent levels.

CHAPTER TWO: THE MANUSCRIPT

Morphological relationship between the superficial cortical and deep gray matter structures in adult human brains: A cadaveric study

Haghegh E.Y, Naidu E.C.S, Azu O.O

Discipline of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences. Nelson R Mandela School of Medicine, University of KwaZulu-Natal, South Africa.

Correspondence: azu@ukzn.ac.za

Abstract

Background: While various neurodegenerative diseases affect the cortical mass and mass of deep gray matter differently, finding an optimal and accurate method for measuring thickness and surface area of the cerebral cortex remains a challenging problem due to the highly convoluted surface of the cortex. We therefore investigated the superficial and deep gray matter thickness and surface area in a sample of cadaveric specimens at the Discipline of Clinical Anatomy, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, South Africa to provide some clue as to possible variations in these parameters.

Materials and Method: With ethical approval, 60 brain samples were uniformly sectioned at 5mm thickness and eight slices containing the deep nuclei were taken from each brain and stained by Mulligan's technique. Thickness was measured at selected angles 0º, 45º, 90º, 135º and 180º for both right and left cerebral hemispheres. The cortical thickness and surface area of selected slices for both the superficial cortex and the corresponding deep nuclei were measured.

Results: Mulligan's stain produced good gray mater differentiation and clear images that enabled manual delineation of structures. There was rightward asymmetry of cortical thickness of the selected slices at the suggested angles which corresponded to structurally and functionally important brain regions. There was a positive correlation between the mean surface area of superficial cortex and deep nuclei across the regions of interest (ROI).

Discussion and Conclusion: Baseline data from 55 brain samples provided a range of means and 95% confidence intervals for the three parameters of cortical thickness, cortical surface area and surface area of deep nuclei to be made for a reference table comprising eight coronal slices taken at five angles. This allows an objective assessment of thinning of the cortex or loss of deep gray matter to be made from measurements of the same parameters for the equivalent slices from a postmortem brain slice or an appropriate radiographic image.

Keywords: Brain, Morphometry, Stains, Gray matter.

Introduction

While the cerebral cortex is largely responsible for higher brain functions, including sensory and motor activity, differential cortical involvement in numerous neurodegenerative pathologies of the brain exists with regards to morphology. For instance, Alzheimer's disease (AD) is known to cause degeneration of limbic and heteromodal regions of the cerebral cortex (Dickerson et al., 2009) whereas in Huntington's disease (HD) progressive striatal degeneration occurs with the level of cortical involvement unknown (Rosas et al., 2002). In addition to this, establishing a clear morphological mapping of brain regions (cortical and deep) that are affected by various diseases remains a challenge. Much of what is known were based on postmortem sections of the brain (Morrison and Hof, 2002) but more sophisticated methods involving magnetic resonance imaging (MRI) *in-vivo* techniques have evolved in recent times, enabling subjective quantitative neuroanatomical assessment of cortical involvement in diseases of the brain (Dickerson et al., 2009).

However, there are more pertinent challenges with regard to assessment of cortical thickness in human brain samples due to the complex folding patterns and regional variability (Rosas et al., 2002). Different methods have been adopted to provide accurate and efficient morphometric measurements, especially for cortical thickness and surface area especially in a cohort of patients (*in vivo* samples). Manual methods are labor-intensive and typically focus on a few *a priori* defined regions (Dickerson et al., 2009) with resultant extensive slices and numerous regions that need to be studied. Therefore, to get accurate and precise results from *in vivo* samples, automated and highly sophisticated method of measurements such as a high-resolution T1-weighted MRI scan which require expensive devices and well trained technicians (Fischl and Dale, 2000) is needed. Other approaches that depend on volume-based deformation described by Talairach (which is a 3-D normalization of cortical surface) are fraught with poor anatomical precision and the metric properties do not reflect the distances along the cortical surfaces (Fischl et al., 1999).

Therefore, it is important to realize a simple and efficient technique that provides better differentiation between the gray and white matter which can be performed in an *in vitro* sample to overcome the measurement errors where the cortical surfaces are not perpendicular to the cardinal axes. The Mulligan's method of staining provides a simple, reliable and efficient procedure that helps to differentiate between the gray and white matter in *in-vitro* samples (Meneses et al., 2004) and also allow for the manual estimation of cortical thickness and surface area confidently. Therefore, our study will investigate cortical thickness difference between right and left cerebral hemispheres in a random sample of adult human

brains and to correlate the superficial cortical surface area with the deep nuclei. This will allow further understanding of how the cortex is affected by diseases and thus may provide important new insights (Rosas et al., 2002).

Materials and methods

Sample of sixty adult cadaveric whole brain specimens was obtained from the Discipline of Clinical Anatomy, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, which represent the 8.4% of total South African white ethnicity population Basic biographical information (e.g., age, sex and race) were recorded as well as any disease condition (if present).

Ethical Approval

The study was approved by the Biomedical Research Ethics Committee (BREC), University of KwaZulu-Natal (Reference BE 134/14). The study was performed at the Department of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal and adhered to regulations guiding the use of human tissues and the South African Human Tissue Act 65 of 1983.

Sample preparation

Each specimen was carefully weighed by a calibrated manual scale (Hanson H005 Mechanical Weighing Scales). These scales weigh up to 3kgs/6.6lbs and in increments of 20g/1oz. Subsequently, each brain sample was photographed using a Sony digital camera (7.2 mega pixels and 4x optical zoom), numbered in order and then transferred into a preservative solution with the following constituents (mixture of 12.5 ml phenol, 50 ml of 40% formalin, 1 liter pine oil, 927.5 ml ethanol and 1 liter glycerin) in 1 liter of water.

Brain slicing

After properly aligning each brain sample, serial coronal sections 5mm thick were obtained from each brain specimen; as shown in appendix 1, using the Russel Hobbs food slicer (Model: RHFS-01/02), as shown in appendix 2. Which is designed to produce slice thickness from 0 - 15mm. 5 mm thickness was chosen to minimize distortions of slices and ensure consistency and stability. Slices were carefully

handled and preserved in labeled plastic containers with the same preservative solution, taking into account the order of consecutive slices. They were approximately 17 - 20 slices per brain sample. These sections were defined carefully by region of interest (ROI) depending on the visual observation of the deep nuclei (caudate nucleus, putamen and globus pallidus). Of note, the selections of those nuclei as (ROI) over others were depending on the size and the location of the nuclei. As the anatomists divided the basal ganglia into four groups; two large nuclei (the striatum and the pallidum) and two smaller nuclei (the substantia nigra and the subthalamic nucleus). That has made the selection of these large nuclei (caudate nucleus, putamen and globus pallidus) more preferable as (ROI), because we mainly relied on visual observation of deep gray matter. In addition, these large, deep nuclei had important participation in normal brain function, behavior and numerous neurological conditions associated with basal ganglia dysfunction. Hence, only eight coronal sections were selected for this study per brain starting from the 6th slice (fronto-occipital direction) with subsequent eight slices carefully chosen.

Staining

After a trial (pilot) phase on the choice of stain between the Barnard and Mulligan's techniques, the latter was adopted due to better differentiate between the gray and white matter (as indicated in Meneses et al., 2004) and a good efficacy of the technique in terms of shorter time, less volume of chemicals and cost effectiveness because each solution can be used more than once and stains 25 to 30 slices.

Mulligan's method (Gregg, 1975): Staining solution is composed of phenol 40 g, copper sulfate 5g and 1.25 ml hydrochloric acid in 1 liter of water. The slices were soaked for 4 minutes at 60-65ºC in a bath warmer LABOTEC (model No. 420), in a Mulligan solution. Then, washed by ice water for 10 seconds and subsequent immersion in 0.4% tannic acid for 1 minute at room temperature. After further soaking in the water for 1 minute, the slices were put in 0.08% ferric ammonium sulfate at room temperature for 10-15 seconds. Then another washing in running water for 8 hours (this was modified to 30 minutes soaking in water in order to cut down on time). This adjustment gave the same result when compared with the 8 hour stated.

Gray matter demarcation

Immediately after the staining, each slice was carefully handled and dried with paper towel and put in transparency file while achieving consistency and stability of each slice with minimal distortion. Under appropriate illumination and good visual observation of gray matter and regions of interest, outlines of

superficial and deep gray matter were manually drawn using a waterproof permanent marker 0.6 mm (Model: edding 141F), as shown in Figure 2.1. Similar steps were followed on the eight slices from each brain. Each transparency was properly numbered according to the sequence of the sample and slice and the image scanned using KONICA MINOLTA Bizhub 211 Office printer scanner. The outlines of each slice drawing were highlighted with BIC chisel tip permanent markers for more demarcation of gray matter, as shown in appendix 3. Thereafter, each scanned copy is saved as a picture file in an organized manner, subsequent to measurement of thickness and surface area of the cerebral gray matter.

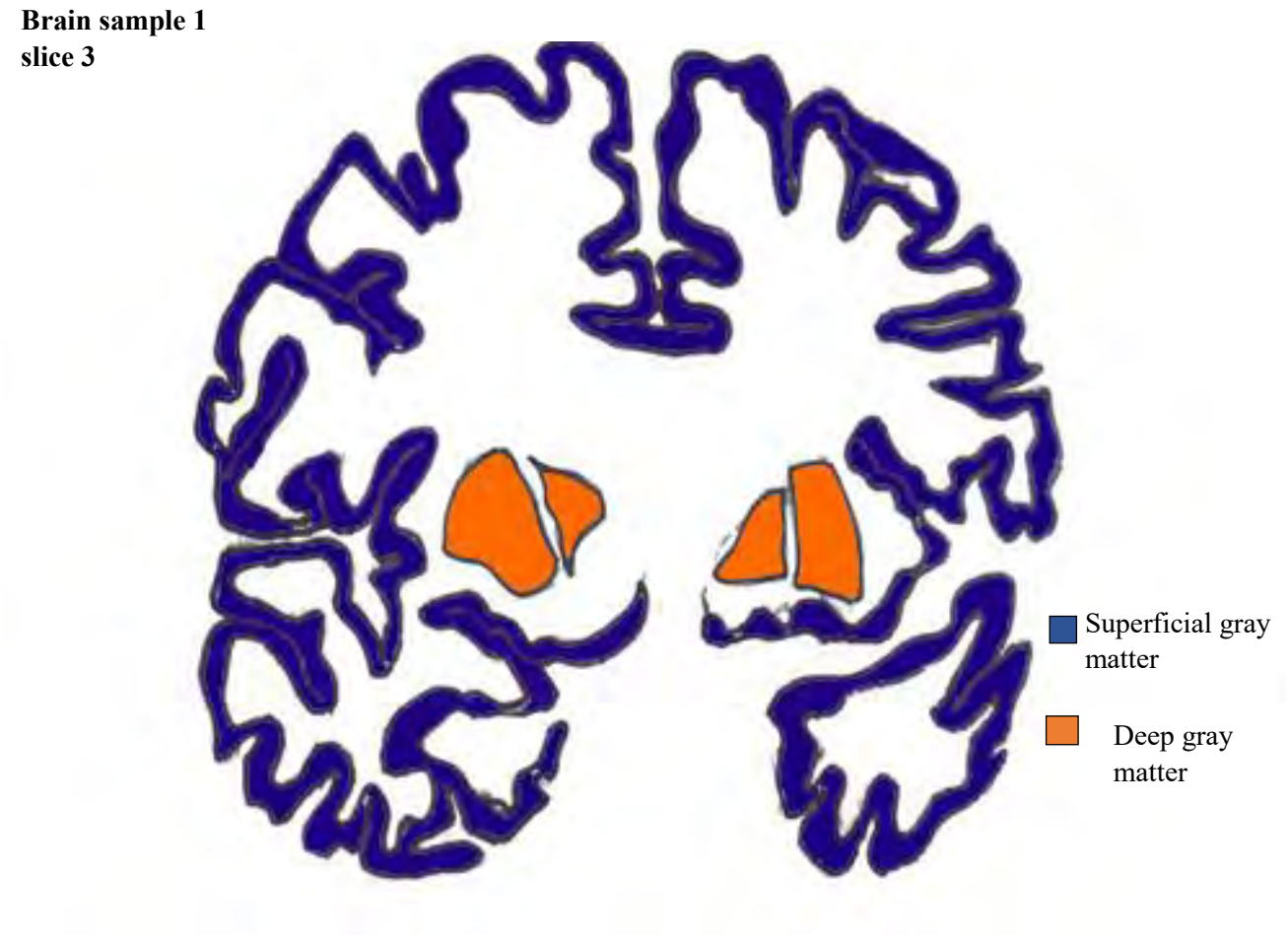

Figure 2.1: Brain sample 1 slice 3 shows the outlines of superficial and deep gray matter using a waterproof permanent marker 0.6 mm (Model: edding 141F).

Gray matter measurements

All saved copies of each slice were printed out for manual measurement of cerebral cortical thickness of both cerebral hemispheres. Cortical thickness was measured at selected area and at chosen angles (0º, 45º, 90º, 135º and 180º) for both right and left hemispheres. These angles were selected because the plans would cross over important gyri and sulci (for example, angle 90º crossed over the lateral sulcus in most of the slice). The angles were marked and measured by positioning the protractor at the center of each slice and carefully noting the intersection of the axes, those angles were presumed as semicircle (180º) rather than circle (360º) because it is more convenient and easier to make a comparison between right and left cerebral cortex. The thickness was then read off at suggested angles (using a metric rule) in millimeters, as shown in figure 2.2. For surface area, both the superficial cortex and deep nuclei were measured using the paint.net software (version 4.0.6, dotPDN LLC and Rick Brewster) which contour and calculates the surface area of selected regions (in mm2) automatically. All data are collated in excel sheet for subsequent analyses.

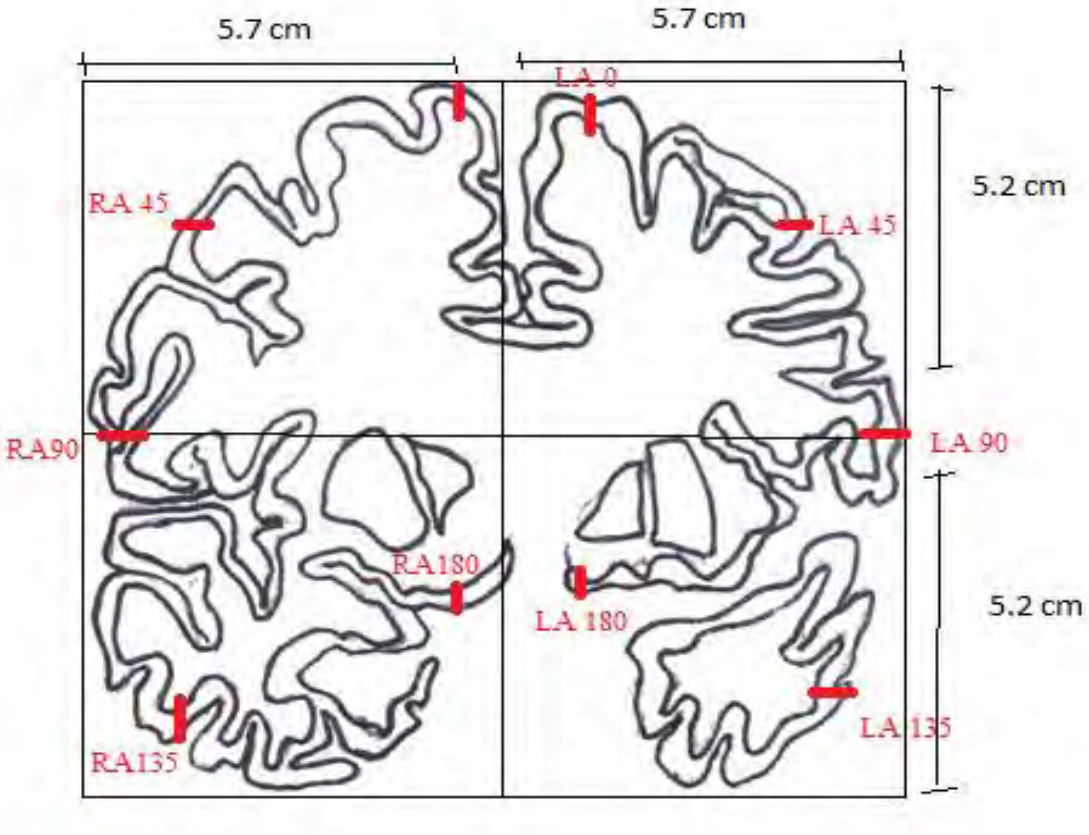

Figure 2.2: Brain sample 1 slice 3 sketch. Measurement of cortical thickness at suggested angles (0°, 45, 90°, 135°, 180°) for both right and left cerebral hemispheres. LA 0º (left angle 0º) = 2 mm, LA 45 (left angle 45º) = 3 mm, LA 90 (left angle 90º) = 5 mm, LA 135 (left angle 135º) = 4 mm, LA 180 (left angle 180º) = 2 mm, RA 0 (right angle 0º) = 4 mm, RA 45 (right angle 45º) = 3 mm, RA 90 (right angle 90º) = 3 mm, RA 135 (right angle 135º) = 5 mm, RA 180 (right angle 180º) = 3 mm.

Statistical analysis

Data were subjected to formal descriptive statistics for cortical thickness at suggested angles (0º, 90º, 135º and 180º) for both right and left cerebral hemispheres. The surface area was calculated for both superficial cortex and deep nuclei. The results were obtained using IBM SPSS Statistics for Windows (Version 23.0. Armonk, NY: IBM Corp.), which are expressed as mean ± SD (standard deviation) with 95% confidence interval.

Limitation of the study

i. This is a random sample represented by the cadavers donated to the Department of Clinical Anatomy between 1990 to 2015
ii. Limited number of slices which represent the regions of interest (ROI).
iii. Limited number of angles which cross structural and functional important regions.
iv. The degree to which the coronal sections are crossing the right and left hemispheres are not at precisely the same levels, due to slight misalignments between the right and left cerebral hemispheres.
v. Cortical surface area was given as a single total. Preferred method of segmentation would involve using the specific angles of the arc as per chosen angles 0º, 45º, 90º, 135º and 180º.

Results

Out of 60 whole cadaveric brain specimen used in this study, 5 (8.33%) were excluded as a consequence of damage either because of pathology 2 (3.3%) or trauma 3 (5%) and 16 (26.7%) had missed the biological information. 22 (36.7%) were males and 22 (36.7%) were females with male to female ratio of 1:1, as shown in figure 2.3. Age was ranged from 47 to 97 years with a median age of 79.5 years and an average of 77.9±11.85 years. All the 44 (73.3%) out of 60 specimens were of white ethnicity.

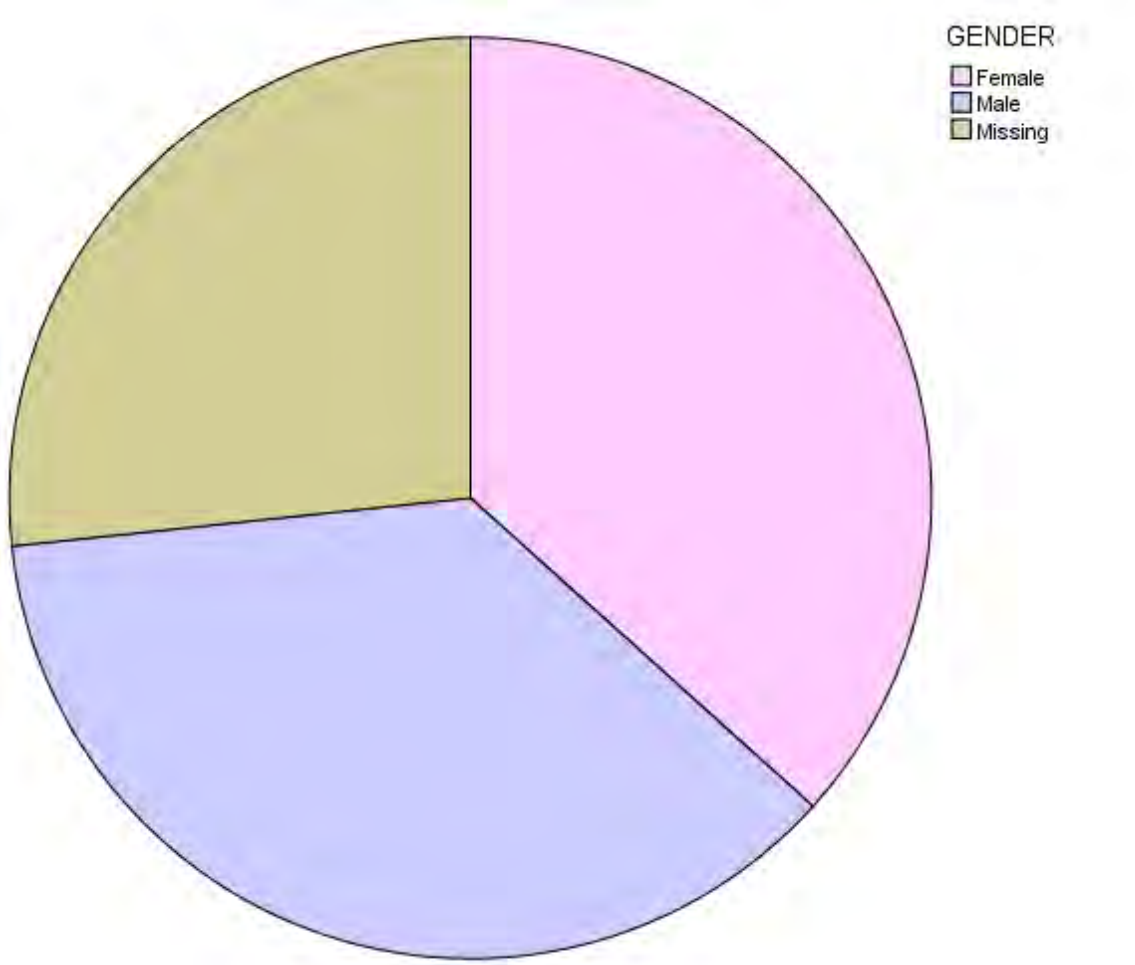

Figure 2.3: The age distribution of the sample population.

Brain weight

Brain weight ranged from 820 - 1540 g with a median of 1190 g and an average of 1186.6±135.5 g.

Cortical thickness

Fifty five specimens (N=55) were analysed for cortical thickness. The thickness of both right and left cerebral cortex were ranged from 1 to 8 mm for slice 1, 1 to 6 mm for slice 2, 4, 6, 7 and 8, 1 to 7 mm for slice 3 and 5, with a median of 4 mm. The average thickness of each slice at the angles 0º, 45º, 90º, 135º and 180º for both right and left cerebral cortex is presented in Table 2.1 and 2.2, respectively.

Figure 2.4 and 2.5 shows the average cortical thickness of the right and left cerebral hemisphere of each slice at angles 0º, 45º, 90º, 135ºand 180º, respectively.

Table 2.1: Cortical thickness of the right cerebral hemisphere of each slice at angles 0º, 45º, 90º, 135ºand 180º.

Slice number and suggested angles	Minimum	Maximum	Mean±SD	95% Confidence Interval for Mean
Slice 1 at				
Angle 0°	1	6	3.78± 1.35	3.42 - 4.15
Angle 45°	1	8	4.05± 1.5	3.64 -4.47
Angle 90°	1	8	4.04± 1.5	3.63 -4.44
Angle 135º	1	8	4.31± 1.7	3.85 -4.77
Angle 180º	1	6	2.87± 1.6	2.42 -3.33
Slice 2 at				
Angle 0°	1	6	3.87± 1.46	3.48 - 4.27
Angle 45°	1	6	4.05± 1.43	3.67 -4.44
Angle 90°	1	6	4.04± 1.4	3.66 -4.41
Angle 135º	1	6	3.91± 1.5	3.50 -4.32
Angle 180º	1	6	2.76± 1.8	2.25 -3.27
Slice 3 at				
Angle 0°	1	7	4.13± 1.41	3.74 - 4.51
Angle 45°	2	7	4.18± 1.47	3.78 -4.58
Angle 90°	1	7	4.18± 1.44	3.79 -4.57
Angle 135º	1	7	4.02± 1.6	3.59 -4.45
Angle 180º	1	6	2.31± 2.2	1.71 -2.91
Slice 4 at				
Angle 0°	1	6	3.69± 1.41	3.31 - 4.07
Angle 45°	1	6	3.89± 1.34	3.53 -4.25
Angle 90°	1	6	3.93± 1.47	3.53 -4.33
Angle 135º	1	6	4.31± 1.27	3.96 -4.65
Angle 180º	1	6	1.64± 1.05	0.61 -1.50

Slice 5 at				
Angle 0°	2	6	4.27± 1.21	3.95 - 4.60
Angle 45°	1	7	4.09± 1.46	3.69 -4.49
Angle 90°	1	6	3.55± 1.67	3.09 -4.00
Angle 135º	2	7	4.11± 1.43	3.72 -4.50
Angle 180º	1	6	0.87± 1.84	0.37 -1.37
Slice 6 at				
Angle 0°	1	6	3.83± 1.49	3.41 - 4.22
Angle 45°	1	6	4.04± 1.38	3.66 -4.41
Angle 90°	2	6	4.11± 1.24	3.77 -4.44
Angle 135º	1	6	3.83± 1.44	3.41 -4.19
Angle 180º	1	5	1.02± 1.58	0.59 -1.45
Slice 7 at				
Angle 0°	1	6	4.15± 1.44	3.75 - 4.54
Angle 45°	1	6	3.93± 1.38	3.55 -4.30
Angle 90°	1	6	3.80± 1.38	3.43 -4.17
Angle 135º	1	6	4.09± 1.40	3.71 -4.47
Angle 180º	1	6	1.15± 1.93	0.62 -1.67
Slice 8 at				
Angle 0°	1	6	3.78± 1.37	3.41 - 4.15
Angle 45°	1	6	3.96± 1.37	3.59 -4.34
Angle 90°	1	6	3.31± 1.13	4.00 -4.62
Angle 135º	1	6	4.16± 1.34	3.80 -4.53
Angle 180º	1	6	2.04± 2.15	1.45 -2.62

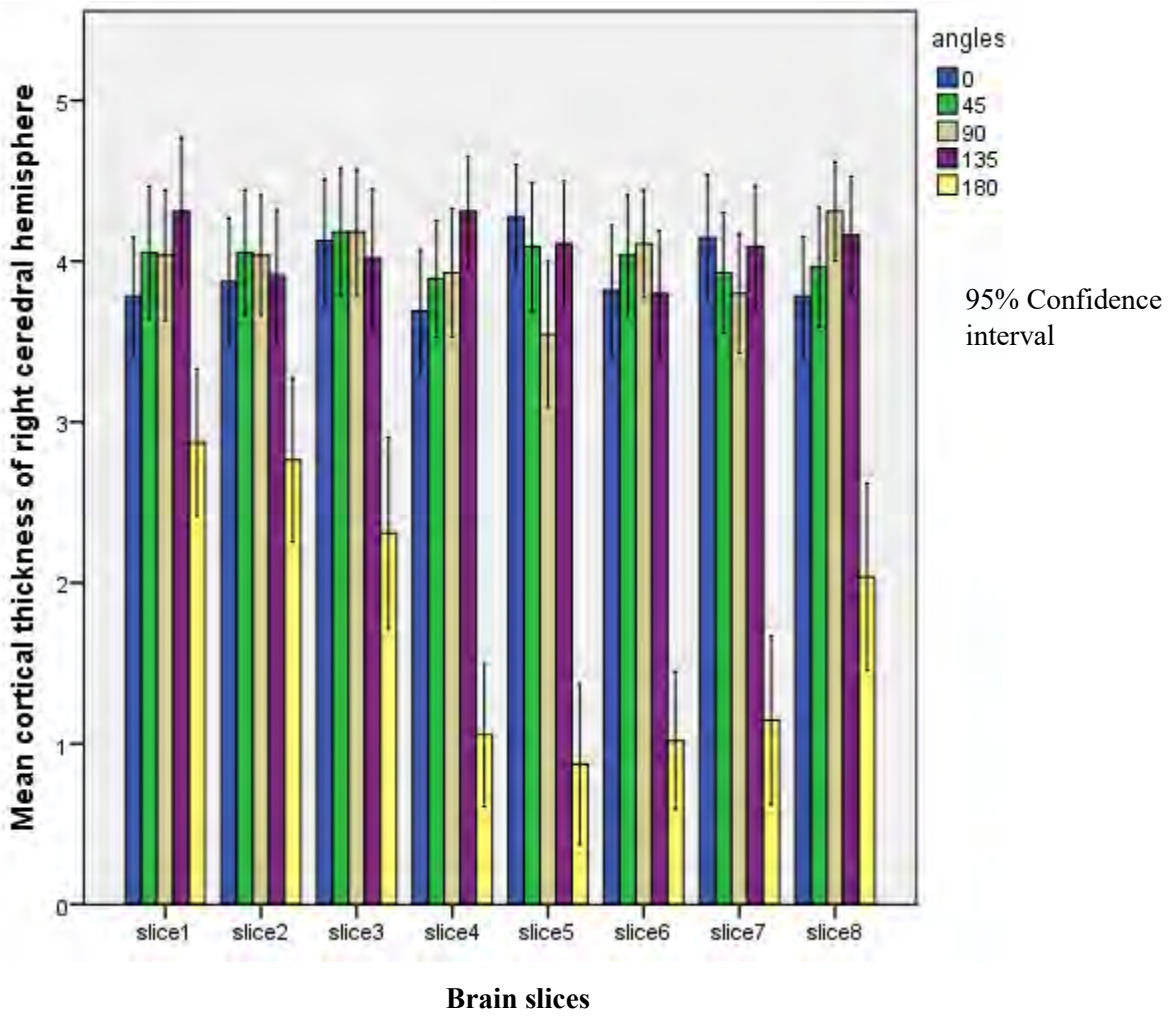

Figure 2.4: Mean cortical thickness of the right cerebral hemisphere at angles 0º, 45º, 90º, 135ºand 180º for slices (1-8).

Table 2.2: Cortical thickness of the left cerebral hemisphere of each slice at angles 0º, 45º, 90º, 135ºand 180º.

Slice number and suggested angles	Minimum	Maximum	Mean±SD	95% Confidence Interval for Mean
Slice 1 at				
Angle 0°	1	8	4.05± 1.5	3.64 - 4.47
Angle 45°	1	8	4.31± 1.7	3.85 -4.77
Angle 90°	1	6	3.53± 1.47	3.13 -4.93
Angle 135º	1	8	4.15± 1.6	3.71 -4.58
Angle 180º	1	6	2.58± 1.7	2.12 -3.04
Slice 2 at				
Angle 0°	1	6	4.5± 1.43	3.67 -4.44
Angle 45°	1	6	3.91± 1.5	3.50 -4.32
Angle 90°	1	6	3.78± 1.27	3.44 -4.13
Angle 135º	1	6	4.16± 1.27	3.82 -4.51
Angle 180º	1	6	2.53± 1.65	2.08 -3.97
Slice 3 at				
Angle 0°	2	7	4.18± 1.47	3.78 - 4.58
Angle 45°	1	7	4.02± 1.59	3.59 -4.45
Angle 90°	1	7	4.29± 1.43	3.90 -4.68
Angle 135º	1	6	3.84± 1.59	3.40 -4.27
Angle 180º	1	5	1.51± 1.84	1.01 -2.01
Slice 4 at				
Angle 0°	1	6	3.89± 1.34	3.53 - 4.25
Angle 45°	1	6	4.31± 1.27	3.96 -4.65
Angle 90°	1	6	3.95± 1.29	3.59 -4.30
Angle 135º	1	6	3.69± 1.37	3.96 -4.65
Angle 180º	1	6	1.16± 1.85	0.66 -1.66
Slice 5 at				
Angle 0°	1	6	4.09± 1.46	3.69 - 4.49

Angle 45°	2	7	4.11± 1.43	3.72 -4.50
Angle 90°	1	6	3.96± 1.26	3.09 -4.00
Angle 135º	1	7	4.16± 1.50	3.76 -4.57
Angle 180º	1	6	1.22± 2.05	0.66 -1.77
Slice 6 at				
Angle 0°	1	6	4.04± 1.38	3.66 - 4.41
Angle 45°	1	6	3.80± 1.44	3.41 -4.19
Angle 90°	1	6	4.02± 1.25	3.68 -4.36
Angle 135º	1	6	3.60± 1.41	3.22 -4.98
Angle 180º	1	6	1.11± 1.93	0.59 -1.63
Slice 7 at				
Angle 0°	1	6	3.93± 1.38	3.55 - 4.30
Angle 45°	1	6	4.09± 1.40	3.71 -4.47
Angle 90°	1	6	4.00± 1.45	3.61 -4.39
Angle 135º	1	6	4.00± 1.36	3.63 -4.37
Angle 180º	1	6	1.09± 1.81	0.60 -1.58
Slice 8 at				
Angle 0°	1	6	3.96± 1.37	3.59 - 4.34
Angle 45°	1	6	4.16± 1.34	3.80 -4.53
Angle 90°	1	6	3.69± 1.39	3.31 -4.07
Angle 135º	1	6	4.29± 1.37	3.92 -4.66
Angle 180º	1	6	1.56± 1.99	1.02 -2.10

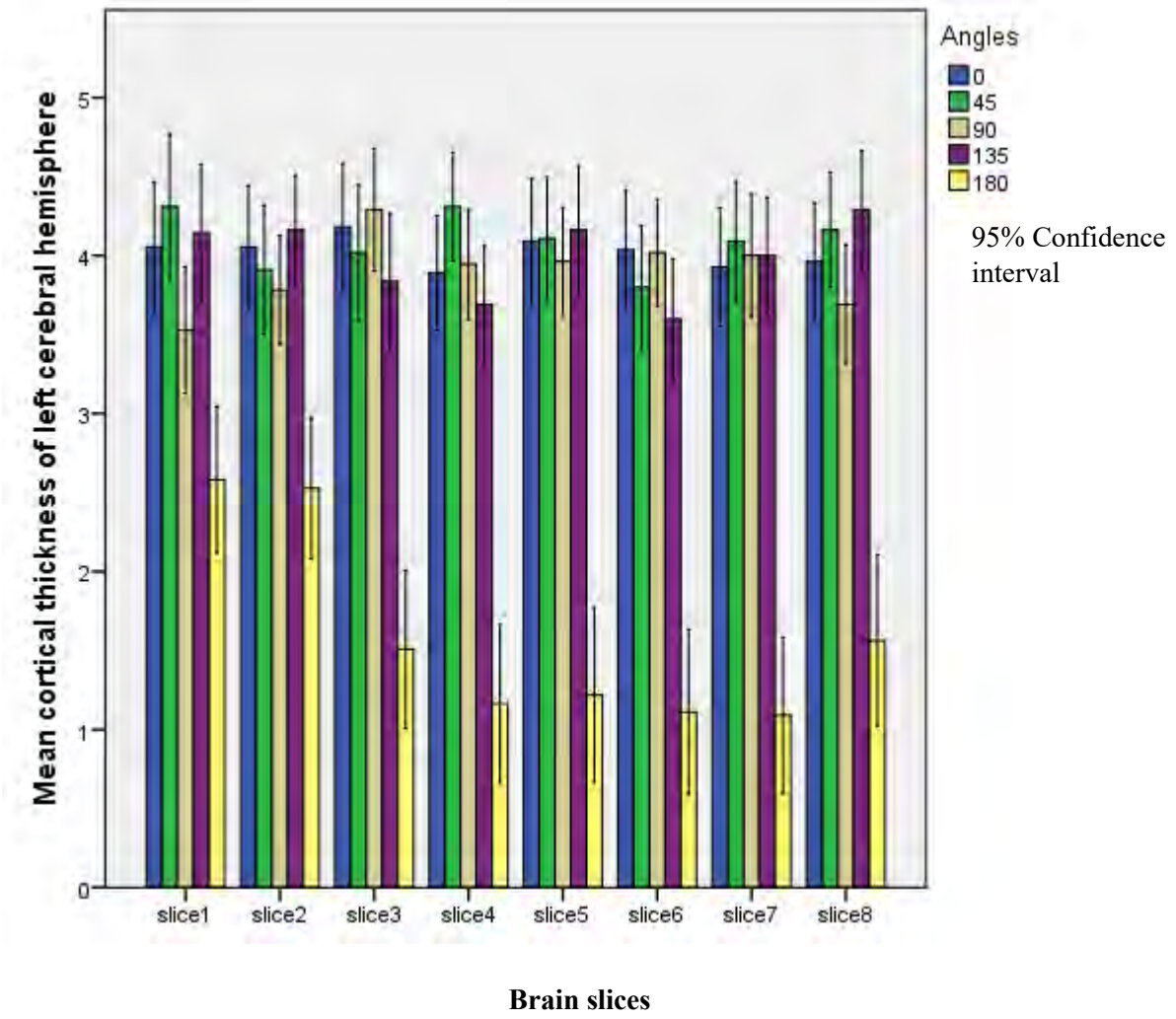

Figure 2.5: Mean cortical thickness of the left cerebral hemisphere at angles 0º, 45º, 90º, 135ºand 180º for slices (1-8).

A comparison of the means of right cerebral cortex showing thickness 4±1.44 mm for slice 1, 4.06±1.35 mm for slice 2, 4±1.47 mm for slice 3, 3.92±1.39 mm for slice 4, 3.99±1.56 mm for slice 5, (4±1.35) for slice 6, 3.93±1.36 mm for slice 7 and 4.13±1.35 mm for slice 8. These values were slightly greater than the corresponding values for the left side as indicated 3.72±1.71 mm for slice 1, 3.69±1.54 mm for slice 2, 3.57±1.89 mm for slice 3, 3.4±1.83 mm for slice 4, 3.51±1.93 mm for slice 5, 3.31±1.86 mm for slice 6, 3.42±1.88 mm for slice 7 and 3.53±1.81 mm for slice 8, as presented in Table 2.3 and Figure 2.6.

On average, the superficial cortical thickness is slightly more for the right cerebral hemisphere in selected slices across the suggested angles which related to structurally and functionally important regions.

Slice number	(Mean±SD)	
	Right cerebral cortex	Left cerebral cortex
Slice 1	(4±1.44)	(3.72±1.71)
Slice2	(4.06±1.35)	(3.69±1.54)
Slice 3	(4±1.47)	(3.57±1.89)
Slice 4	(3.92±1.39)	(3.4±1.83)
Slice 5	(3.99±1.56)	(3.51±1.93)
Slice 6	(4±1.35)	(3.31±1.86)
Slice 7	(3.93±1.36)	(3.42±1.88)
Slice 8	(4.13±1.35)	(3.53±1.81)

Table2.3: Comparative mean cortical thickness between right and left cerebral hemisphere.

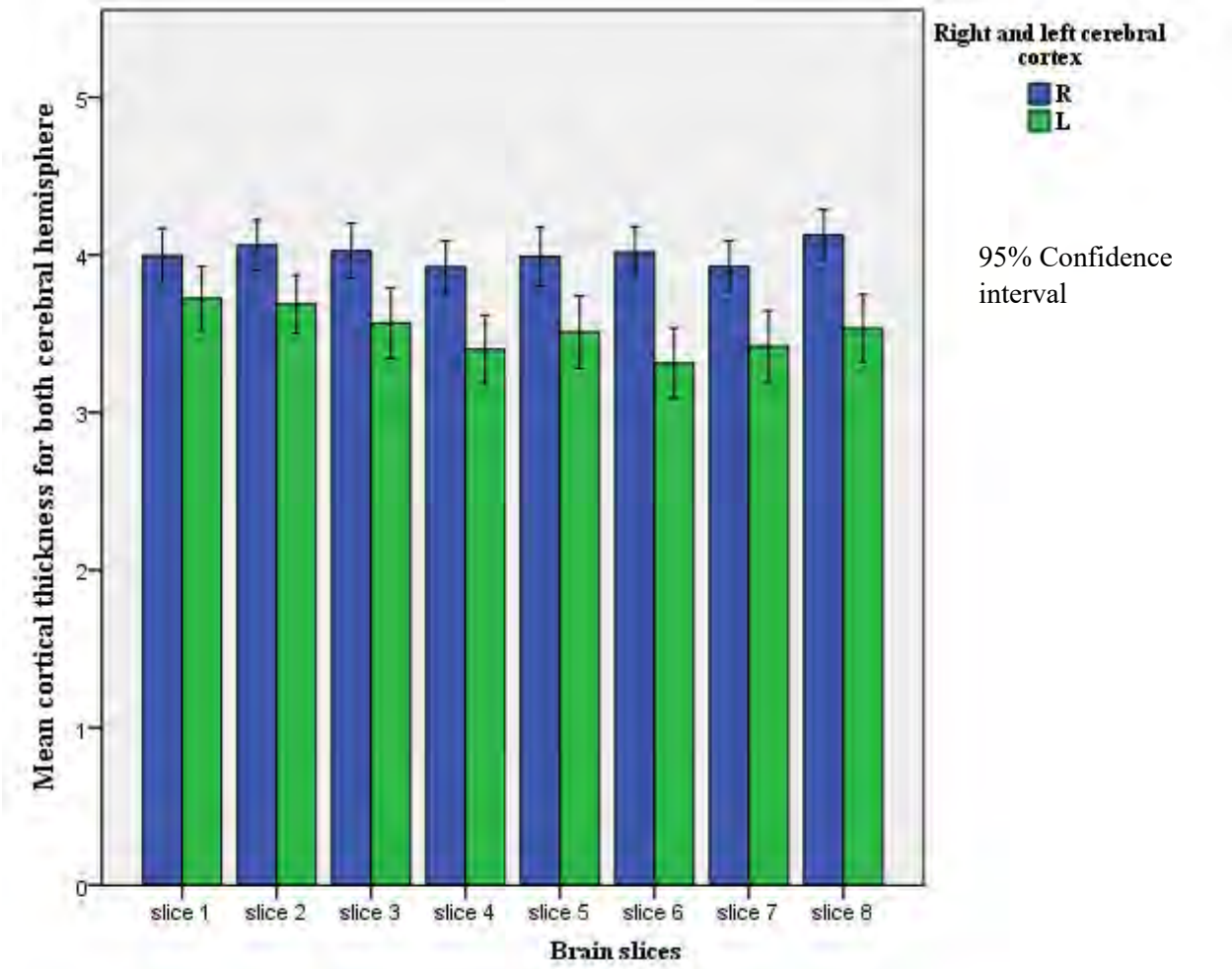

Figure 2.6: Comparative mean cortical thickness between right and left cerebral hemisphere.

Inter-observer errors:

Total number of staining slices is 440 slices and the cortical thickness was measured by 3 observers for each angle. Therefore, the total number of measurements is 3 times x 10 angles x 440 slices =13200 measurements. In collecting the data, a manual measurements are more susceptible to errors, therefore, an inter observer repeated measure ANOVA was conducted to estimate whether the differences between the observers was reasonably acceptable. Table 2.4 shows the mean, standard error and 95% confidence interval for mean.

Number of observers	Mean	Standard error	95% Confidence Interval for Mean
Observer 1	3.541	.027	3.607 – 3.881
Observer 2	3.493	.027	3.604 – 3.876
Observer 3	3.557	.027	3.535 – 3.803

Table 2.4: Descriptive statistics of the 3 observer measurements of cortical thickness.

The Greenhouse Geiser test did not reach statistical significance. However as Mauchly's Test of Sphericity was violated (Mauchly's W = .783, P < .001), and Wilk's Lambda was used for the subsequent interpretation. A statistical difference between the 3 observer's measurements ($\Lambda\lambda = .997$, $F(2, 4391) = 7.212$, $p = .001$, $\eta^2 < .003$) was found, however the error was of no practical relevance due to the manual measurement having a resolution of 1 mm (see figure 2.7).

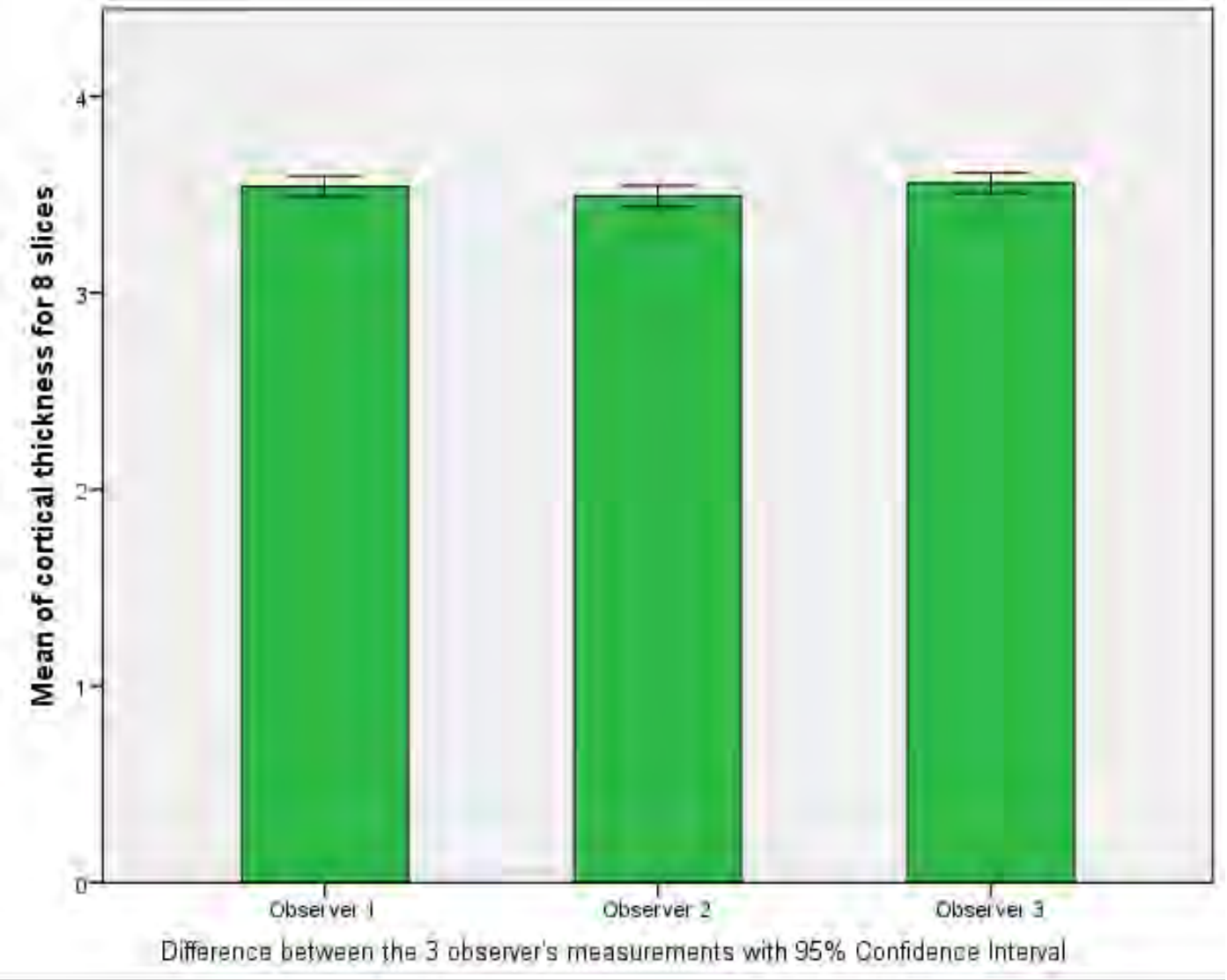

Figure 2.7: The difference between the mean of the 3 observer's measurements with 95% confidence interval.

Surface area of superficial cortex and deep nuclei

The mean of the superficial cortical surface area is 8730±1353 mm² for slice 1, 9253±1294 mm² for slice 2, 9900±1098 mm² for slice 3, 9837±1199 mm² for slice 4, 1126± 414 mm² for slice 5, 9754±1764 mm² for slice 6, 9751±1083 mm² for slice 7 and 9595±1274 mm² for slice 8. The means surface area of the cortex is larger than the deep nuclei surface area as follow: 730±206 mm² for slice 1, 927±396 mm² for slice 2, 1000± 368 mm² for slice 3, 1059± 338 mm² for slice 4, 1126± 414 mm² for slice 5, 1239± 1243 mm² for slice 6, 1230± 1234 mm² for slice 7, 940± 992 mm² for slice 8, as presented in Table 2.5.

Slice number	Mean±SD	95% Confidence Interval for Mean
Slice 1		
Superficial cortical surface area	(8730±1353)	8328 - 9132
Deep nuclei surface area	(730±206)	675 - 786
Slice 2		
Superficial cortical surface area	(9253±1294)	8999 -10063
Deep nuclei surface area	(927±396)	820 - 1034
Slice 3		
Superficial cortical surface area	(9900±1098)	9574 – 10226
Deep nuclei surface area	(1000± 368)	901 - 1100
Slice 4		
Superficial cortical surface area	(9837±1199)	9481 – 10194
Deep nuclei surface area	(1059± 338)	967 - 1150
Slice 5		
Superficial cortical surface area	(9882±1246)	9511 – 10252
Deep nuclei surface area	(1126± 414)	1014 - 1238
Slice 6		
Superficial cortical surface area	(9754±1764)	9230 - 10278
Deep nuclei surface area	(1239± 1243)	902 - 1557
Slice 7		
Superficial cortical surface area	(9751±1083)	9429 - 10073
Deep nuclei surface area	(1230± 1234)	896- 1564
Slice 8		
Superficial cortical surface area	(9595±1274)	9217 - 9973
Deep nuclei surface area	(940± 992)	672 - 1208

Table 2.5: the average surface area of superficial cortex versus the deep nuclei.

The mean surface area of deep nuclei for each slice is related to the mean value of the superficial cortical surface area. For example, the mean value of surface area of deep nuclei for the fifth slice is 1126 mm² with a 95% confidence interval range of 1014 – 1238 mm², while the mean value of surface area of cortex for the same slice is 9882 mm² with a 95% confidence interval range of 9511 – 10252 mm² as shown in Table 2.5 and Figure 2.8. Given an unknown brain slice from an equivalent location, if the absolute value of the cortical surface area falls below the 95% confidence interval indicated above while the surface area of deep nuclei lie within the 95% confidence interval, then there is objective significant thinning of the cortex at P<0.05.

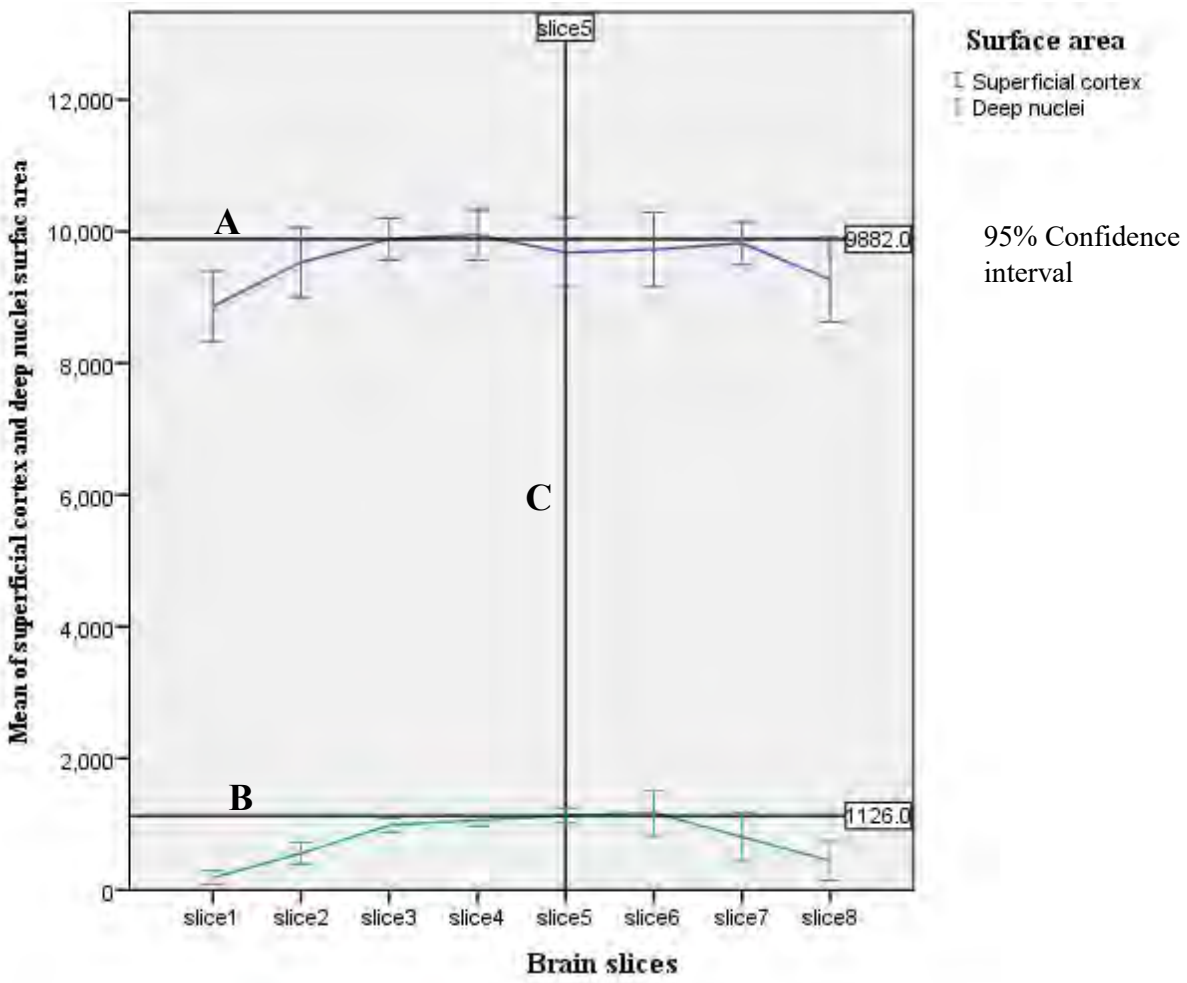

Figure 2.8: Comparative mean of superficial cortex and deep nuclei. **A** represents the mean value of superficial cortex surface area and **B** represents the mean value of the deep nuclei surface area. Both **A** and **B** lies within the 95% confidence interval. **C** represents the fifth slice which crosses A and B at their mean values.

Discussion

The study of the human cerebral cortex and the estimation of its thickness and surface area based on manual measurements can provide insight into normal brain development and neurodegenerative disorders. Accurate and proper measures of the cortical thickness and surface area are desired for sensitive detection of any changes can occur in the cerebral cortex. Especially, investigation of the cortical surface area and thickness may provide a useful understanding of normal brain development and brain aging, (De Leon et al., 1997, Jack et al., 1997), as well as structural effects of neurodegenerative disorder such as Huntington's disease (HD) (Vonsattel and DiFiglia, 1998, Walker, 2007), schizophrenia (Van Os, 2009), amyotrophic lateral sclerosis (ALS) (Kiernan and Hudson, 1994), as well as in Alzheimer's disease (AD) (Thompson et al., 2003). In contrast cortical thickness measurements provide beneficial information about neuronal loss or degradation indicated by thinning of the cortex which is of great benefit in assessing the efficacy of a wide range of treatments for such diseases.

The difficulty of properly measuring the thickness and surface area of the cortex without clear demarcation between white and gray matter is the key point to obtain efficient and easy way of measurements. Therefore, to overcome the measurement errors, we used staining technique that allows clear differentiation between the gray and white matter which is Mulligan's technique of staining. . This differentiation is preferably performed with *in-vitro* samples as morphometric data obtained from postmortem cadaveric brains provide significantly more detailed structural information from direct visual observations of the regions of interest (ROI). In addition, there is less structural resolution in the regions where the gyri and sulci are buried (Van Essen et al., 1998, Zilles et al., 1988; Deng et al., 2014, Fischl and Dale, 2000) which manual measurements can significantly improve.

As mentioned, many other studies tried to do such measurements in *in-vivo* samples through different suggestions such as using "high - dimensional warping" by recording two volumes (Christensen et al., 1995, Evans et al., 1994, Joshi et al., 1997, Miller et al., 1993). This does not guarantee the sulcal and gyral alignment, which is the accurate landmark for the location of functional areas (Fischl et al., 1999). While other technique depended on transformation and reconstruction of the cortical surface to measure the distance between only two structural or functional points on the cortical surface, this technique had poor accuracy because of distortion of the anatomical structure of the cortical surface (Fischl et al., 1999, Miller et al., 1993, Talairach et al., 1967). In general the cortical thickness and surface area obtained from such technique of measurements will not be accurate and precise to distinguish the site and progress of cortical changes, where this level of precision is important in early detection of regionally specific cortical atrophy associated with early manifestations of neurodegenerative disorders such as Alzheimer disease (AD) (Thompson et al., 2003).

Here, we present the first study in Africa that provides the basis for setting up a reference database of information on the thickness of the cerebral cortex and studying the relationship between the surface area of superficial and deep gray matter. This is a useful first step in objectively quantify the changes associated with the specific diseases. Often these changes are usually subjectively determined clinically by visual inspection of radiographs such as MRI. However, for reliable and repeatable comparisons, measures of cortical thinning or loss of deep gray matter need to be objectively quantified. This will result in increased understanding of how the cortex is affected by diseases and thus provide important new insights (Rosas et al., 2002. Morphometric measurement of the cortex is considered one of the key methods that help in understanding brain structure and function.

Thus, we focused on evaluating the superficial cortical thickness of certain specific regions of interest (ROI) at angles 0º, 45º, 90º, 135º and 180º for both right and left cerebral hemispheres, these angles represent the most important areas of structure and function. For example, angle 90º represents the lateral sulcus for most of the slices. In order, to address the fact that there is a slight difference between the right and left cortical thickness of cerebral hemispheres at these angles 0º, 45º, 90º, 135º and 180º and investigating the correlation of the surface area of the superficial cortex versus the deep nuclei, we conducted a comprehensive descriptive statistical analysis on the thickness and surface area of the cerebral cortex for the selected slices with underlying deep nuclei.

Comparing the means of the right cerebral cortex, a thickness of 4±1.44 mm for slice 1, 4.06±1.35 mm for slice 2, 4±1.47 mm for slice 3, 3.92±1.39 mm for slice 4, 3.99±1.56 mm for slice 5, 4±1.35 mm for slice 6, 3.93±1.36 mm for slice 7 and 4.13±1.35 mm for slice 8 was found. These values were slightly greater than the corresponding values for the left side with 3.72±1.71 mm for slice 1, 3.69±1.54 mm for slice 2, 3.57±1.89 mm for slice 3, 3.4±1.83 mm for slice 4, 3.51±1.93 mm for slice 5, 3.31±1.86 mm for slice 6, 3.42±1.88 mm for slice 7 and 3.53±1.81 mm for slice 8, which reflect the relationship between cortical thickness and functional specialization of each hemisphere (Paus et al., 1996, Watkins et al., 2001) for these slices. For example, in most specimens, the fourth and fifth slices represented the postcentral gyrus.

The average surface area of the cerebral cortex for the combined eight slices is 9756±1391 mm^2 and that of the deep nuclei is 1004±387 mm^2. With respect to the cortex, the surface area is a function of volume and due to the generally uniform thickness of the cortex; cortical volume is also a function of the cortical thickness. This is the basis for using cortical thickness and cortical surface area as a surrogate marker of cortical volume. Loss of cortical neurons ultimately leads to shrinkage in cortical volume, which may be detected by a reduction in cortical thickness, which in turn also manifests itself as a reduction in cortical surface area for a given slice due to the above relationships. Loss of neurons affecting the deep nuclei

correspondingly also results in a decrease in deep gray matter volume. This can be detected in individual slices as a reduction in the total surface area of deep nuclei in a given brain slice.

Establishing a normal range of cortical thickness, cortical surface area and deep nuclei surface area for a specific coronal brain slice will enable one to objectively evaluate to some degree whether there is indeed a significant loss of cortical gray mater or loss of deep gray matter upon inspection of an equivalent slice from an unknown subject where this pathology is suspected.

Although, there is a large absolute difference, there is a close relationship between the surface area of the superficial cortex and the deep nuclei of the same slice when the relative sizes are compared. For example, the mean value of surface area of deep nuclei for the fifth slice is 1126 mm^2 with a 95% confidence interval range of 1014 – 1238 mm^2, while the mean value of surface area of cortex for the same slice is 9882 mm^2 with a 95% confidence interval range of 9511 – 10252 mm^2 as shown in Table 3.6. Given an unknown brain slice from an equivalent location, if the absolute value of the cortical surface area falls below the 95% confidence interval indicated above while the surface area of deep nuclei lies within the 95% confidence interval, then there is objective significant thinning of the cortex at $P<0.05$

Conclusion

Structural and functional features of the cortex are still under much study, due to the complexity of the cortical sheet. Therefore, a large number of methods have been used to elucidate and understand the relationship between the structural and functional localization of the cerebral cortex. These methods rely on finding an efficient and accurate technique of measurement, which is a powerful tool to detect any cortical gray matter changes associated with neurodegenerative and developmental disorders.

The results obtained from this study were achieved by combining a simple, efficient and reliable method for measuring cortical thickness to enable an investigation into the relationship between the surface area of the superficial versus the deep gray matter. This can be useful in studying diseases affecting the cerebral cortex and deep nuclei. This study provides an objective quantification and lays the foundations for developing a reference database of information on the thickness and surface area of cerebral cortex and deep nuclei.

Recommendations

This study emphasized the need to develop a reference database of information on the thickness and surface area of the cerebral cortex, which will provide further opportunity to determine any cortical changes associated with normal and diseased conditions. Further comparative studies of average cortical thickness and surface area across specific structural and functional regions of interest are needed. These

comparisons can be done with patients and control groups to validate and verify the findings of this study. In addition, comparisons can be taken between gender, race and age differences of the cortical thickness and surface area of superficial and deep gray matter make this study provide more extensive informative database on South African population.

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CHAPTER THREE: SYNTHESIS

3.1 Synthesis

Different methods have been adopted to provide accurate and efficient morphometric measurements especially for cortical thickness and surface area, which provide a powerful tool for diagnosing and studying a variety of neurological and psychiatric diseases that affect the cortical ribbon in different ways. Interestingly, the cortical thickness is not distributed uniformly for most of the functional regions (Kandel et al., 2000) and is proportional to the number of neurons that form the columns of the cortical sheet. Therefore, the assessment of structural and functional changes of the human brains with *in vitro* samples allows insight into mechanisms of normal brain development such as during aging or any pathological processes such as Alzheimer's disease (AD) that cause degeneration of the limbic and heteromodal regions of the cerebral cortex (Dickerson et al., 2009a), whereas gross striatal atrophy is the prominent feature of Huntington's disease (HD) with the level of cortical involvement unknown (Rosas et al., 2002). In addition to this, it is hard to find the optimum and accurate method for measuring the thickness and surface area of cerebral cortex because such measurements remain a challenging problem due to the highly convoluted surface of the cortex (Rosas et al., 2002).

Therefore, Staining by Mulligans method can overcome these difficulties and provides an efficient and reliable method for measuring cortical thickness, allowing a clear differentiation between the gray and white matter architecture. This differentiation is preferably performed with *in-vitro* samples as morphometric data obtained from postmortem cadaveric brains provide significantly more detailed structural information from direct observations of the regions of interest (ROI). In addition, there is less structural resolution in the regions where the gyri and sulci are buried (Van Essen et al., 1998; Zilles et al., 1988; Deng et al., 2014; Fischl and Dale, 2000), which manual measurements can significantly improve.

While the approach described by Talairach which is a 3-D normalization of cortical surface has significant drawbacks. This is because of poor anatomical precision and the metric properties do not reflect the real distances along the cortical surfaces (Fischl et al., 1999). For example; the distance between the lateral tip of the central sulcus and the superior temporal gyrus is a centimeter distance. But when measure the distance between the same two points along the actual cortical surface in the Cartesian embedding space, it was 10 cm because of the Sylvian fissure depth. So, the Talairach coordinate system is not adequately accurate to distinguish between neighboring cortical areas especially where some

functional areas are less than 2 cm wide such as in the visual area. (Fischl et al., 1999; Miller et al., 1993; Talairach et al., 1967).

This is the first study in Africa that specifies the basic ground to setting up a reference database of data on the thickness of the cerebral cortex and considering the relationship between the surface area of superficial and deep gray matter. This is a valuable initial phase in unbiased measuring the progressions associated with particular diseases. Often these progressions are normally subjectively determined clinically by visual examination of radiographs such as MRI. In any case, for reliable repeatable comparisons, measures of cortical diminishing or loss of deep gray matter should be objectively evaluated. This will bring about expanded comprehension of how the cortex is influenced by diseases and hence give imperative new bits of knowledge (Rosas et al., 2002). Besides, considering the cortical thinning is possibly valuable matter in surveying the adequacy of a wide variety of treatments. Morphometric measurement of the cortex is considered as one of the key methods that help us better understand brain structure and function.

Consequently, we focused on evaluating the superficial cortical thickness of certain specific regions of interest (ROI) at angles 0º, 45º, 90º, 135º and 180º for both right and left cerebral hemispheres, these angles represent the most important areas of structure and function. For example, angle 90º represents the lateral sulcus for most of the slices. altogether, to address the way that there is a slight difference between the right and left cortical thickness of cerebral hemispheres at these angles 0º, 45º, 90º, 135º and 180º and investigating the correlation of the surface area of the superficial cortex versus the deep nuclei, we conducted a comprehensive descriptive statistical analysis on the thickness and surface area of the cerebral cortex for the selected slices with underlying deep nuclei.

Comparing the means of the right cerebral cortex, a thickness of 4±1.44 mm for slice 1, 4.06±1.35 mm for slice 2, 4±1.47 mm for slice 3, 3.92±1.39 mm for slice 4, 3.99±1.56 mm for slice 5, 4±1.35 mm for slice 6, 3.93±1.36 mm for slice 7 and 4.13±1.35 mm for slice 8 was found. These values were slightly greater than the corresponding values for the left side with 3.72±1.71 mm for slice 1, 3.69±1.54 mm for slice 2, 3.57±1.89 mm for slice 3, 3.4±1.83 mm for slice 4, 3.51±1.93 mm for slice 5, 3.31±1.86 mm for slice 6, 3.42±1.88 mm for slice 7 and 3.53±1.81 mm for slice 8, which reflect the relationship between cortical thickness and functional specialization of each hemisphere (Paus et al., 1996, Watkins et al., 2001) for these slices. For example, in most specimens, the fourth and fifth slices represented the postcentral gyrus.

The average surface area of the cerebral cortex for the combined eight slices is 9756±1391 mm^2 and that of the deep nuclei is 1004±387 mm^2. With respect to the cortex, the surface area is a function of volume and due to the generally uniform thickness of the cortex; cortical volume is also a function of the cortical

thickness. This is the basis for using cortical thickness and cortical surface area as a surrogate marker of cortical volume. Loss of cortical neurons ultimately leads to shrinkage in cortical volume, which may be detected by a reduction in cortical thickness, which in turn also manifests itself as a reduction in cortical surface area for a given slice due to the above relationships. Loss of neurons affecting the deep nuclei correspondingly also results in a decrease in deep gray matter volume. This can be detected in individual slices as a reduction in the total surface area of deep nuclei in a given brain slice.

Establishing a normal range of cortical thickness, cortical surface area and deep nuclei surface area for a specific coronal brain slice will enable one to objectively evaluate to some degree whether there is indeed a significant noteworthy loss of cortical gray matter or loss of deep gray matter upon inspection of an equivalent slice from an unknown subject where this pathology is suspected.

Although there is a large absolute difference, there is a close relationship between the surface area of the superficial cortex versus the deep nuclei of the same slice when the relative sizes are compared. For example, the mean value of surface area of deep nuclei for the fifth slice is 1126 mm^2 with a 95% confidence interval range of 1014 – 1238 mm^2, while the mean value of surface area of cortex for the same slice is 9882 mm^2 with a 95% confidence interval range of 9511 – 10252 mm^2. Therefore, given an unknown brain slice from an equivalent location, if the absolute value of the cortical surface area falls below the 95% confidence interval indicated above while the surface area of deep nuclei lies within the 95% confidence interval, then there is objective significant thinning of the cortex at $P<0.05$.

3.2 Conclusion

Structural and functional features of the cortex are still under much study, due to the complexity of the cortical sheet. Hence, countless methods have been utilized to elucidate and comprehend the relationship between the structural and functional localization of the cerebral cortex. These methods rely on finding an efficient and accurate technique of measurement, which is a powerful tool to detect any cortical gray matter changes associated with neurodegenerative and developmental disorders.

The results obtained from this study were achieved by combining a simple, efficient and reliable method for measuring cortical thickness to enable an investigation into the relationship between the surface area of the superficial versus the deep gray matter. This can be useful in studying diseases affecting the cerebral cortex and deep nuclei. This study provides an objective quantification and lays the foundation for developing a reference database of information on the thickness and surface area of cerebral cortex and deep nuclei.

3.3 Recommendations

This study emphasised the need to develop a reference database of information on the thickness and surface area of the cerebral cortex, which will provide further opportunity to determine any cortical changes associated with normal and diseased conditions. Further comparative studies of average cortical thickness and surface area across specific structural and functional regions of interest are needed. These comparisons can be done with patients and control groups to validate and verify the findings of this study. In addition, comparisons can be taken between gender, race and age differences of the cortical thickness and surface area of superficial and deep gray matter make this study provide more extensive informative database on South African population.

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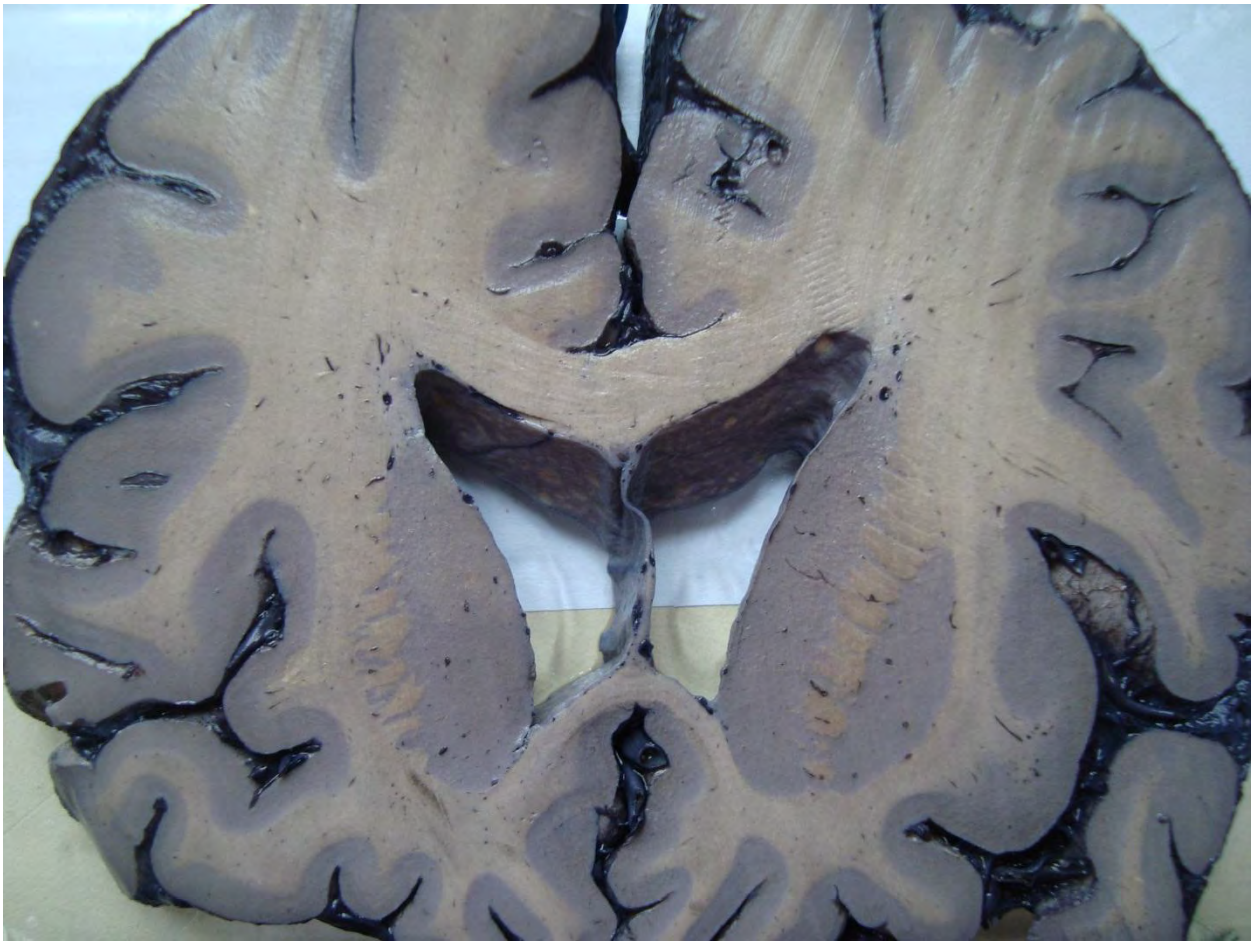
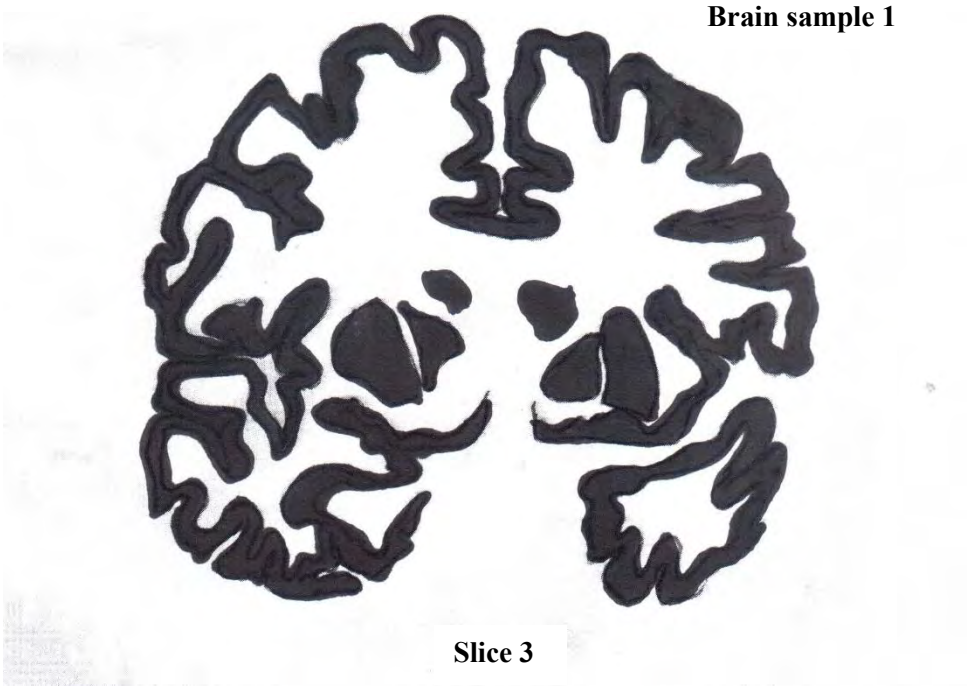
Appendices

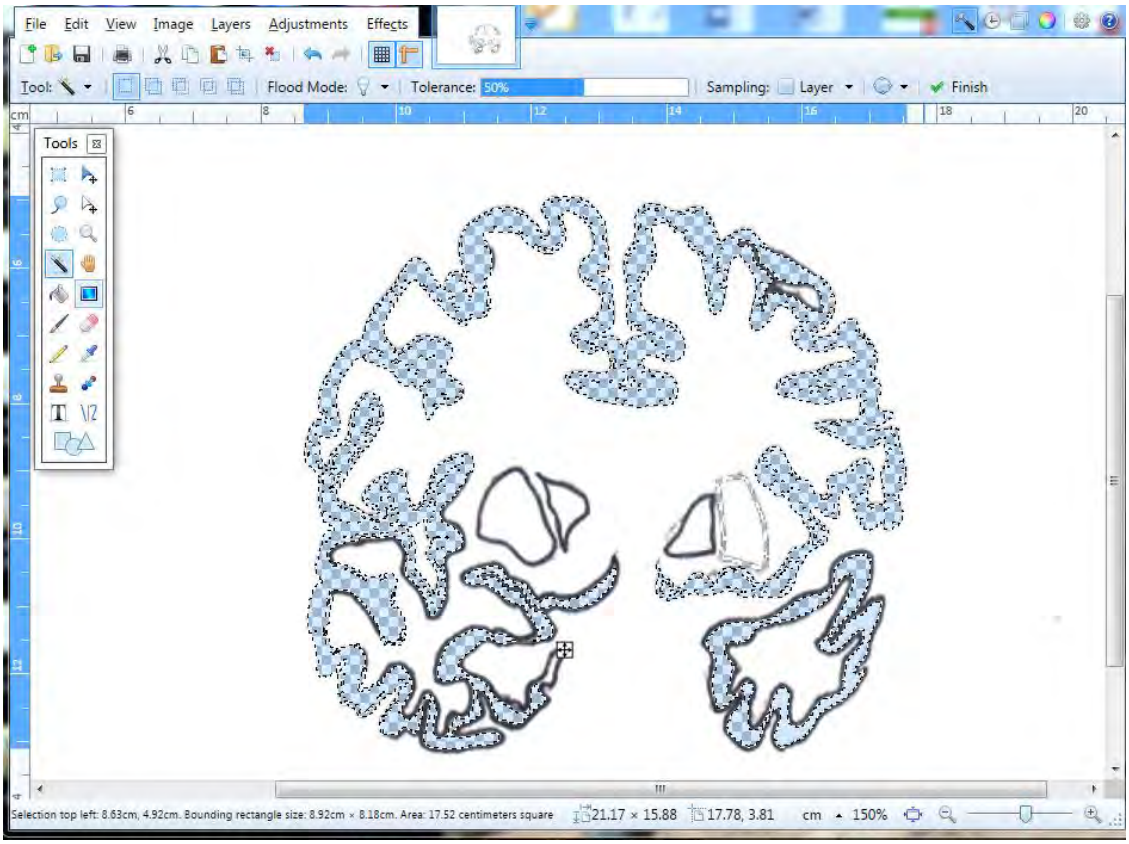


Appendix 1: Brain slice stained by the Mulligan's technique. There is clear demarcation between the white and gray matter.

Appendix 2: Russel Hobbs food slicer (Model: RHFS-01/02) that used for brain slicing.



Appendix 3: Brain sample 1 slices 3. The outlines are highlighted with BIC chisel tip permanent marker.



Appendix 4: Brain sample 1 slice 3. Measurement of the surface area on Paint.net software

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

25 April 2014

Dr Eman Haghegh
P O Box 13816
Cascades
PMB
3202
emanyacob@yahoo.com

Dear Dr Haghegh

PROTOCOL: A comparative cross sectional study of the morphological relationship between the superficial and deep grey matter structures in a random sample of cadaveric adult human brains in the discipline of anatomy at UKZN. REF: BE134/14

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 31 March 2014.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 22 April 2014 to queries raised on 03 April 2014 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 25 April 2014.

This approval is valid for one year from **24 April 2014.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on **10 June 2014.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

Professor D Wassenaar (Chair)
Biomedical Research Ethics Committee
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban, 4000, South Africa
Telephone: +27 (0)31 260 2384 **Facsimile:** +27 (0)31 260 4609 **Email:** brec@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx
Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

Appendix 5: Ethical approval letter

Amaechi Okpara <amechi2@yahoo.com> Dec 7 (1 day ago)

to me

On Sunday, December 6, 2015 5:39 PM, Amaechi Okpara <amechi2@yahoo.com> wrote:

On Sunday, December 6, 2015 5:37 PM, vitJournals <noreply@vitjournals.com> wrote:

Dear Dr. Azu

Your manuscript entitled "Morphological Relationship between the superficial cortical and deep grey matter structures in adult human brains: A cadaveric study " (manuscript reference number: eja.150391oa) has been successfully submitted online and is now being examined by our editorial staff. You can follow the status of your paper by logging in to vitJournals at http://www.vitjournals.com/eja

Please mention the above manuscript reference number in all future correspondence concerning this manuscript.

Thank you for submitting your manuscript to "European Journal of Anatomy".

Yours sincerely
Teresa Vazquez

Editorial Administrator.

European Journal of Anatomy
Editorial Office
Teresa Vazquez
tvazquez@ucm.es

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E-37007 Salamanca, Spain
Tel.: +34 923 294546; Fax: +34 923 294559
Web page: http://www.eurjanat.com
