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SEX DIFFERENCES IN THE CORPUS CALLOSUM OF *MACACA FASCICULARIS* AND *PAN TROGLODYTES*

By

Douglas C. Broadfield

A Dissertation submitted to the Graduate Faculty in Anthropology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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Abstract

SEX DIFFERENCES IN THE CORPUS CALLOSUM OF *MACACA FASCICULARIS* AND *PAN TROGLODYTES*

By

Douglas C. Broadfield

Advisor: Professor Jeffrey T. Laitman

As the primary commissure in the brain, the corpus callosum has been an area of intense investigation in humans. Further, studies in humans have shown that this structure is sexually dimorphic. While the extent and meaning of sexual dimorphism in the human corpus callosum has been investigated, what this structure is like in our closest relatives, the living apes, has not been approached. This dissertation investigates whether sex differences are present within two primate species, *Pan troglodytes* and *Macaca fascicularis*, addressing several issues important to neurology, paleoneurology, and human evolution.

The corpus callosum was examined morphologically and histologically in two different primate species. In the morphological phase, the midsagittal area of the corpus callosum was examined in *Pan troglodytes* (12 females, 11 males) and *Macaca fascicularis* (20 females, 20 males). Measurements included total callosal midsagittal area and area measurements of callosal regions (splenium, isthmus, anterior and posterior midbody, genu). Two techniques were used to divide the corpus callosum into regions, a radial-line method and a straight-line method. For both methods, the area of the corpus

callosum and each region was calculated to assess statistically significant differences in absolute area and relative callosal area between males and females.

In the second phase the most posterior one-fifth of the corpus callosum, the splenium, was examined in *Macaca fascicularis* (2 females, 2 males) and *Pan troglodytes* (1 male, 1 female) in order to elucidate its axonal composition. The splenium of each individual was thin sectioned (0.5μm), stained with toluidine blue, and examined using light microscopy (100x).

The results obtained from the morphological and histological aspects of this dissertation demonstrate that there is not a statistically significant difference between males and females of *Pan troglodytes* and *Macaca fascicularis* with regard to total and regional midsagittal area of the corpus callosum; or with regard to axon density/100μm^2, overall axon numbers, or within any of the axonal diameter classes in the splenium of the corpus callosum in either species. These results strongly suggest that dimorphism of the brain and corpus callosum arose later in hominin evolution, possibly not until the arrival of *Homo sapiens*.

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I am grateful to many people who have supported me throughout my graduate career. I could not have completed this dissertation without their support. First and foremost, I would like to thank my friend and advisor Jeffrey T. Laitman. He took me on as his student, although he knew he would be guiding me in an area that he admitted at the time he was not an expert. Nevertheless, he gave me a place to hang my hat and study the aspects of human evolution that interested me the most. He has guided and supported me throughout my graduate career, always willing to lend a helpful hand or a kind word where needed. Moreover, Jeff enthusiastically shared his knowledge in all areas of anatomy and anthropology for which I will be eternally grateful. It is rare to find an advisor who cares more about his students and his field. My approach to every aspect of academics and research will be forever influenced by him. Thank you Jeff for being the friend, advisor and person you are. I could not have accomplished any of my successes without you.

Another person who has given me more than he will ever know is Ralph L. Holloway. As an undergraduate and in my first few years of graduate school I had never intended to make a career of studying the brain. Indeed, when I first got to know Ralph, while taking his skeletal biology course, we never had one conversation about the brain. Afterwards during the now traditional Wednesday lunches at Columbia, Ralph and I began to discuss various topics in brain evolution. It was through these conversations that my interest in the corpus callosum and brain evolution was hatched. However, he knew the brain was not the focus of my studies when I entered graduate school, and thus, I was required to do a yearlong tutorial with him before I could even begin to study my topic of interest, sex

differences in the corpus callosum. It is that kind of dedication and demand for excellence for which I am grateful to him. I am honored to be able to count Ralph among my closest and dearest friends. He has always made himself available at whatever hour and location, be it New York or Cape Cod, to listen to my dilemmas, frustrations, and elations. His loyalty, frankness, and compassion enabled me to finish this dissertation. I am forever indebted to him for this and more.

In addition to Jeff and Ralph there have been many other people who have helped, guided, and advised me during my graduate career. Most notable among these people is Dr. Eric Delson, who has stood beside me and supported me from the day I arrived in New York. At a time I considered leaving New York, Eric convinced me to stay, and for that I am grateful. The resources available to a physical anthropologist in New York and NYCEP are unparalleled in the United States. When I came into the C.U.N.Y. program I was interested in studying early primate evolution and locomotion. Now I leave having done my dissertation on a topic that could not be more removed from my early interests. I could only have made such an alteration in my studies in New York. It is through Eric's vision to create the New York Consortium in Evolutionary Primatology that I have been able to study a topic in which I am truly interested.

Other NYCEP faculty members have been important to me during my graduate career. I would like to thank Drs. Sara Stinson, Tim Bromage, John Oates, Fred Szalay, Don Melnick, Todd Disotell, and the late Pat Bridges and Warren Kinsey for sharing their immense knowledge with me. These individuals also are important to me, because each has played an important role in shaping my career.

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nonhuman anatomy on many levels. For this I would like to thank Max Levitan, Ki Mak, and Marilyn McGinnis. One member of this group who deserves a special thanks is Joy Reidenberg. Joy has been invaluable friend during my graduate studies, giving me advice and support on everything from teaching to research methodology.

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In addition to the above individuals there have been other individuals who have helped me through this dissertation and life's triumphs and tragedies. These individuals mean a great deal to me, and I will never be able to repay them for all that they have given me over the years. Despite all that Jeff, Ralph and the others mentioned here have done for me, this dissertation could not have been possible without the support of two very

important people, my parents. They have always and continue to be my biggest fans. The things I have learned from them are too numerous to mention here. Everything I am and will be is because of them, and for that I am forever indebted to them. This dissertation is in honor of them. I do not believe, though, it is possible to thank my parents alone, since I have been blessed to grow up with three siblings. I also want to thank my sisters Marilyn and Bonnie who were always there to lend a sympathetic ear or a spontaneous plane ticket at a time of need. Above all this dissertation may not have ever occurred without my brother, Don. He set off my interest in science and comparative anatomy when I was six and he decided he, his friend Jamie and I should dissect several fish taken from the creek behind our house. He also introduced me to the theory evolution when I was 12 years old and he was a high school student finishing a biology paper late into the night. I would not have thought that those two events would have such a profound effect on my life, but like most younger brothers, I grew up in awe of my older brother. I still am.

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CHAPTER 1

INTRODUCTION, SCOPE OF RESEARCH, AND AN INTRODUCTION TO THE ANATOMY OF THE CORPUS CALLOSUM

1.1 INTRODUCTION AND SCOPE OF RESEARCH

This section presents a brief overview of the organization and direction of this dissertation. A complete discussion of the literature and each topic is presented in the subsequent chapters.

With the exception of cetaceans, the human and nonhuman primate brain is unique among mammals for its volume relative to body size. There is little argument over the complexity of the human brain, yet its underlying nature, which determines human uniqueness, is a mystery. In addition, humans appear to possess sex differences in cognitive ability. This dissertation contributes to the hypothesis that: *sex differences in the human brain occurred within the hominin lineage, and were not present in the ancestor of modern apes and humans*. While sex differences may occur in more primitive brain structures, this dissertation focuses on structures related to the cerebral hemisphere, that portion of the brain which has undergone the most change during the course of human evolution.

The cerebral cortex has undergone a dramatic evolution during hominin history. Progressing from a small, chimpanzee-like brain in *Australopithecus*, the human brain has come to be capable of linguistic, mathematical, abstract, and behavioral elements apparently unobtainable by other primate groups. An additional aspect of this evolution has been the emergence of sex differences in cognitive behaviors. The existence of sex

differences is not unheard of in primates, but it has been difficult to document in primate cognition. Anatomical distinctions between nonhuman primates and modern humans have become more difficult as we have come to appreciate our short evolutionary history. It is possible that during the course of primate evolution sex differences in the brain developed in early sexually dimorphic clades such as the cercopithecoids. This scenario is plausible due to the presence of sexually dimorphic skeletal morphology and group behaviors. Females behave differently from males, possibly due to different reproductive strategies. If sex differences occur in such phylogenetically distant taxa such as *Macaca* and *Papio*, it is possible that sex differences became even more distinct in a more recent common ancestor to humans such as *Pan*. The presence of sex differences in the brain of modern humans closest living relative would indicate that sex differences were already present in the earliest hominins. This would suggest that sex differences exhibited in modern humans are not unique, but merely an extension of *Homo*'s evolutionary past.

An alternative hypothesis suggests that sex differences in the modern human brain are unique to modern humans and did not occur until late in hominin evolution, possibly not until the advent of our own species, *Homo sapiens*. Although some similarity exists in the brains of *Pan* and modern humans, these similarities have not exposed any common sex differences between these two groups. Studies on modern human brains, however, have exposed a number of sex differences, albeit these discoveries occurred within non-neocortical structures. The presence of sex differences in cognition has also been observed. These results suggest that sex differences in the brain and cognition did not occur until late in human evolution.

This study focuses on the second hypothesis that sex differences in the telencephalon occurred late in hominin evolution. The cerebral cortex represents one of the most complex and costly structures humans possess. The complexity of this structure has evolved over 3-5 million years of hominin history to allow modern humans to perform complex cognitive tasks not seen in other animal groups. In addition, humans have evolved the cerebral areas responsible for these tasks such that males excel at certain tasks while female excel at others. Males for example perform better at tasks of mental rotation while females do better on tests of verbal richness. There is little information about how these differences develop or within which specific cerebral structures they reside. One cerebral structure, however, has shed light on the presence of sex differences in the brain, the corpus callosum (Fig. 1.1). As the major interhemispheric pathway of the brain, the corpus callosum provides a point at which to begin to examine cerebral sex differences. Since morphological sex differences have been noted in this structure, the question of when these sex differences developed in human evolution can be asked. If the presence of sex differences in the corpus callosum represents an epiphenomenon of primate brain evolution associated with the advent of the Catarrhini, then sex differences in this structure should manifest in *Macaca*. If these differences don't occur until the evolution of the Hominoidea, then *Pan* would exhibit this trait. If sex differences in this structure did not occur until after the ape-human split, then it would represent an autapomorphic character of the hominin clade.

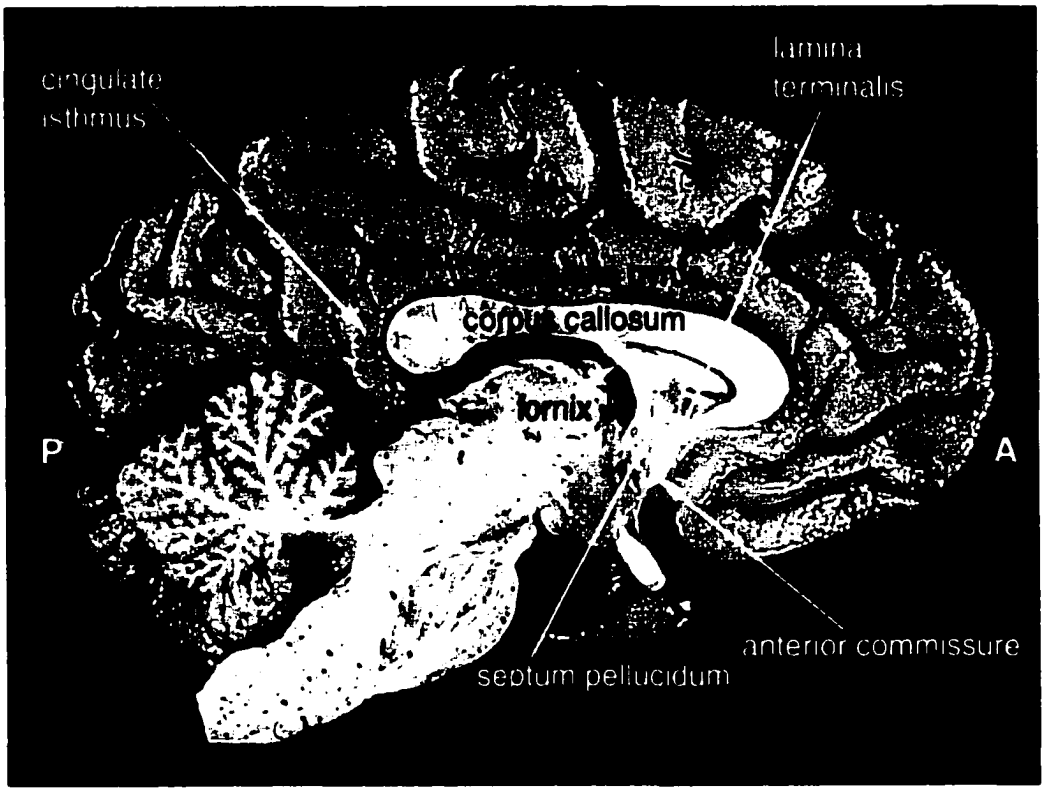

Fig. 1.1. Midsagittal view of brain of *Pan troglodytes*. A: anterior. P: posterior.

Using quantitative analysis of the midsagittal area of the corpus callosum in *Macaca fascicularis* and *Pan troglodytes*, this dissertation addresses several issues that are of fundamental importance to neurology and paleoneurology. It adds to current knowledge on the nerve fiber composition of the corpus callosum in primates, and clarifies our understanding of the fiber composition of this structure in nonhuman primates, including *Pan* for which no information is available. Further, the description of gender differences in the corpus callosum based on the fiber composition of the splenium addresses the problems created by morphological measurements. These fiber differences can be incorporated into the existing knowledge base regarding cognitive differences between male and female primates to understand the nature of these differences. Results used to

assess sex differences in the fiber composition of the splenium of the corpus callosum in *M. fascicularis* and *P. troglodytes* were used to construct evolutionary models explaining the development of sex differences in the brains of humans.

Section 1.2 gives an overview of the discovery and early descriptions of the anatomical structure of the corpus callosum as well as suggestions posited throughout history concerning the function of the corpus callosum. Section 1.2.2 presents the embryology of the corpus callosum in both human and nonhuman primates. Section 1.3.1 describes the basic evolution of the corpus callosum, while section 1.3.2 gives an overview of the anatomical structure of the corpus callosum in placental mammals. Section 1.3.3 discusses the topographic projection sites of interhemispheric axons and callosal function. Section 1.4 discusses sex differences in the primate brain. Finally, section 1.5 describes sex differences in the corpus callosum of rodents and human and nonhuman primates.

Chapter 2 reports the materials, specimen acquisition, specimen preparation and anatomy of the corpus callosum in Macaca fascicularis and Pan troglodytes. Chapter 3 reports the morphological phase of the dissertation research. It reports the methods, measurements and results for total and regional area of the corpus callosum in the above species. Chapter 4 reports the histological methods and analysis of the splenium of the corpus callosum for the above species. Finally, chapter 5 provides a discussion of the results of the dissertation research.

1.2 INTRODUCTION TO THE CORPUS CALLOSUM

The telencephalon is comprised of two large hemispheres (cerebral hemispheres) which are separated from each other by a deep longitudinal fissure. In humans the telencephalon accounts for approximately 76% of human brain volume (Stephan et al., 1981). Information passing into or out of the cerebral hemispheres must traverse the subcortical white matter. The myelinated fibers forming the white matter are organized into three types of fiber bundles: (i) arcuate bundles that connect adjacent or widely separated gyri in the ipsilateral hemisphere; (ii) internal capsule that connects the cerebral cortex with downstream nuclei (corticofugal fibers) and fibers conveying information to the cerebral cortex (corticopetal fibers); (iii) commissural bundles that connect the two cerebral hemispheres. Of the commissural bundles in the brain, the corpus callosum is the largest. In the human it contains approximately 300 million axons (Aboitiz et al., 1992a) that interconnect portions of the cerebral cortex. While each hemisphere can perceive and emote separately and simultaneously (Zaidel et al., 1990), interhemispheric connections are crucial for unified sensory, motor and cognitive performance (Onufrowicz, 1887; Suitsu, 1920; Sperry et al., 1969; Kimura, 1980; Witelson, 1985). In general these fibers connect homotopic areas; however, some end in areas different from those in which they arise (e.g., Brodmann's area 17 of one hemisphere connects to areas 18 and 19 of the contralateral hemisphere) (Aggoun-Zouaoui and Innocenti, 1994). Most cerebral areas receive callosal projections with the exception of the hand area in the somatosensory cortex and area 17 not representing areas adjacent to the vertical midline. In addition, fibers running between the temporal lobes, in particular the middle and inferior temporal gyri, run instead through the anterior commissure (Nolte, 1993).

1.2.1 A brief history of the discovery of the corpus callosum

Galen first mentioned the corpus callosum, literally "callous body", in the second century, using the Latin *corpus* or "body" and the Greek term *tulos* meaning hard - later translated to the Latin, *callus* (Kanne and Finger, 1999). Galen's description, however, was lacking details. This, though, was the only available description of the corpus callosum or any other brain structure until the 16th century when Vesalius in "De Humani Corporis Fabrica" presented a more complete description of the corpus callosum (1543, translated by Singer, 1952; Clarke and O'Malley, 1968; Harris, 1995). While Vesalius' description provided a better anatomical map of this structure, it would be another hundred years before researchers began to examine the corpus callosum in detail and speculate on its function (Njiokiktjien, 1991).

At the time Rene Descartes (1664) was promoting the pineal gland as "the seat of the soul", Thomas Willis in his *Cerebri Anatome* (1664) proposed that the corpus callosum was a more likely candidate for this honor. It was Willis who first suggested that the corpus callosum may serve an interhemispheric communicative role (Harris, 1995). Later, a papal physician named Giovanni Maria Lancisi speculated that Willis' position on the corpus callosum as the "seat of the soul" may be correct. In addition, a contemporary of Lancisi's, Francois Gigot de La Peyronie (1741) observed that while injuries to the cerebrum involving the pineal gland did not result in death, those involving the corpus callosum invariably did. As he viewed it, the corpus callosum was essential for life (Neuburger, 1897; Finger, 1994; Harris, 1995; Kanne and Finger, 1999). Several years after this proposal, though, in some of the first known experiments on the corpus callosum Johann Zinn (1749) damaged this commissure in cats and dogs, demonstrating

that it was not essential for life. Had Zinn observed his subjects more closely before they died, he may have shown what Karl Burdach hypothesized in the early 19th century, that the corpus callosum acted to unite the two hemispheres, albeit he like others before him also felt the corpus callosum was capable of reason. After Zinn's experiments other researchers conducted commissurotomy studies on animals, demonstrating evidence of motor, memory and attentional deficits (Finger, 1994; Kanne and Finger, 1999).

Conjecture concerning the function of the corpus callosum continued through to the early 20th century. In the 1920s Ivan Pavlov (1927) observed that dogs conditioned to salivate in response to a stimuli presented to a specific location on one side of the body responded with a similar response when the contralateral location was stimulated. Konstantin Bykov, a colleague of Pavlov's, conducted a series of commissurotomy experiments on dogs that were then conditioned using Pavlov's techniques (Bykov and Speranski, 1924). From these experiments he found that there was no bilateral transfer effect in dogs that had undergone commissurotomy. These experiments demonstrated for the first time the role of the corpus callosum in interhemispheric transfer (Finger and Kanne, 1999; Kanne and Finger, 1999). Since the time of Bykov's experiments, researchers have continued to study the nature and variability of the corpus callosum in normal functioning, disease, and development.

1.2.2 Development of the corpus callosum in human and nonhuman primates

The prosencephalon or forebrain develops from the telencephalic vesicles around the 5th week of gestation, and is derived from neural plate cells (embryologic ectoderm). These vesicles soon differentiate into a caudal diencephalon and a rostral telencephalon.

While the diencephalon continues development along the midline of the growing brain corresponding to most of the third ventricle, the telencephalon develops around the lateral ventricles and partially along a forward extension of the third ventricle. The precursors of the corpus callosum begin to develop along the roof of the ventricles from the lamina terminalis (the membrane connecting the cerebral hemispheres along the rostral midline) as a structure initially composed of astrocytic processes around the 8th week of gestation (Silver et al., 1982; Sidman and Rakic, 1982). It does not, however, present a distinct form until some time between human prenatal weeks 11 and 12 (Rakic and Yakovlev, 1968), being anchored anteriorly by the lamina terminalis and posteriorly by the developing fornix with the first callosal fibers appearing just superior to the hippocampal commissure (Wahlsten, 1981). While some researchers suggest that the rostrum of the corpus callosum is the last segment to develop (Rakic and Yakovlev, 1968; Hansen et al., 1993), others provide provocative evidence suggesting that the rostrum, which may arise as an extension of the lamina terminalis as the lamina rostralis, develops before the genu and splenium (Bull, 1967; Kier and Truwit, 1997). Although this suggestion is still debated, there is general acceptance that by the 16th week of gestation a rudimentary genu and body are present (Fig. 1.2).

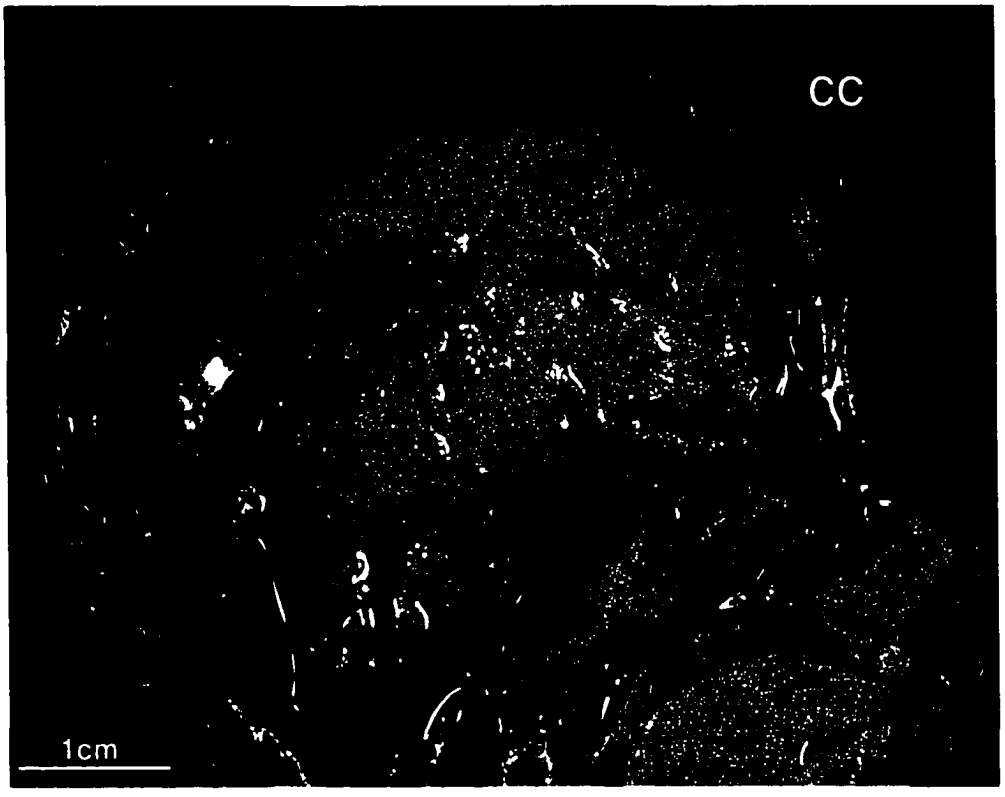

Fig. 1.2. Midsagittal section of the head of a 16 week old human fetus. CC: corpus callosum.

The corpus callosum grows posteriorly away from the lamina terminalis as axons from developing neurons in each hemisphere traverse its glial structure. It has been suggested that the astrocytic composition of the developing corpus callosum may provide molecular clues to guide axons across the midline to access the contralateral side (Silver et al., 1982). As astrocytes guide axons across the developing corpus callosum the corpus callosum continues to grow posteriorly; by the 20th week of gestation (Fig. 1.3) the genu is prominent, and the midbody and splenium have become elongated, albeit not well differentiated (Kier and Truwit, 1996). The upper end of the developing corpus callosum soon migrates posteriorly as more axons invade the area to eventually form the callosal midbody, isthmus and splenium of the adult. In addition, the corpus callosum expands

anteriorly as the genu continues to develop (Sidman and Rakic, 1982; Kier and Truwit, 1996). With its posterior advancement the corpus callosum extends above the choroid fissure, carrying the fornix with it. In addition, as the corpus callosum arches over the roof of the developing third ventricle, the remains of the lamina terminalis which lies between the corpus callosum and the fornix become stretched to form the septum pellucidum (Hewitt, 1962). The callosum then invades the vestiges of the hippocampal formation, which once occupied the area. These hippocampal vestiges form the induseum griseum that sits on top of the corpus callosum and the medial and lateral longitudinal stria. Soon by the 22nd week of human gestation the corpus callosum reaches its caudal limit as the growing dentate gyrus and hippocampus prevent further extension in the median plane. The corpus callosum along with other neural components continues to develop through birth as neurons grow and establish connections.

Fig. 1.3. Midsagittal section of the brain of a 20 week old human fetus. CC: corpus callosum, S: splenium of the corpus callosum, M: midbody of corpus callosum, G: genu of corpus callosum. Anterior end is to the right of the picture.

At birth the human brain is not completely developed. The components of the central nervous system continue to develop as they modify their water, lipid, and protein concentration due to progressive myelination and synapse development (Holland et al., 1986). The corpus callosum is no exception as it continues to expand from the passing of fibers across the midline to connect homotopic areas. Upon birth the corpus callosum is thin and flat with a symmetrical appearance (Fig. 1.4). It lacks the characteristic bulbous splenium seen in adults such that the thickness of the callosum is consistent through its length from the genu to the splenium. Despite this lack of an adult morphology, the corpus callosum of the neonate, like other areas of the brain, possesses more axons than the adult. In a study on *Macaca mulatta*, LaMantia and Rakic (1990b) found that

approximately 4 million axons pass through the corpus callosum in a developing rhesus monkey at embryonic day 65 (approximately week 15 – 16 in humans). This number increases to 188 million axons at the time of birth (embryonic day 165).

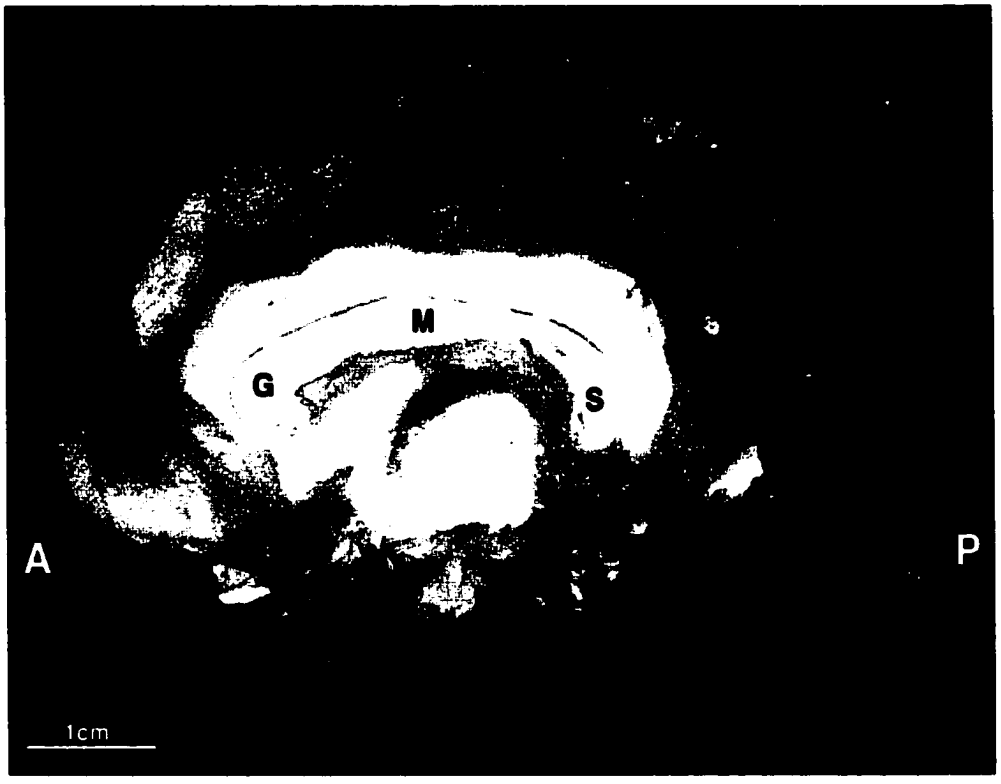

Fig. 1.4. Midsagittal section of a human neonatal brain. A: anterior, P: posterior, G: genu, M: midbody, S: splenium.

With regard to axonal proliferation, postnatal development of the corpus callosum proceeds in the opposite direction. A decline in the number of axons observed in the corpus callosum can be seen as early as postnatal day 5 in the rhesus macaque. Within 6 months after birth the macaque corpus callosum contains only 30% of its original axonal

compliment, having gone from 188 million axons at birth to 56 million. A consequence of neuronal elimination is an increase in myelination. At birth few axons are seen to have undergone myelination, while approximately 90% have done so by adulthood (Suitsu, 1920; LaMantia and Rakic, 1990b). The function of axon proliferation and subsequent elimination has been difficult to determine. The difference, nevertheless, between the number of cells present in a newborn versus the adult indicates the inevitable presence of apoptosis and paring of synaptic connections in the postnatal individual (Rakic and Goldman-Rakic, 1982; Innocenti, 1986; Rakic, 1986; Pandya and Seltzer, 1986).

Once formed, the corpus callosum expands in absolute size from 20 weeks of gestation to 15 years (Hayakawa et al., 1989; Ferrario et al., 1996; Rajapaske et al., 1996). Throughout this period modifications in the shape of the callosum take place. The genu grows more quickly during the fetal stage of development than the midbody and splenium (Rakic and Yakovlev, 1968). This difference in rate of growth continues postnatally as the genu continues to expand in the first few months of life, while the splenium and midbody begin to do so months later. Soon the growth of these two components exceeds that of the genu.

By 8 months to 1 year of age the corpus callosum has undergone an increase in its antero-posterior curvature and assumed the adult shape, albeit not adult proportions (Rakic and Yakovlev, 1968; Valk and van der Knapp, 1989). As mentioned above, morphogenesis of the corpus callosum coincides with myelination of the central nervous system. A second stage of callosal development takes place during the completion of myelination in the central nervous system. From 2-5 years of age the human corpus callosum witnesses an expansion of the genu followed by moderate expansion in the

midbody and splenium (Ferrario et al., 1996). From childhood to adolescence (6-15 years) the corpus callosum comes to resemble the adult shape, size and form (Fig. 1.5). Areas of principle growth include the isthmus, splenium and the midbody, while the genu continues to exhibit linear growth (Ferrario et al., 1996; Rajapaske et al., 1996). Brain size remains unchanged during this time, implying that the shape changes occurring in the corpus callosum during this period are not dependent on brain size (Giedd et al., 1996). The size changes witnessed in the corpus callosum from childhood through adulthood are the result of myelination and the acquisition of additional lamellae around axons (Yakovlev and Lecours, 1967). While many studies have suggested that this development ceases in late adolescence (Yakovlev and Lecours, 1967; Jernigan and Tallal, 1990, Ferrario et al., 1996; Rajapaske et al., 1996; Giedd et al., 1999), it has been conjectured that the corpus callosum continues to show an increase in size through early adulthood (20-25 years) (Pujol et al., 1993). Regardless of the age at which growth in the corpus callosum ceases, the area occupied by the corpus callosum begins to decline later in life (Salat, 1997).

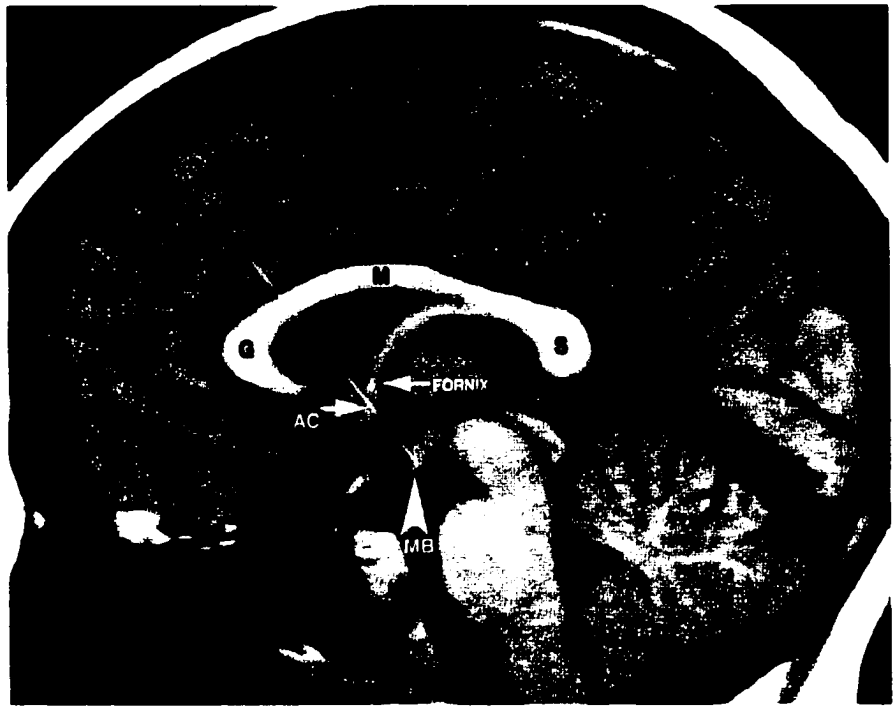

Fig. 1.5. Midsagittal magnetic resonance image of a 15 year old human. G: genu, M: midbody, S: splenium, AC: anterior commissure, MB: mammillary body. (after Kier and Truwit, 1996)

By 6 – 15 years of age the human corpus callosum attains an adult thickness (Hayakawa et al., 1989; Ferrario et al., 1996). Following the suit of other components of the brain, the size of the corpus callosum remains unchanged after it has reached adult proportions (Fig. 1.6) until the 31 – 40 year age range (Pujol et al., 1993). After this time brain size begins to decrease presumably due to the loss of neural circuitry (Dekaban, 1978). The corpus callosum also shows similar age related changes, beginning to decrease in thickness with significant changes in size having occurred by 60 years of age (Hayakawa et al., 1989). In addition, females, but not males, show a loss of area within certain sections of the corpus callosum (Salat, 1997). These age-related changes are

manifest in increased interhemispheric transfer times for certain cognitive tasks (Jeeves and Moes, 1996).

Fig. 1.6. Midsagittal section of an adult human brain. A: anterior, P: posterior, G: genu, M: midbody, S: splenium.

Aboitiz et al. (1996) examined the fiber composition of the corpus callosum in adult individuals. This study found that while there was a minor loss in the number of fibers passing through the corpus callosum with age, there was an increase in the number of fibers above 1μm in diameter. The function of this age related change, which has also been confirmed in rats (Godlewski, 1991), is uncertain. It is, however, possible that the brain increases the efficiency of interhemispheric connections as the brain's synaptic networks are lost. Alternative explanations suggest, though, that the brain does not

actively recruit larger myelinated axons as a result of function, but instead the development of these large axons is the result of hormonal influences (Juraska and Kopcik, 1988; Gravel and Hawkes, 1990; Aboitiz et al., 1996). This last hypothesis gains support from the findings of Aboitiz et al. (1996) demonstrating an increase the number of myelinated fibers above 1μm in diameter in certain areas of the corpus callosum in females but not in males. These sex-related changes will be discussed further below.

1.3 THE MAMMALIAN CORPUS CALLOSUM

1.3.1 Evolution of the corpus callosum

The corpus callosum, while it is unique to mammals, can trace its origins to the earliest animals. With the development of a second opening for the mouth, the deuterosomes began a phase of neural evolution that would lead, eventually, to the complex neural system of extant mammals. Until the advent of the deuterosomes, which include echinoderms (e.g., starfish), hemichordates (e.g., acorn worms) and chordates, the nervous system had been limited to a simple network of a few nerves (Butler and Hodos, 1996). However, the subsequent predatory phase of evolution that followed selected for more advanced and differentiated sensory systems. It is at this point in time that the evolution of the vertebrate brain begins (Northcutt, 1981; Karten and Shimizu, 1989; Butler and Hodos, 1996).

Vertebrates unlike their chordate ancestors are uniquely derived by possessing two ectodermal tissues, neural crest cells and ectodermal placodes. These tissues are crucial for the development of the anterior part of the head and the special sense organs. While

neural crest cells give rise to neural tissue of the anterior vertebrate head, ectodermal placodes, which overlay migrated neural crest tissue, are responsible for most of the special sense organs (Gans and Northcutt, 1983; Noden, 1991; Northcutt, 1995, 1996). The chordate brain is derived from these embryological components.

The mammalian brain has evolved into a prominent structure despite the fact that mammals have existed for a relatively short time, and make up only a small percentage of the known animal species on earth (Kuan et al., 1997). The ability to understand the mammalian brain, nevertheless, extends to understanding its origin, which can be found in the closest living relative of chordate ancestors. Cephalochordates such as *Brachiostoma*, and primitive vertebrates, such as bony fish, possess a brain with constituents homologous to mammals, albeit diffuse and poorly differentiated (Butler and Hodos, 1996; Northcutt, 1996). The constituents in these ancestral groups which apparently evolved in response to a predatory lifestyle include the rhombencephalon (medulla, pons and cerebellum), mesencephalon (midbrain), and prosencephalon, which includes the diencephalon (thalamus and hypothalamus), and the telencephalon (cerebrum, olfactory bulbs, and hippocampus) (Butler and Hodos, 1996). Of the components of the brain found in the ancestor of mammals, the telencephalon has received the greatest attention (Kuan et al., 1997; Striedter, 1997; Aboitiz, 1999).

Primitive vertebrates live according to sensory cues, responding to them instinctually. As such, higher order processing of information is not within their capability (Butler and Hodos, 1996; Hodos and Butler, 1997). Information, however, must still be processed and sent from one side of the brain to the other. To allow the two sides of the telencephalon to communicate with each other nonmammalian amniotes have evolved

two nerve pathways, the anterior and posterior pallial commissures, which permit portions of the medial pallium (hippocampus and subicular cortices) and dorsal pallium (cerebral cortex) to communicate with their contralateral compliment. In higher nonmammalian amniotes (reptiles and birds) the anterior pallial commissure is well developed interconnecting medial and dorsal pallial regions. This rudimentary interhemispheric fiber tract is homologous with the hippocampal commissure of mammals (Bruce and Butler, 1984; Neary, 1990; Butler and Hodos, 1996). The mammalian telencephalon, however, relies on a number of interhemispheric commissures, including the anterior and hippocampal commissures, and the corpus callosum. While it is possible to conjecture that the anterior and hippocampal commissures find their homologues in the anterior pallial and posterior pallial commissures, respectively, in the brain of nonmammalian amniotes, the corpus callosum is an enigma (Butler and Hodos, 1996). It has been suggested that the corpus callosum derives its' origin from either the hippocampal commissure or the anterior commissure. New evidence, however, suggests that the corpus callosum arose *de novo* and as such has no homologue (Katz et al., 1983), but a consensus has not been achieved.

Brain evolution within the three major groups of mammals, the prototheria, metatheria, and eutheria, has been dramatic compared to that of other vertebrate groups. The principle result of brain evolution within mammals has been expansion of the isocortex (cerebral cortex), which has evolved as a result of mosaic evolution in the various extant mammalian groups (Barton and Harvey, 2000). In particular, the result of this mosaic evolution has been the unequal development of association and

somatosensory areas, and the interhemispheric pathways connecting them (Krubitzer, 1995; 1998; Hodos and Butler, 1997).

Long classified as primitive mammals, the monotremes possess a brain that lacks many of the neural structures associated with higher mammals. One structure in particular lacking in the monotremes is the corpus callosum. Thus, while this group has evolved elaborate motor and sensory maps in the brain (Krubitzer et al., 1995), it does not possess a pathway directly linking the higher cortical areas of the cerebrum. The absence of a direct cortical link via the corpus callosum, however, is not unique to the monotremes, since this structure is absent in the marsupials (metatheria) as well (Smith, 1894; Abbie, 1939).

In the marsupials the anterior commissure has been adapted to act as the major interhemispheric pathway for the hippocampal formation and the cerebral hemispheres (Smith, 1897, 1902; Johnston, 1913; Abbie, 1939; Ebner, 1969; Heath and Jones, 1971). Unlike the placental anterior commissure, the anterior commissure of marsupials develops postnatally, albeit the course of development is similar between the two groups (Cummings and Brunjes, 1993). Studies on the development of this commissure in *Macropus* (wallaby) and *Monodidelphis* (opossum) indicate the development of axonal connections in this structure is similar to that of the anterior commissure and corpus callosum in placentals (Silver at al., 1982; Ashwell et al., 1996; Cummings et al., 1997). In addition, the anterior commissure in marsupials connects areas of the cerebrum that in placentals are connected via the corpus callosum. Although this relationship between anterior commissure and callosal axons could lead to speculation that the corpus callosum

evolved from the anterior commissure, this conjecture would be incorrect (Katz et al., 1983).

The placental corpus callosum is likely an autapomorphic structure. While the anterior commissure does connect left and right halves of the brain like the corpus callosum, many of its axons do not completely cross it (Katz et al., 1983). The corpus callosum, however, does not appear to possess any axons which decussate within its structure, indicating it has a separate embryological development from other commissures (LaMantia and Rakic, 1990a,b). Embryological studies indicate that there are two critical steps to the development of the corpus callosum, which indicate that it is not a derivative of the anterior commissure. The first is the fusion of the two cerebral hemispheres rostral to the lamina terminalis. In marsupials the anterior commissure arises as an extension of the lamina terminalis, while in placentals the corpus callosum develops independently of the lamina terminalis. The second critical step in formation of the corpus callosum is development of the glial sling. This sling aids the interhemispheric crossing of developing axons (Silver et al., 1982). Damage to the glial sling results in agenesis of the corpus callosum, while other commissures develop normally (Wahlsten, 1982; Silver et al., 1982). In addition, cases of acallosal mutations have shown that the glial sling fails to develop despite the presence of callosal axons, albeit these axons exist as nonfunctional bundles (Silver et al., 1982; Silver and Ogawa, 1983; Wahlsten, 1982). Thus, while many interhemispheric axons are common to montreme, marsupial, and placental mammals, only the placentals have evolved a separate pathway for many of these interhemispheric axons to travel. The corpus callosum represents a new interhemispheric pathway in placental mammals, and it is likely its development is not the result of a

concordant gene, but instead a pleiotropic response corresponding to other changes in the cerebrum (Katz et al., 1983; Ashwell et al., 1996; Butler and Hodos, 1996).

1.3.2 The corpus callosum in different mammalian groups

1.3.2.1 The corpus callosum in non-primate mammals

Within the placental mammals the corpus callosum has achieved a recognizable position as a midline structure. Prominently involved in communication and coordination in the frontal, parietal, temporal, and a small part of the occipital cortices, the size of the corpus callosum varies as a function of brain size and species (Cameron, 1917; Myers and Sperry, 1958, Sperry, 1962, Gazzaniga, 1966; LaMantia and Rakic, 1990a,b). As mentioned above the corpus callosum is comprised of different regions (rostrum, genu, midbody, isthmus, and splenium). The shape and area occupied by these regions, however, are not consistent across species. Rabbits possess the most amorphous corpus callosum. In this species the rostrum is not well defined and does not recurve beneath the genu (Fig. 1.7). In addition, the splenium is reduced in size, making the midbody appear expanded (Olivares et al., 2000). The rat also possesses a corpus callosum with diminutive features. Although the genu and rostrum are more developed in this group than they are in the rabbit, the splenium lacks the prominent bulbous appearance seen in some higher mammals. Animals such as cats, dogs, horses, and cows all possess a corpus callosum with a well-developed genu and splenium, and the rostrum is recurved (Fig. 1.8). While general differences in shape occur between mammalian species, other differences can be found with regard to particular body plans.

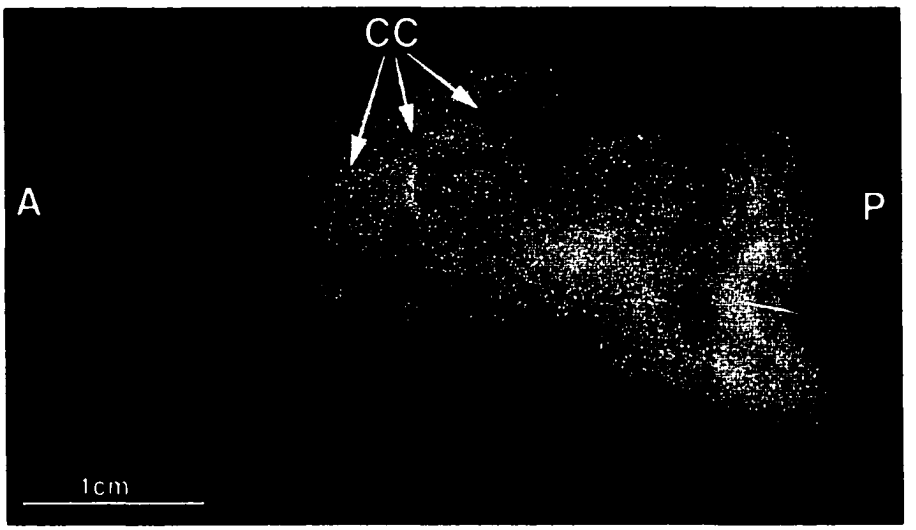

Fig. 1.7. Midsagittal view of the brain of a rabbit (*Oryctolagus cunculus*). A: anterior, P: posterior, CC: corpus callosum.

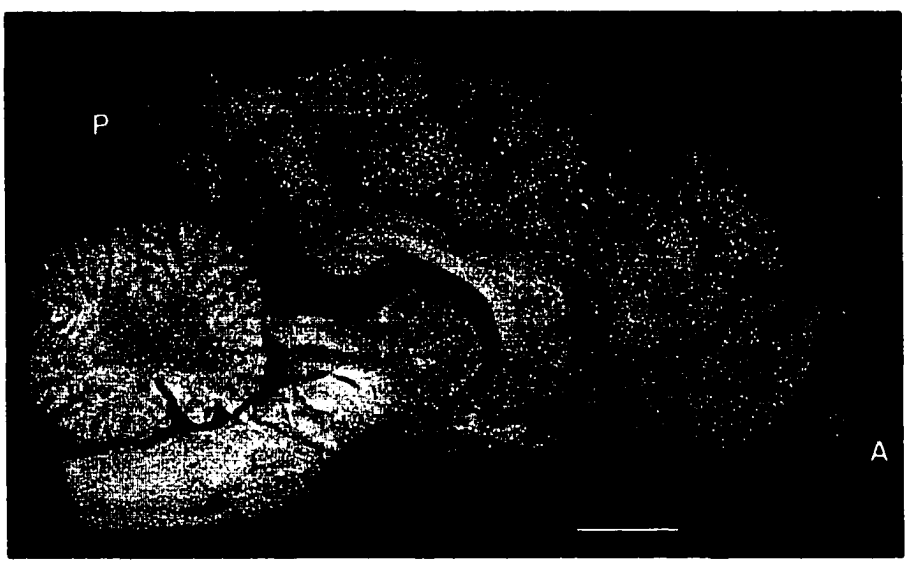

Fig. 1.8. Midsagittal view of the brain of a domestic dog (*Canis familiaris*). A: anterior, P: posterior, S: splenium, M: midbody, G: genu.

Mammals that are classified as frontal-looking species (human, cat, and dog) tend to have a midbody and splenium that are relatively larger than those of lateral-eyed species (cow, horse, rabbit, and rat) (Olivares et al., 2000). In addition, the shape of the corpus callosum in cetaceans is similar to that of lateral-eyed land mammals (Fig 1.9) (Tarpley and Ridgway, 1994). This suggests that differences in the splenium may be due to variations in visual fibers passing through the corpus callosum (Pandya and Seltzer, 1986; Bourdet et al., 1996; Kim et al., 1996). Variation in callosal shape also maybe related to feeding adaptations (i.e., predatory lifestyle versus herbivory), although this hypothesis has yet to be investigated. As such, functional interpretations of callosal shape based on morphologic or trophic levels are difficult to assess.

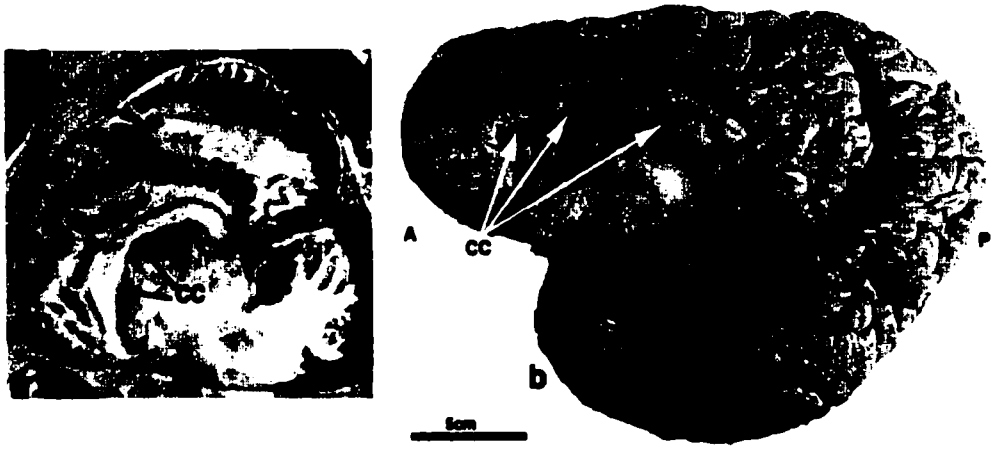

Fig. 1.9. Midsagittal view of the brains of **a)** Risso's Dolphin (*Grampus griseus*) and **b)** infant sperm whale (*Physeter macrocephalus*). A: anterior, P: posterior, CC: corpus callosum.

For the mammalian species that have been examined the area of the corpus callosum demonstrates a linear correlation with brain weight (Anthony, 1938; Nieto et al., 1976; Jancke et al., 1997; Rilling and Insel, 1999; Olivares et al., 2000). In addition, the callosal-cortical area allometric exponent is approximately 0.75 based on brain weight (Haug, 1987; Olivares et al., 2000). Although callosal area scales with brain size, it, thus, does not keep up with the increase in cortical surface area observed in larger brains (Olivares et al., 2000). A mammalian example, which asserts the validity of this hypothesis, can be found in the odontocete cetaceans. This group (and, preliminarily, other cetacean groups) possesses the smallest corpus callosum relative to brain size for any species (see Fig. 1.9) (Anthony, 1938; Nieto et al., 1976; Tarpley and Ridgway, 1994). For example, the corpus callosum of a killer whale (*Orcinus orca*) is approximately the same size as a human corpus callosum, despite having a brain that is five times larger (Ridgeway, 1986). A comparison of cerebral structures, however, between the cetaceans and other mammals is difficult, since cetacean cerebral morphology is distinct from that of other human and nonhuman mammals (Marino, 1998). In addition, a comparison of callosal areas between species is confounded by the taxon-level effect, which says that the value of the allometric exponent decreases when drawing comparisons between lower taxonomic levels (i.e., there is more variation within a family, genus or species than there is in an order or phylum) (Pagel and Harvey, 1989). This makes comparisons between mammalian orders more difficult to interpret.

While it is possible to compare callosal areas between orders, it difficult to describe the functional differences that exist. Aboitiz (1996) suggests that as brain size increases there is an expected tendency to exponentially increase the number of neuronal

connections. Since this would result in a decrease in computational efficiency, natural selection would favor a decrease in callosal connections (Ringo, 1991; Mitchison, 1991; Aboitiz, 1996). As it relates to large mammals such as cetaceans, an increase in brain size would be followed by hemispheric isolation due to longer interhemispheric communication delays (Ringo et al., 1994; Hopkins and Rilling, 2000). As such, large brains like those found in cetaceans would not profit from a proportionally sized corpus callosum. Nevertheless, the cetacean and other mammalian examples that are available do provide valuable information about the corpus callosum. Data suggest that across species the corpus callosum is a conservative structure, despite the presence of minor species differences in shape. This is possibly the result of callosal connections (discussed in section 1.3.3.3) presenting homotopical projections as evidenced in topographic studies in rats (Juraska and Kopcik, 1988; Kim et al., 1996), cats (Nakamura and Kanaseki, 1989), primates (LaMantia and Rakic, 1990a,b) and humans (Giroud and Dumas, 1995) with the exception of dermopterans, which lack some of the homotopic connections present in the above groups (Krubitzer et al., 1998).

1.3.2.2 The corpus callosum in primates

A conservative mammalian structure, the shape of the corpus callosum in primates is not remarkably different from other orders. In the strepsirhines the rostrum is only slightly recurved, and the midbody is thickened. In addition, the splenium is narrow in appearance, possibly as a result of the thicker midbody. In platyrrhines, the splenium is slightly bulbous, and the isthmus and posterior midbody are comparably thinner. Some South American species such as *Saimiri*, *Cebus*, and *Ateles*, which has the largest brain

relative to body size for this group, possess an anterior midbody and genu that are more expanded compared to some other groups, especially the callitrichids (Fig. 1.10) (Huxley, 1861; Herskovitz, 1977; Rilling and Insel, 1999). In the cercopithecoids the splenium appears more bulbous and the genu is more expanded than the above groups (Fig. 1.11) (LaMantia and Rakic, 1990a,b; Rilling and Insel, 1999; Franklin et al., 2000). This is also the case for *Pongo* (Fig. 1.12a), *Gorilla*, *Pan* (Fig 1.12b), and *Homo* (Fig. 1.12c). Each of these genera is characterized by an expanded genu, narrow midbody, and bulbous splenium (Anthony, 1938; de Lacoste and Holloway, 1982; Holloway, 1990; Holloway et al., 1993; Rilling and Insel, 1999), albeit the degree of bulbousness in the splenium is highly variable within species.

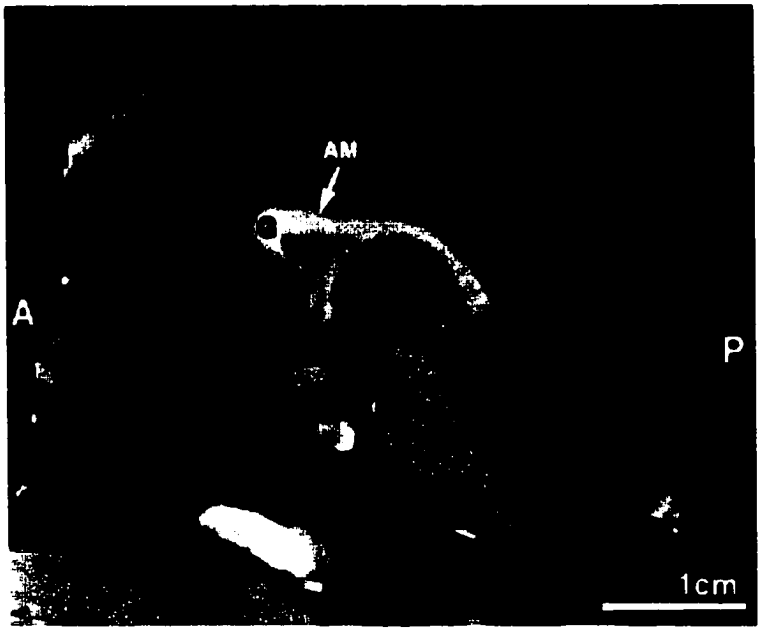

Fig. 1.10. Midsagittal MR image of the brain of *Cebus apella*. A: anterior, P: posterior, G: genu, AM: anterior midbody. (courtesy of Dr. J. Rilling)

Fig. 1.11. Midsagittal view of the brain of *Macaca fascicularis*. A: anterior, P: posterior, S: splenium, G: genu.

Fig. 1.12a. Midsagittal view of the brain of *Pongo pygmaeus*. A: anterior, P: posterior, S: splenium, M: midbody, G: genu.

Fig. 1.12b. Midsagittal view of the brain of *Pan troglodytes*. A: anterior, P: posterior, S: splenium, M: midbody, G: genu.

Fig. 1.12c. Midsagittal section of a human neonatal brain. A: anterior, P: posterior, G: genu, M: midbody, S: splenium.

As mentioned above, it is difficult to determine the cause of shape variations in the corpus callosum for different species. Measurements on the corpus callosum using MRI on live animals by Rilling and Insel (1999) suggest that some shape changes may occur as a result of increasing brain size. For example, their results indicate that while the corpus callosum scales with increasing primate brain size in general, the size of the splenium increases disproportionately to other callosal regions. This increase appears to result from an increase in fiber diameter and myelination (LaMantia and Rakic, 1990a,b; Aboitiz et al., 1992a; Ringo et al., 1994). Since interhemispheric transmission times seem to decrease with increasing brain size, this augmentation in fiber composition of the splenium may be due to the need for the visual areas to maintain rapid integration (Aboitiz et al., 1992a; Tarpley and Ridgway, 1994; Rilling and Insel, 1999). With regard to the corpus callosum as a whole, callosal midsagittal area increases with increasing brain size in primates. Yet as a relative value to brain size, callosal area decreases, particularly in the great apes and humans (de Lacoste and Woodward, 1988; Holloway et al., 1993; Rilling and Insel, 1999; Hopkins and Rilling, 2000). This difference in the relative size of the corpus callosum may be due to a decrease in interhemispheric connectivity and concordant increase in intrahemispheric connectivity (Aboitiz, 1996; Tarpley and Ridgway, 1999). Moreover, this might explain the evolution of functional cerebral lateralization in the great apes and humans (Witelson, 1985; Zaidel et al., 1995; Hopkins and Rilling, 2000); however, this has yet to be thoroughly investigated. Thus, while the corpus callosum in primates is not morphologically different from other mammals, it does stand out as a structure with emergent properties with regard to the evolution primate brain lateralization.

1.3.3 Topographic projection sites of interhemispheric axons and callosal function

An objective of evolutionary neuroanatomy is to determine form-function relationships. As the principle interhemispheric fiber tract in the brain, the corpus callosum plays a critical role in the coordination of certain cognitive functions (Gazzaniga, 1989). In addition, it has evolved in such a way as to assist cognitive lateralization (Witleson, 1985). The specificity, though, of fiber tracts passing through the corpus callosum is not well understood. Several studies have been performed on rodents (Noonan et al., 1996; Norris and Kalil, 1992; Lewis and Olivarria, 1995) and primates (LaMantia and Rakic, 1990a,b; Seltzer and Pandya, 1983; Gould et al., 1986), yet the corpus callosum has never been similarly studied in humans. Investigations in humans have instead relied on deficit studies on patients with callosal agenesis (Aglioti, 1996; Jancke, 1997; Taylor, 1998), or split-brain studies on individuals who have undergone complete or partial callosotomy (de Lacoste, 1981; Gazzaniga, 1983; Gazzaniga et al., 1984, 1989, 1996; Marzi et al., 1991, 1999; Faber 1993; Funnell et al., 1999, 2000a,b). The heterogeneity of these approaches, though, has contributed more to understanding the topographical arrangements of axons and callosal function than has any single approach.

1.3.3.1 Projection studies in non-primate mammals

Primates are unique among mammals in possessing certain cortical structures such as a prefrontal lobe (Preuss, 1995). Yet even within primates variation occurs. While nonhuman haplorhine primates possess a prefrontal cortex similar in structure to humans, the prefrontal cortex of strepsirhines is arranged differently (Preuss and Goldman-Rakic,

1991a,b,c). Despite differences in certain higher cortical areas, though, the composition and function of the telencephalon is generally similar across mammalian taxa. Due to this similarity in the make up of the brain, it is possible to perform interspecies comparisons using anatomical, neurochemical and behavioral study results. Two types of non-primate mammals that have been particularly useful in understanding the development and function of the corpus callosum are rodents and cats.

Despite their small size, rats and other rodents have been useful for understanding the topography of callosal axons. Like other mammals the callosal connections in rodents are distributed in discrete patches. In addition, the callosal topographical map is homotopic with projections connecting reciprocal regions of the cortical hemispheres (Yorke and Caviness, 1975; Wise and Jones, 1978; Miller and Vogt, 1984; Lewis and Olavarria, 1995). In examining the growth of axons through the corpus callosum, Norris and Kalil (1992) observed that axons projecting through the dorsal portion of the midbody in developing hamsters connect homotopic areas of the sensorimotor cortex. This confirmed the results of an earlier study on adult rats (Wise and Jones, 1976), suggesting that afferent callosal connections are established early on instead of in adulthood. Variations in somatosensory callosal connections do occur in other mammalian species. In the large gliding mammal *Pteropus poliocephalus*, the somatosensory callosal connections are more dense for certain body areas and less dense for others. In addition while most sensory callosal projections are to matched body part representations, some projections are mismatched (Krubitzer et al., 1998). Krubitzer et al., (1998) found that callosal projections in Brodmann's area 1/2 for the first digit in this species project not only to the somatosensory area corresponding to the first digit on the

ipsilateral side, but also to somatosensory representations of the ipsilateral wing, head neck and face. Such a projection pattern for axons passing through the corpus callosum indicates that definite variations do occur in callosal topographic maps. Thus, while it may be possible to derive a general topographic map for mammals, possible interspecies variations in this map must be considered.

The visual system is organized in such a way that a stimulus perceived by one eye is interpreted simultaneously by both hemispheres. The transfer of this information is accomplished by the optic chiasm (thalamo-cortical pathway) and corpus callosum (Purves et al., 1997; Olavarria, 2001). In a series of landmark experiments, Myers(1956, 1962) demonstrated that information can be successfully passed to the ipsilateral hemisphere in cats trained to learn a discrimination task with one eye, despite having complete sectioning of the optic chiasm. This experiment clearly established the role of the corpus callosum in visual perception. Other experiments (Ptito et al., 1986, 1991; Timney and Lansdown, 1989; Wang and Dreher, 1996) on cats have indicated that both the optic chiasm and corpus callosum are involved in depth perception to varying degrees (i.e., depth perception ability was better in split-callosum subjects versus split-chiasm subjects). Other studies dealing with the visual cortex discovered that unlike the axons connecting sensorimotor areas, those invading the striate cortex do so homotopically and heterotopically (Cusik and Lund, 1981; Olavarria and van Sluyters, 1983; Lewis and Olavarria, 1995). While callosal axons found in the medial portion of the rat striate cortex connect homotopic areas, those located in Brodmann's region 17/18a connect heterotopic areas (Lewis and Olavarria, 1990; Olavarria, 2001).

The visual map in the corpus callosum has been further refined in the cat. Within this mammalian group axons projecting between the marginal gyri, where the lower visual fields are represented, have been shown to pass through the posterior midbody of the corpus callosum, while fibers connecting the ventral portions of the postero-lateral gyri, where the upper visual fields are represented, pass through the dorsal portion of the splenium (Payne, 1990; Payne, 1991; Payne and Siwek, 1991). In addition, Payne and Siwek (1991) demonstrated that fibers connecting the ventral portions of the postero-lateral gyri, where the central visual fields are represented, pass through the posterior and ventral portions of the splenium. Moreover, Bourdet et al. (1996) have shown that callosal connections may not only be important for development of the visual system, but that there may also be a reciprocal response such that development of the binocular visual mechanism may play an important role in callosal development (see also Hubel and Wiesel, 1967).

Callosal projections to other portions of the cerebral cortex have been investigated as well. Lomber et al. (1994) examined callosal connections in the cat and determined that callosal projections connecting auditory areas pass through the posterior two thirds of the midbody and the dorsal part of the splenium. In the motor cortex callosal axons project through the rostrum and genu. In addition like the visual cortex, the callosal connections of the motor cortex are uneven (Ebner and Myers, 1965; Jones and Powell, 1968; Spidalieri et al., 1996) with the representations for the face, torso and proximal portion of the limbs being highly homotopically connected, and the representations for the distal limbs being relatively devoid of callosal connections (Ebner and Myers, 1965; Kawamurra and Otani, 1970). Spidallieri et al. (1996) suggest that this lack of callosal

connectivity in homotopic contralateral motor areas representing the distal portions of the limbs contributes to the coordination of motor cortex activity in some types of movement, albeit in their experiments this was only shown for eye blinking.

While a basic topographic map of the corpus callosum can be derived from the studies mentioned above, a conclusive map of callosal projections is still elusive. Echoing the above results, Noonan et al. (1996) have demonstrated in the rat that the genu of the corpus callosum connects the medial prefrontal cortex; the midbody connects frontal and parietal areas; and the splenium connects occipital areas. The specific callosal fiber maps presented by Noonan et al. (1996), however, give a more detailed concept of callosal topography. Based on these maps callosal fibers do not pass through distinct portions of the corpus callosum. Instead callosal projections overlap and intermingle extensively, especially in the posterior half of the corpus callosum. Although the particular fiber groups were determined based on general cerebral regions and not specific Brodmann areas, these data suggest that it is not possible without retrograde tracing to view two different fibers or fiber groups as they pass through the corpus callosum and determine to which cortical region they correspond.

1.3.3.2 Projection studies in primates

Non-primate mammals such as the rat and cat have provided insights into the workings of the brain not yet achieved in human and nonhuman primates. As such the use of these mammals has been invaluable to the advancement of neuroscience. The direct application of findings from these mammals, however, is confounded by a lack of homologous features in their brains versus primates. For example, although rats, cats,

and other placental mammals possess a corpus callosum, they lack the true prefrontal cortex of primates (Preuss and Goldman-Rakic, 1991a,b,c; Preuss, 1995). While it is possible then to study callosal development in these non-primate groups, it is not possible to devise callosal topographic projection maps that could be applicable to humans.

A general feature of brain expansion in primates has been a reduction in the interhemispheric connectivity of the corpus callosum (Rilling and Insel, 1999). When looking at separate callosal regions, however, it is apparent that reduction of the corpus callosum through the course of evolution has been heterogeneous. While the area of the splenium has increased concordantly with increasing brain size (slope = 0.77), the area of the midbody has increased at slower pace (slope = 0.67) (Rilling and Insel, 1999). The differential increase in callosal size through primate evolution may relate to the need to maintain fast interhemispheric connections in some projection areas and less in others.

Several studies have examined callosal connections within primates (Curtis, 1940; McCulloch and Garol, 1941; Ebner and Myers, 1969; Jones and Powell, 1969; Pandya et al., 1969; Pandya and Vignolo; 1969). These early studies, however, concentrated more on the location of these fibers within the cerebral cortex than their location within the corpus callosum. In an examination of the location of projection fibers in the corpus callosum of the rhesus macaque (*Macaca mulatta*), Pandya et al. (1971) suggested that fibers travelling through the anterior half of the corpus callosum connect areas in the frontal lobe, while those passing through the posterior half connect parietal, temporal, and occipital lobes and areas within the insula. While this study went on to describe more specific locations of these various interhemispheric fibers, it failed to describe the location of these fibers in terms of current callosal regions. Later studies on this primate

species, though, have gone on to describe the specific location of interhemispheric fibers (de Lacoste, 1981; Seltzer and Pandya, 1983; Pandya and Seltzer, 1986; LaMantia and Rakic, 1990a).

Examining parietal interhemispheric connections, Seltzer and Pandya (1983) found that interhemispheric fibers arising from the anterior portion of the inferior parietal lobule are situated in the posterior midbody, and those from the posterior parietal lobule are situated within the anterior isthmus. They also found that fibers arising from the superior and medial parietal lobe pass through the anterior border of the splenium. While the labeling in this study covered restrictive areas, the location of the interhemispheric fibers was not restrictive. Instead some overlap was viewed in each callosal region. Further investigations have added to these early studies.

Pandya and Seltzer (1986) and LaMantia and Rakic (1990a) examined callosal topography and axonal composition, respectively. The combined results of these efforts produce a more complete view of the topography of callosal axons (see LaMantia and Rakic, 1990a). The anterior region of the corpus callosum constituting the genu and rostrum is characterized as having the highest callosal axon density with many small (diameter 0.08 – 0.4μm) and medium (diameter 0.4 – 1.0μm) myelinated axons and a significant percentage (>20%) of unmyelinated axons (diameter < 0.4μm). This axonal group projects interhemispheric fibers to the prefrontal cortex, connecting both homotopic and heterotopic regions (Barbas and Pandya, 1984). One area in particular harboring heterotopic fibers is the orbitofrontal cortex, an area important in both memory and behavior (Parker et al., 1997; Hasegawa et al., 1998; Bechara et al., 2000; Cavada et al., 2000). The anterior midbody with an intermediate axon density possesses small,

medium, and large (1.0 – 2.5μm) myelinated axons and a small percentage (< 10%) of unmyelinated axons (LaMantia and Rakic, 1990a). This particular group of axons contains interhemispheric fibers connecting the primary, secondary and supplementary motor cortices (Pandya et al., 1969; Pandya et al., 1971; Pandya and Seltzer, 1986). The mid-portion of the midbody, which connects primary and secondary somatosensory areas, has a lower number of axons than the other callosal areas mentioned above (Pandya and Seltzer, 1986; LaMantia and Rakic, 1990a). The low axon density in this area is possibly due to the presence of gigantic (> 2.5μm) myelinated axons, which would prevent more unmyelinated and small, medium and large myelinated axons from occupying the area (LaMantia and Rakic, 1990a). This heterogeneous mix of axons continues posteriorly in the posterior midbody. The fibers in the rostral half of this division connect areas in the postcentral and posterior parietal lobe, while those in the caudal half apparently connect both portions of the posterior parietal and the superior and inferior temporal lobes (Seltzer and Pandya, 1983; Pandya and Seltzer, 1986; LaMantia and Rakic, 1990a).

Like the posterior midbody, the isthmus (see Bean, 1906; and section 2.3.2.4 for definition) connects varying areas. In addition, it also possesses a mix of fibers. The anterior isthmus is composed of a varying mix of small, medium, large and gigantic myelinated axons with a few unmyelinated axons dispersed throughout (LaMantia and Rakic, 1990a). This section connects portions of the superior and inferior temporal lobe along with a small portion of the posterior parietal lobe. The posterior part of the isthmus apparently lacks the gigantic axons found in the anterior end. This part interconnects portions of the superior and inferior temporal lobe (Pandya and Seltzer, 1986).

The most caudal section of the corpus callosum, the splenium has a mix of fibers like the last two sections mentioned. Its rostral half has an axonal composition similar to the posterior portion of the isthmus, and like the isthmus also connects portions of the superior and inferior temporal lobes. The caudal half of the splenium, however, possesses many gigantic myelinated axons, along with small, medium and large myelinated axons and a few unmyelinated axons (Pandya and Seltzer, 1986; LaMantia and Rakic, 1990a). This part of the corpus callosum is primarily responsible for connecting homotopic as well as heterotopic areas within the primary and secondary visual cortex (Pandya et al., 1971; Pandya and Seltzer, 1986).

While most of what is known about the topographic distribution of interhemispheric fibers in the corpus callosum of primates comes from studies on *M. mulatta*, studies on other primate species such as *Aotus* (Cusick et al., 1984; Gould et al., 1986; Beck and Kaas, 1994), *Galago* (Weyland and Swadlow, 1980; Cusick et al., 1984; Beck and Kaas, 1994), and *Saimiri* (Gould et al., 1987) have produced similar results. While some of these studies do not present topographic maps of the corpus callosum, information gleaned from their results indicate that some primate groups lack callosal projection patterns that mimic those seen in cercopithecoids. A particular exception is *Galago*. Callosal connections studied in the visual striate cortex suggest that this species and possibly all strepsirhines have large numbers of callosal fibers deep within Brodmann's area 17 (Beck and Kaas, 1994), while callosal axons are completely absent from area 17 except at the border in New World monkeys such as *Aotus* (Beck and Kaas, 1994) and *Saimiri* (Gould et al., 1987) and cercopithecoids such as *Macaca* (Winfield et al., 1975; Van Essen at al., 1982). The types of myelinated axons traversing the corpus callosum in

these species, however, is unknown. Nevertheless, the studies that have been performed on various primate genera indicate that the topographic projection pattern in the splenium, and possibly isthmus, of the corpus callosum in strepsirhines is a plesiomorphic character, while the topography of these structures in haplorhines represents an apomorphic character, albeit the pattern in *Tarsius* is unknown.

1.3.3.3 The function of the corpus callosum in humans: Implications from functional imaging and deficit studies

Topographic projection studies on the corpus callosum in the various genera mentioned above have provided a wealth of information concerning the function of this structure. These studies, however, only provide a partial picture of callosal function. Humans may be unique among mammals for possessing highly lateralized brains (Holloway, 1968, 1979, 1995; Vallortigara et al., 1999). The role of the corpus callosum in cerebral lateralization is not fully understood, but its function in certain cognitive processes is decipherable (Wong, 2000). It is not possible to obtain projection maps of callosal fibers in humans like it is in some other animals, so the majority of information on the corpus callosum in humans must come from imaging, deficit and postmordem studies (de Lacoste and Holloway, 1982; Hines et al., 1992; Witelson, 1995; Gazzaniga et al., 1996; Jancke, 1997; Funnell et al., 1999).

The earliest studies on the role of the corpus callosum in the brain come from studies on split-brain individuals or individuals with callosal agenesis. Over forty years ago Sperry et al. (1956) recognized that cats who had their corpus callosum severed developed a visual recognition deficit, unable to transfer what was viewed by one visual

field to the other. This contradicted the precept of the time that proposed callosotomy produced little if any cognitive deficits (Trendelburg and Hartman, 1927; Akelaitis, 1943). However, subsequent experiments by Sperry (1959), and Hubel and Wiesel (1967) set about altering the perception of the corpus callosum as merely a support structure to an interhemispheric pathway integral to cognition. Additional visual system experiments on *Macaca* (Downer, 1959), *Papio* (Trevarthen, 1978) and *Pan* (Black and Myers, 1964) confirmed these results, strongly suggesting that the corpus callosum plays an important role in the transfer of functionally organized sensory information between the cerebral hemispheres. These results, however, only apply anecdotally to humans, since the corpus callosum in humans apparently functions differently from cats and primates (Gazzaniga, 1989).

Human studies have found that the corpus callosum is crucial for the interhemispheric transfer of not only visual information, but other forms as well, including language and tactile/sensory information (Gazzaniga, 1995). Some of the information gleaned from these studies has shown that functional loss from callosotomy is more complete in humans than other mammals. For example, Gazzaniga (1989) reports that individuals who had undergone callosotomy lacked functional overlap at the visual midline.

Earlier in the century, Akelaitis (1941, 1942, 1943, 1944) conjectured that the corpus callosum did not serve a functional role in the brain. This was deduced from a series of studies on patients that had undergone callosotomy; severing the corpus callosum, but leaving the anterior commissure intact. These results can be understood better when examining Akelaitis' patients. All of the patients suffered from low IQ prior to callosotomy, which may have prevented Akelaitis from having them perform more

complex cognitive task. While this is conjecture, Akelaitis may have been biased by his patients low IQ, assuming they could not perform a task simply because they lacked the basic intelligence to understand it. Regardless, as a result of these experiments the function of the corpus callosum would remain unknown until Gazzaniga et al. (1962) studied another patient that had undergone callosotomy, and for the first time described the clinical diagnosis of split-brain.

The patient studied by Gazzaniga et al., (1962) was a World War II veteran who had suffered head trauma and endured subsequent grand mal seizures. To relieve this individual of these life-impairing seizures he underwent a complete commissurotomy. This first patient of Gazzaniga et al. (1962) was tested for tactile, language and visual deficits. The results from tactile experiments echoed the cliché that "the left hand did not know what the right hand was doing". For example, the patient, W.J., could put together a puzzle with either hand but not both. In addition, W.J. also exhibited serious agnosia and agraphia when performing a task exclusive to the left hand. Visual tasks produced similar results with the left visual field performing more poorly than the right, and W.J. demonstrated profound agnosia for objects and stimuli presented to the left visual field. This occurred despite the fact that stereoscopic vision and depth perception were preserved (Trevarthen and Sperry, 1973). From this Gazzaniga et al. (1962) were able to conclude that the corpus callosum is integral to the interhemispheric transfer of information, not to mention the proposition that the cerebral hemispheres are specialized and independent in function. Later studies, involving other split-brain patients found that visual, tactile, olfactory, auditory and proprioceptive information pass through the corpus

callosum to the contralateral hemisphere (Gordon and Sperry, 1969; Gazzaniga and Freedman, 1973, Gazzaniga, 1987, 1989, 1995; Intriligator et al., 2000).

Most split-brain studies are performed on individuals who have undergone either partial or complete callosotomy to relieve persistent and untreatable symptoms of epilepsy (Liederman, 1995; Gazzaniga, 1995). An immediate side effect of the surgery can be muteness. Sullivan and McKeever (1985) found that whether a patient was mute for a period after surgery or not depended on whether the patient's dominant hand was controlled by the same hemisphere as speech. In the cases they studied, they found that left-handed individuals who also had speech controlled by the left hemisphere suffered a profound speech deficit post-callosotomy. In addition, individuals who were concordant for speech and handedness had normal speech after surgery (Sullivan and McKeever, 1985; Censori et al., 1989). The degree of language deficit in split-brain patients is dependent on not only the type of task being performed, but also by how much of the corpus callosum has been severed (Zaidel, 1995).

Based on the examination of several split-brain patients Zaidel (1995) posits that while such studies do attest to the functional concept of hemispheric independence, individual variability abounds. Some individuals with split-brain perform better on certain stimulus tasks than others. Within split-brain patients it is possible that disconnected hemispheres may behave differently from normal hemispheres, which most likely continually share information while independently processing information. Nevertheless, certain consistencies can be found among various split-brained studies. For example, patients with speech located in the left hemisphere are unable to identify stimuli presented to the left ear, left hand or left visual hemifield (Mohr et al., 1994; Zaidel, 1995; Marzi et al.,

1999). In addition, the interhemispheric transfer time is pronounced in split-brain patients.

Other studies comparing patients who have undergone complete callosotomy to those that have undergone only partial callosotomy have also aided in understanding the role of the corpus callosum in humans. As with topographic maps studied in other mammals, patients who have undergone only partial callosal section provide clues to the topographic map of the human corpus callosum. In one case Foxman et al. (1986) examined a female patient who had only the posterior half of the corpus callosum sectioned. Tactile tests revealed that while this individual could indicate the point of stimulus received by the right hand with the left hand, she was unable to perform the task in the contralateral direction. This would indicate that left to right interhemispheric fibers passing through the anterior portion of the posterior midbody were intact while those sending signals from the right hemisphere to the left had been severed. While this image of the topographic projections of the interhemispheric fibers connecting the somatosensory areas is incomplete, it does indicate that the position of these fibers in the corpus callosum is similar to that of other mammals (Pandya and Seltzer, 1986). In addition, a study on a patient with a similar morphology to the patient studied by Foxman et al (1986), Intriligator et al (2000) found that while their patient retained the ability to name objects, faces, textures and colors presented to the left and right visual hemifields separately and together, he had lost the ability to compare stimuli, except line drawings of objects, presented between the two hemifields. Thus, this would suggest that one of the regions in the posterior corpus callosum is essential for comparing stimuli between the two visual fields as well as tactile comparisons.

In another patient where only the caudal portion of the splenium was spared, Gazzaniga (1989) demonstrated that the corpus callosum in humans, like other mammals, was divided into functional zones. With only this fraction of this corpus callosum present, this patient possessed normal transfer of visual information, allowing this patient to match case, pattern, color, and linguistic information presented to the other side of the brain. This patient, however, was unable to determine an object based on touch alone. In addition, this patient exhibited left ear suppression when information was presented to both ears simultaneously. Again this information is consistent with that derived from animal models.

In a final example, Gazzaniga et al., (1989) observed that a patient who had the corpus callosum severed, save the rostrum and the caudal most portion of the splenium, was able to carry out a highly specific task. In a task that required the patient to determine whether two words rhymed, the patient was able to perform at better than chance when the printed words both looked alike and sounded alike, albeit the patient performed as well as other split-brain patients on other variations of the task. Gazzaniga et al. (1989) reasoned that the rostrum preserved some of the interhemispheric fibers involved in language. In a follow-up study, however, Funnell et al. (2000a,b) found that the splenium in this patient may actually be responsible for the transfer of word specific information.

Split-brain studies have provided insight into the function and topography of the human corpus callosum. While other studies such as those on callosal agenesis (Lessonde et al., 1995; Aglioti et al., 1996; Jancke et al., 1997) confirm the results of cerebral lateralization provided by split-brain studies (Levy et al., 1971; Gazzaniga, 1989), they do not provide any evidence for specific callosal function. What is evidenced

by split-brain studies is that the corpus callosum provides interhemispheric connections integral for higher cognitive processes involving concepts, memory, language, and visual and mental images (Levy et al., 1971; Levy and Trevarthen, 1976; Galaburda, 1995; Harris, 1995; Witelson, 1995; Zaidel, 1995; Arguin et al., 2000; Forster et al., 2000), not to mention some basic cognitive processes such as sustained attention (Rueckert and Levy, 1996; Rueckert et al., 1999), auditory integration (Jancke and Steinmetz, 1994) and integration of tactile stimuli (Mason and Geffen, 1996; Velay and Benoit-Dubrocard, 1999). Despite the confidence of the data derived from split-brain studies for understanding callosal function, the results should be reviewed with caution. Although the studies on split brain patients have predominantly occurred shortly after the corpus callosum was severed, many patients have limitedly recouped some functions over the course of time, especially if they were young at the time of surgery (Gazzaniga and LeDoux, 1978; Gazzaniga, 1988). Thus, such studies indicate that while the corpus callosum may be divided into functional regions, the callosal axons projecting through those areas do so within sometimes far reaching territories. In addition, split-brain studies not only attest to the functional importance of the corpus callosum in connecting the cerebral hemispheres, allowing the left hemisphere to contribute functions to the right and vice versa, but also to the plasticity of the brain.

1.4 SEX DIFFERENCES IN THE PRIMATE BRAIN

Ever since serious study on the brain began, researchers have sought to identify sex and racial distinctions in brain size and organization. Huschke (1854) was among the first to suggest that males possessed larger brains than females. Subsequent to Huschke others investigated this question among others of whether or not sex differences existed in the brain. In the course of this ambition it was concluded that males do indeed have larger brains than females not only in humans, but also in nonhuman primate species. However, the meaning, functional aspects, and true influence of these differences do not appear to be well understood, and are still debated. Within primates while males tend to have larger brains than females, the differences are principally allometric (Mall, 1909; Haug, 1987). Nevertheless, sexual dimorphism does manifest in the primate brain on many levels, including the corpus callosum (Mall, 1909; Papez, 1927; de Lacoste and Holloway, 1982, 1996 - appendix; Holloway and de Lacoste, 1986; Holloway et al., 1993; de Courten-Myers, 1999).

1.4.1 Sex differences in the nonhuman primate brain

It has been shown that sex differences in brain size occur among many primate species. In particular, polygynous haplorhine species show a degree a sexual dimorphism in the brain concordant with sexual dimorphism in body size (Holloway, 1980; Stephan et al., 1981; Smuts et al., 1987; Haug, 1987; Heilbroner and Holloway, 1988; Holloway and Heilbroner, 1992; Sawaguchi, 1997). Cercopithecoids tend to show greater sexual dimorphism than New World monkeys (Herskovitz, 1977; Smuts et al., 1987; Holloway and Heilbroner, 1992; Sawaguchi, 1997; Falk et al., 1999; Falk, 2000) and possibly

strepsirhines (Gilissen et al., 1999). In addition, *Pongo*, *Pan troglodytes* (but not *Pan paniscus*) and *Gorilla* exhibit greater sexual dimorphism in these areas than most other primate species (Holloway, 1980; Bramblett, 1994; Sawaguchi, 1997; Herndon et al., 1999; Falk, 2000). While basic information on the gross differences in brain size and body size is available for almost all known primates, there is a paucity of information on the relative size of the brain for each species.

Measurements of the relative size of the brain between males and females for various primate species indicate that the resultant absolute values for brain size and body size are often negligible. For example, when body size or other allometric variables are taken into account, the degree of sexual dimorphism in the species that have been studied is either reduced or reversed. Falk et al. (1999) have suggested that in *M. mulatta* males have larger brains than females (102.85cc vs. 94.66cc). While this is consistent with other studies that have measured brain size in *M. mulatta* (e.g., Holloway, 1980; Cheverud et al., 1990; Holloway and Heilbroner, 1992), results of relative brain size for their sample demonstrate that the degree of sexual dimorphism is reversed with females having relatively larger brains compared to males. This reversal effect in relative brain size has also been reported for *M. fascicularis*, *Saguinus*, *Saimiri*, *Pongo*, *Pan troglodytes*, and *Gorilla* (Holloway, 1980, but particularly in humans; Holloway and Heilbroner, 1992). In addition to general sexual dimorphism in brain size, sexual dimorphism has been observed in the corpus callosum (discussed below), amygdala (Franklin et al., 2000), medial preoptic area (Ayoub et al., 1983; Byne, 1998), and the anterior hypothalamus (Byne, 1998).

It is uncertain why relative differences in brain size occur in primates and other mammals. At this point the various proposals concerning the evolution of this phenomenon are merely speculative. It has been suggested though that sexual dimorphism in the brain may be the result of either inter- (niche expansion hypothesis) or intrasexual (mate choice) selection, or developmental influences of gonadal hormones unrelated to sexual selection (see Martin et al., 1994 for review). Falk et al. (1999) have suggested that male brains are absolutely larger than female brains (in *M. mulatta* and humans) due to enhanced visuospatial abilities in males. While the evidence for male visuospatial abilities in *M. mulatta* (Georgopoulos et al., 1989), *Papio* (Vauclair et al., 1993), and humans (Linn and Peterson, 1985) is substantial and provocative, there is little evidence that the visual system is the sole reason for sexual dimorphism in absolute brain size. In addition, Falk et al. (1999) did not measure any component of the visual system to support their proposal.

How sexual selection, reproductive strategy and foraging have come to influence brain size and sexual dimorphism in primates continues to be debated. Martin et al. (1994) favor the hypothesis that females have relatively larger brains than males due to phylogenetic inertia favoring a reduction in female body size, possibly to conserve resources. In addition, they suggest that males from polygynous species have relatively smaller brains due to a slight increase in general body size. Regardless of the evolutionary mechanisms selecting for females with relatively larger brains or males with relatively smaller brains, it is generally accepted that the physiological mechanism producing such results concerns gonadotropic hormones. Studies that have sought to control the influence of gonadotropic hormones during development have produced clear

and consistent results supporting the theory that sexual dimorphism in brain size (and possibly sexual dimorphism in the brain in general) is due to circulating androgens and to a lesser degree estrogens (Phoenix et al., 1959; Goldman, 1974; Bachevalier and Hagger, 1991; Gahr, 1994; Brown et al., 1996; Cooke et al., 1999).

1.4.2 Sex differences in the human brain

Sexual dimorphism in the brain has possibly been studied more in humans than in any other primate species. Since Mall (1909) researchers have proposed that sexual dimorphism exists in the human brain in general, specific brain areas, and specific cognitive functions. Studies of this nature have been possible in humans because of an individual's ability to provide definitive answers to certain cognitive tasks. In addition, deficit studies often provide precise information about a damaged area within the brain (e.g., Damasio and Tranel, 1993). In nonhuman primates, however, such tasks may provide intriguing data on counting, visuospatial skills, and even language, but they may raise more questions than they answer (e.g., Boysen et al., 1996; Brannon and Terrace, 1998; Savage-Rumbaugh et al., 1998; Barbas, 2000; Hauser et al., 2000; Ramus et al., 2000; Zuberbuhler, 2000).

Von Bichoff (1880), Marchand (1902) and Mall (1909) found that males possessed larger brains than females (Kretschmann et al., 1979). In addition, Mall (1909) found that the frontal lobe is relatively larger in men than women. While Mall did not standardize his results according to body size, his results nevertheless were well accepted and provided the impetus for future research in this area. Studies subsequent to Mall (1909) reported similar results for brain size and other areas in the brain. A basic tenant

of these studies is that morphological differences underlie the various cognitive differences between males and females. For example, the brains of males are assumed to be more asymmetrically organized for verbal functions (McGlone, 1977; Kimura, 1980, 1983, 2000) and/or spatial functions (Witelson, 1976), albeit skeptics exist (e.g., Fairweather, 1976). These cognitive differences may manifest in the size and organization of the brain and its constituents.

Although anatomical sex differences in the human brain had been observed in part since Heschl (1878) and Mall (1909), specific study of this topic has occurred only recently, beginning with de Lacoste and Holloway's (1982) examination of the corpus callosum. Like other primates, human males possess absolutely larger brains than females (Pakkenberg and Voigt, 1964); however, females possess relatively larger brains (Holloway, 1980). As mentioned above this disparity between absolute and relative values for brain size is not likely due to allometry (Holloway, 1980). However, there is significant sexual dimorphism in cognitive functions in humans, which may be due to males having absolutely larger brains or females having relatively larger brains. One cognitive area that has received significant attention has been language.

Females tend to possess greater verbal fluency than males (Hutt, 1972). For example, females have a larger vocabulary. In addition, they seem to have fewer problems recovering language abilities following insult to related temporal areas (Edwards et al., 1976). As such it has been suggested that areas corresponding to speech and language in the female brain are organized differently from males (McGlone, 1977; Kimura, 1980, 1983, 2000). Unlike the female brain, the male brain is more lateralized for speech (Kimura and Harshman, 1984; Eviatar et al., 1997). In an examination of the

cytoarchitecture of Brodmann's areas 44 and 45, Amunts et al. (1999) found that males have higher cell densities on the left side in area 44, albeit there was less dimorphism in area 45. Harasty et al. (1997) found sex differences in the relative volume of the region corresponding to the orbital, triangular and opercular parts of the left inferior frontal lobe, the planum temporale, and the superior temporal lobe. In addition, Frederikse et al. (1999) have found that males tend have a significantly larger left but not right inferior parietal lobule than females. The inferior parietal lobule encompasses portions of Wernicke's area and that plays an important role in processing visual, auditory, attentional and somatosensory information.

Males tend to be more asymmetric in the inferior frontal lobe of the brain than females. This asymmetric morphology corresponds with a strong lateralization of language in males, especially for phonological tasks (Shaywitz et al., 1995). In addition, while males may possess higher cell densities on the left side, females tend to have a higher percentage of gray matter ventral to the dorsolateral cortex (Schlaepfer et al., 1995). In other areas suggested to be indicative of language lateralization, males tend to be distinctly asymmetric. For example, males are more asymmetric than females in the posterior part of the Sylvian fissure, albeit they are not asymmetric nor different from females in the anterior portion of the fissure (Witelson and Kigar, 1992). In addition, there is a robust dimorphic activation of the prefrontal lobe of males for verb generation subtraction image tasks (Buckner et al., 1996). Thus while both males and females tend to be asymmetric in the volume of the inferior frontal lobe and the cellular densities within this area, males are absolutely more lateralized morphologically and functionally for speech and language (Amunts et al., 1999; Uylings et al., 1999).

A second principle cognitive difference between males and females is visuospatial ability. As with speech and language males tend to be more lateralized for visuospatial skills (Levy, 1974; Witelson, 1985). These skills include maze learning, tracing an object through space, aiming, recall of geometric shapes, locating objects embedded within a picture, mental rotation, and perception of geometric illusions (Kimura, 1993, 2000; Levy and Heller, 1992; Rasmjou et al., 1999; Joseph, 2000). Unlike the language areas discussed above, cortical areas corresponding to vision and visual perception do not appear to offer any volumetric size differences between males and females. The one distinction that does exists is that males tend to possess more white matter relative to gray matter than females (Gur et al., 1999), and possible greater hemispheric specialization (Witelson, 1976). Gur et al. (1999) interpret the sexual dimorphism of spatial abilities as requiring greater interhemispheric transfer, while language may require less. One task requiring both visual and linguistic interpretation is the recognition of facial expression. While males and females tend to possess the same ability to recognize faces (Hugdahl et al., 1993; Leveroni et al., 2000), females appear to be more sensitive to facial emotional expressions (Natale et al., 1983; Burton and Levy, 1989). This indicates that while certain visual information requires rapid interhemispheric transfer, other forms require hemispheric specialization.

In addition to the morphological and functional dimorphisms discussed above, other brain areas that have been shown to be dimorphic include, but are not exclusive to, the anterior hypothalamus, which is two times larger in size and neuronal numbers in males than females (M > F) (Swaab and Fliers, 1985); the caudate of the basal ganglia (F > M) (Giedd et al., 1997); the hippocampus (F > M) (Filipek et al., 1994); the central sulcus in

which males but not females have a sulcus that is asymmetric for depth, where the deeper sulcus occurs in the hemisphere controlling the dominant hand (M > F) (Amunts et al., 2000); and the corpus callosum (F > M) (de Lacoste and Holloway, 1982), which will be discussed below. The elucidation of these various sexually dimorphic nuclei, gyri and sulci attest that sexual dimorphism in the human brain is extensive. The interpretation of the development and possible function of these dimorphic areas, however, is difficult to determine.

The above and other studies suggest that the underlying cause for many of the dimorphic differences in the brain are the result of not simply the relative area occupied by a structure or the ratio of white to gray matter, but instead the neuronal composition of those structures. Pakkenberg and Gundersen (1997) and Rabinowicz et al. (1999) each stereologically examined the cerebral cortex of males and females. Their results show that male brains have more neurons (Pakkenberg and Gundersen, 1997; Rabinowicz et al., 1999), while female brains have more neuronal processes (Rabinowicz et al., 1999). Witelson et al. (1995), however, found that females have more neurons in the posterior temporal cortex. While this appears to contradict the results of Rabinowicz et al. (1999), they, instead, merely suggest that the neuronal composition of the cortex varies between males and females in certain areas. The development of sexually dimorphic differences in the human brain and cognitive functions is still being elucidated, although preliminary information suggests that these features are likely the result of epigenetic phenomena dependant not only on control genes, but also on factors of hormonal influence and environment (Koenigsknecht and Friedman, 1976; McGlone, 1980; Juraska and Kopcik,

1988; Fitch et al., 1991; Bishop and Wahlsten, 1999; Joseph, 2000); and possibly race (Klekamp et al., 1991).

1.5 SEX DIFFERENCES IN THE CORPUS CALLOSUM

Considerable current controversy surrounds the existence of identifiable sexual differences in the nonhuman primate and human brain. An area that has come under increasingly greater focus is the corpus callosum, the principal neocortical commissure. For example, many recent studies on humans have demonstrated morphological differences between the sexes in callosal measures (de Lacoste-Utamsing and Holloway, 1982; Wium, 1984; de Lacoste et al., 1986; Holloway and de Lacoste, 1986; Holloway, 1990; Holloway et al., 1993; Davatzikos and Resnick, 1998; Oka et al., 1999) as well as fiber composition of the corpus callosum (Tomasch, 1954; Aboitiz et al., 1992a,b,c). Comparable data from nonhuman primates has, however, been generally lacking (e.g., Le May, 1976; de Lacoste and Woodward, 1988; LaMantia and Rakic, 1990a,b). The paucity of information on the primate corpus callosum has prevented further exploration of the origin, evolution, and functional significance of sex differences in the primate brain. Nevertheless, the limited information that is available for human and nonhuman primates provides provocative data concerning the above issues. In addition, the present study adds to the current knowledge of and provides new information on sex differences of this structure in *Macaca fascicularis* and *Pan troglodytes*. These results will be discussed in Chapter 5 of this dissertation.

1.5.1 Sex differences in the corpus callosum of humans

1.5.1.1 Sex differences in the shape and size of the human corpus callosum

The human corpus callosum has been the subject of extensive study relating to its involvement in a number of diseases such as: Down's syndrome (Wang et al., 1992; Kivitie-Kallio et al., 1998), epilepsy (e.g. Khanna et al., 1994), amyotrophic lateral sclerosis (Yamauchi et al., 1995), Alzheimer's (Vermersch, 1996; Thompson et al., 1998), attention-deficit hyperactivity disorder (Baumgardner et al., 1996; Lyoo et al., 1996), autism (Piven et al., 1997; Manes et al., 1999), schizophrenia (e.g., Coger and Serafetinides, 1990; Raine et al., 1990; Hoff et al., 1994; Cowell et al., 1996; McCarley et al., 1999; Meisenzahl et al., 1999; Narr et al., 2000), Williams syndrome (Schmitt et al., 2001), Marchiafava-Bignami disease (Shiota et al., 1996), Tourette syndrome (Baumgardner et al., 1996; Mostofsky et al., 1999), dyslexia (Rumsey et al., 1996; Robichon and Habib, 1998) and other speech associated deficiencies seen when the corpus callosum is sectioned (Kaga et al., 1990; Davidson and Hugdahl, 1995). These studies, however, have done little to discern the sex differences associated with this structure.

Early studies on the corpus callosum found no differences in sex based on size and shape (Bean, 1906; Mall, 1909). However, subsequent research of this kind on the corpus callosum remained dormant until de Lacoste-Utamsing and Holloway (1982) re-addressed the issue. De Lacoste-Utamsing and Holloway took into account what Mall (1909) had stressed earlier, namely that brain size must be considered when suggesting dimorphism in brain morphology. They concluded that while the area differences between males and females may be small, they are nevertheless significant. Subsequent

studies in this area have produced varying results. Some studies have suggested that there is sexual dimorphism in the corpus callosum (de Lacoste, 1981; de Lacoste-Utamsing and Holloway, 1982; Witelson, 1985; Holloway and de Lacoste, 1986; Yoshii et al., 1986; Reinarz et al., 1988; Clarke et al, 1989; Hayakawa et al., 1989; Witelson, 1989; Holloway, 1990; Elster et al., 1990; Allen et al., 1991; Clarke and Zaidel, 1994; Johnson et al., 1994, 1996; Driesen and Raz, 1995; Steinmetz et al., 1992, 1995; 1996; Salat et al., 1997; Davatzikos and Resnick, 1998; Oka et al., 1999). Others report that it lacks dimorphism (Bell and Variend, 1985; Weber and Weis, 1986; Kertesz et al., 1987; Oppenheim et al., 1987; Byne et al., 1988; Demeter et al., 1988; O'Kusky et al., 1988; Weis et al., 1989; Going and Dixson, 1990; Prokop et al., 1990; Denenberg et al., 1991a,b; Emory et al., 1991; Habib et al., 1991; Aboitiz et al., 1992c; Steinmetz et al., 1992; Zaidel et al., 1995; Constant and Ruther, 1996; Koshi et al., 1997; Matano and Nakano, 1998). However, several of these latter studies did not consider sexual dimorphism in brain size, and thus did not analyze relative callosal measurements (i.e., taking brain size into account). Holloway et al. (1993) reexamined some of these results, and concluded that when brain size is taken into account sexual dimorphism in the corpus callosum is indeed indicated by such studies as: Witelson (1985), Weber and Weis (1986), Yoshii et al. (1986), Kertesz et al. (1987), Oppenheim et al. (1987), Byne et al. (1988), Demeter et al. (1988), Elster et al. (1990), Going and Dixson (1990), Habib et al. (1991), and Steinmetz et al. (1992).

In a recent meta-analysis, Bishop and Wahlsten (1997; see also Fitch and Denenberg, 1998) suggested that there are no sexual differences in callosal shape or size. It should be noted, though, that Bishop and Wahlsten downplay the effect of allometric scaling in the

brain, proposing that it is not an appropriate way to analyze cortical data. In addition, their use of specific individual data sets in their meta-analysis is exceptional as their measurements are based on means derived from a number, albeit not all, studies examining sex differences in the corpus callosum. Thus, a central issue in many studies on the size or shape of the corpus callosum concerns the use of standardized results. This continued disagreement between these two camps has failed to provide a resolution to the issue of whether or not there is actually sexual dimorphism in the size and fiber composition of the corpus callosum.

Reviews by McGlone (1980), Kimura (1980, 1983, 1987, 2000), Witelson (1983), and Davidson and Hugdahl (1995), among others, confirm that there are sex differences in the brains of humans. Through cognitive studies on visuospatial tasks (see McGlone, 1980) and speech tasks such as speed of articulation, fluency within a language, and grammar (Hutt, 1972; LeDoux, 1982; Ross et al., 1997), it has been suggested that the adult male brain is more asymmetrical than the adult female brain with regard to verbal functions (Hutt, 1972; McGlone, 1977; LeDoux, 1982; Zaidel et al., 1995; Grimshaw, 1998), spatial functions (Witelson, 1977, 1983; Corsi-Cabrera et al., 1997), or both (Hutt, 1972; Springer and Deutsch, 1989). This information has led to the suggestion that the structure of the corpus callosum, one of the areas of the brain which allows the two hemispheres to communicate, is responsible for certain sex differences in cerebral lateralization (de Lacoste-Utamsing and Holloway, 1982; Witelson and Kigar, 1987; Witelson, 1989; Holloway, 1990; Pulvermuller and Mohr, 1996; Funnell et al., 2000b).

While debate about sex differences in the corpus callosum continue, studies published since the meta-analysis by Bishop and Wahlsten (1997) have shed new light on

morphological differences in the corpus callosum between males and females. Early in development (20 – 40 weeks gestation) there is little difference in callosal size and shape between males and females (Koshi et al., 1997). Later in development, however, males and females begin to differentiate more from each other, and exhibit sex differences in several brain areas, including the corpus callosum (de Lacoste and Holloway, 1982; see section 1.4.2). Yet the degree of dimorphism is often difficult to establish. In an examination of the corpus callosum in Japanese autopsied brains Matano and Nakano (1998) found that males have an absolutely larger corpus callosum than females. However, when they standardized their results to the area of the sagittal view of the cerebrum, they discovered that the distinct sex differences in the corpus callosum faded. In addition, the area of the splenium was larger in females than males when the results were standardized, albeit the difference was not statistically significant. It should be noted though that the measurements where obtained on older adults and the differences or lack thereof may be the result of aging and not developmental sexually dimorphic differences.

Salat et al. (1997) have found that the anterior and middle portions of the corpus callosum atrophy more in women with age than men. This distinct and statistically significant selective atrophy in females calls into question the use of older individuals for assessing sex differences in the corpus callosum and other brain structures. Of the studies that have limited their sample to younger individuals (18 – 39 years), most have found statistically significant sex differences in the shape or size of the corpus callosum. The disparity between the results of these two groups appears to be the consideration of brain size in their results. For example, those studies that found significant size

differences in the corpus callosum standardized their results to brain size (Kertesz et al., 1987; Steinmetz et al., 1995; Johnson et al., 1996), while the studies that found no sex differences failed to standardize their results (Allen et al., 1991; Denenberg et al., 1991a,b; Rajapakse et al., 1996).

There are, nevertheless, contradictory results even when studies that have standardized callosal size to brain size are examined (Bishop and Wahlsten, 1997; Peters et al., 2000). Whether these differences are merely due to age (Salat et al., 1997), race (Klekamp et al., 1991) or procedural issues (Giedd et al., 1995; Rauch and Jinkins, 1996), is difficult to discern. However, an additional explanation is that sex differences in the human corpus callosum may not manifest equally throughout the entire corpus callosum or a particular subsection. Instead, the differences may occur within a subsection on a level that fails to significantly alter the overall shape of the callosal subsection.

In an examination of the corpus callosum in which the data were analyzed using deformation functions[1], Davatzikos and Resnicke (1998) found that there is a statistically significant sex difference in the splenium with females having a proportionally larger and more bulbous splenium. However, Davatzikos and Resnicke did not standardize total callosal area to brain size, and thus, found no overall significant difference in callosal size. The use, however, of deformation functions permits the comparison of regional areas between males and females without the consideration of brain size. The principle

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[1] The deformation function is used to compare an individual's corpus callosum to a template. The template is "deformed" to fit the shape of the subject's corpus callosum. The resulting shrinkage or expansion of the template is quantified at each point by a deformation function. The deformation function is a collection of coefficients measuring how much the template had to be altered or deformed around each point to equal the size and shape of the subject's corpus callosum. These values can then be compared to demonstrate differences between individuals or groups (Davatzikos and Resnick, 1998).

finding of the study concluded that the male – female difference in the splenium is not manifest in the overall size of the splenium, but instead, is concentrated in the central portion along the posterior isthmus – posterior splenial border.

The use of morphometrics in brain research has occurred only recently (Thompson et al., 1996, 1997; see also Toga and Thompson, 2001). This methodology is benefited by its ability to use a standardized coordinated system that allows cross group comparisons while preserving subtle intragroup variability (Thompson et al., 2000). In a provocative study to assess morphometric changes in the corpus callosum between normal and schizophrenic subjects, Narr et al. (2000) found that the corpus callosum of schizophrenics is statistically significantly different from normal subjects. Schizophrenic subjects had a corpus callosum that was significantly vertically displaced in the middle portion such that the curvature of the corpus callosum along its length was noticeably distinct from normal subjects. Moreover, Narr et al. (2000) were able to establish that there is a statistically significant difference in the shape of the corpus callosum between male and female schizophrenics, and schizophrenic and normal subjects. They did not find, however, that there is a significant difference in the shape of the corpus callosum in normal male and female subjects, albeit slight differences in size were observed in the splenium and midbody. It should be noted, though, that the study did not focus on sex differences in normal subjects, and that Narr et al. (2000) suggested that further research be done on this area using these techniques. Thus, it can be assumed that newer techniques involving deformation variables and morphometrics have done little to resolve the issue of sex differences in the corpus callosum at this time. Nevertheless, as Holloway et al. (1993) stressed any assessment of sex differences in the corpus callosum

or other brain structure must consider allometric variables, namely brain size. When brain size is taken into account robust sex differences in callosal shape and size generally emerge. Once this is done the subsequent questions of what do these sex differences mean and how are they created can be addressed. Obtaining a clear answer is important, since the corpus callosum plays such an important role in functions that are lateralized in the brain, such as vision (Demeter et al., 1990; Payne, 1990; Krubitzer and Kaas, 1993; Vercelli and Innocenti, 1993) and speech (Kertesz et al., 1987; O'Kusky et al., 1988; Kaga et al., 1990; Galaburda, 1995; Aboitiz and Ide, 1998).

1.5.1.2 Sex differences in the fiber composition of the human corpus callosum

Few studies have addressed the nature of the human corpus callosum on a histological level (Tomasch, 1954; Aboitiz et al., 1989, 1992b; Highley et al., 1999). Tomasch (1954) conducted the first study focused on the fiber composition of the corpus callosum. While he did not include any females in his study, Tomasch established the corpus callosum as the primary interhemispheric pathway. He also showed for the first time that it is composed of large numbers of axons of various sizes. While Tomasch's study would provide new and valuable information on the fiber composition of the human corpus callosum that would alter previous notions of this structure, further study of this interhemispheric highway would remain latent for forty-five years.

More recently, in a human study, Aboitiz et al. (1989, 1992a,b,c) reexamined the topic of fiber composition of the human corpus callosum. Unlike Tomasch (1954), Aboitiz et al. (1989, 1992a,b) included females in their sample. This allowed for a comparison of fiber numbers and types between sexes. From their examination of ten

males and ten females they concluded that any differences in either total fiber number or fiber type were not statistically significant. While it was found that females posses more large myelinated fibers (> 3μm) than males, this difference was statistically insignificant. In addition, males were found to have more small myelinated (< 3μm), yet this difference was also statistically insignificant. These results suggest that sex differences in the corpus callosum are not evident in the overall fiber composition of this structure. Although, they do not specifically propose that sex differences in fiber composition may occur within certain callosal subsections, their data suggest that such differences may occur within certain regions such as the isthmus and midbody (Aboitiz et al., 1992a, 1996). While the above studies by Tomasch (1954) and Aboitiz et al., (1992a,b,c, 1996) have led to a greater understanding of the neuronal contribution to the corpus callosum, there is still a gap in studies on sexual differences that explain the cognitive differences seen between human males and females.

In a recent study on sex differences in schizophrenia, Highly et al. (1999) found that there is a significant sex difference in the density of callosal fibers in normal and schizophrenic subjects. In the normal sample midsagittal area of the corpus callosum was not significantly different between males and females. However, normal females had a statistically significant greater density of callosal axons than males, especially in the splenium. The converse was found in schizophrenics. Male schizophrenics had a greater axon density in all callosal regions, especially in the splenium. A sex specific trend that occurs in schizophrenia is that along with a general reduction in brain size females exhibit a concordant reduction in fiber density in the corpus callosum, while males do not show a significant change. Why females show a dramatic reduction in the density of

fibers passing through the corpus callosum, although the overall size of the corpus callosum, save the splenium, is not reduced from normal subjects, is difficult to discern. Highly et al. (1999) and Crow et al. (1998) conjecture that one variable that may account for the differences discussed above (i.e., the significant reduction in the size of the corpus callosum and fiber density in schizophrenic females) is the presence of increased lateralization or impairment of hemispheric communication in schizophrenia. Moreover, the significant difference in fiber density between normal males and females may explain certain cognitive differences between the sexes.

1.5.2 Sex differences in the corpus callosum of nonhuman primates and rodents

While studies such as Aboitiz et al. (1992a,b,c) and Highly et al. (1999) on humans have begun to address the question of the reality of gender-related differences in the corpus callosum, they have not completed the journey. Beginning with deLacoste-Utamsing and Holloway (1982), there have been many studies coming down on either side of the question. Obtaining an unambiguous answer is important, since the corpus callosum plays such an important role in lateralization of function in the brain, most importantly vision (Demeter et al., 1990; Payne, 1990; Krubitzer and Kaas, 1993; Vercelli and Innocenti, 1993; Intriligator et al., 2000) and speech (Kertesz et al., 1987; O'Kusky et al., 1988; Kaga et al., 1990; Galaburda, 1995). While all aspects of the human corpus callosum can not be gleaned from studies on other mammals, examinations of this structure, however, in two particular mammalian groups, rodents and nonhuman primates, have provided clues about the function of the corpus callosum and its regions.

1.5.2.1 Sex differences in the corpus callosum of rodents

In response to the supposition of sex differences in the splenium of the corpus callosum in humans by de Lacoste and Holloway (1982), Juraska and Kopcik (1988) began to examine the development of sex differences in the corpus callosum of rats to determine the stimuli required to produce sex differences in this structure. In the first of a series of studies on the rat corpus callosum, Juraska and Kopcik (1988) found no sex differences in the size of the corpus callosum in rats that had either been raised in a complex environment or isolation, albeit they used only gross measurements. They did, however, find that females possessed more unmyelinated axons than males regardless of environment. In addition, females that were raised in a complex environment had more myelinated axons than similarly raised males, although males tended to have larger myelinated axons passing through the corpus callosum. The relevance of this study was to show that although morphological sex differences may not exist in the midsagittal area of the corpus callosum in humans, it is possible that axonal differences do exist. It also demonstrated that environmental conditions may influence the composition of this structure.

Subsequent studies on the corpus callosum have revealed sex differences in the fiber composition of the splenium. While there are no significant sex differences in the total number of axons passing through the splenium, there are sex differences in the types of axons in it. Females tend to possess more unmyelinated axons than males. In contrast males possess larger myelinated axons than females (Kopcik et al., 1992; Mack et al., 1995; Kim et al., 1996). The production of the differences is currently a subject of debate. Are the differences merely environmental and thus developmental (Juraska and

Kopcik, 1988; Kopcik et al., 1992; Kim and Juraska, 1997; Nuñez et al., 2000), or are they based purely on hormonal influences (Fitch et al., 1991; Mack et al., 1996; Bishop and Wahlsten, 1999; Bimonte et al., 2000)?

Juraska and Kopcik (1988) found that rats raised in complex environments had a larger number of myelinated axons than those raised in isolation. In addition females possessed more axons overall, albeit statistically insignificant, and more myelinated axons than males when raised in a complex environment. In contrast females had fewer myelinated axons when raised in isolation, while there was little difference between males of the two environmental groups. From these results they suspected that environmental influences may play a role in callosal development as well as sex differences within this structure. Subsequent studies by this lab recapitulated the results of the initial study, finding that males possessed significantly larger myelinated axons than females (Kim et al., 1996; Kim and Juraska, 1997). In addition, Kim and Juraska (1997) and Nuñez et al. (2000) report an increase in the number and size of myelinated axons continues well into adulthood. The most dramatic expansion of myelinated fibers occurs in males. The presence of larger myelinated axons in males than females and the withdrawal of axons in females into adulthood, when environmental conditions are similar, suggests that sex differences in this area may primarily be the result of hormonal influences (Kim and Juraska, 1997).

To elucidate the underlying mechanisms for sex differences in the corpus callosum, Fitch et al. (1991a) examined the development of the corpus callosum in ovariectomized rats. They show that ovariectomized females demonstrated a masculinization of the corpus callosum as late as postnatal day sixteen. The corpus callosum of ovariectomized

females was larger than non-ovariectomized females (Fitch et al., 1991a; Fitch and Denenberg, 1998). These results occurred whether the animal was ovariectomized prenatally or postnatally (Fitch and Denenberg, 1998; Bimonte et al., 2000). In addition, males that had been demasculinized prior to birth had reduction in the size of the corpus callosum over normal males (Fitch et al., 1991b). These data would suggest that feminization or masculinization of the corpus callosum is sensitive to gonadal steroids until adulthood (Mack et al., 1996), although Fitch and Denenberg (1998) concede that environment may partially influence the development of this structure.

1.5.2.2 Sex differences in the corpus callosum of nonhuman primates

Many studies have focused on the sexual dimorphism of the human brain (Mall, 1909; Kimura, 1992; see McGlone, 1980; Falk, 1997 for reviews), but few have examined the issue in nonhuman primates (Le May, 1976; de Lacoste and Woodward, 1988; Falk et al., 1999; Franklin et al., 2000). At the same time most of the research that has been performed on sexual dimorphism in the brain of primates has had more to do with morphology than with the actual composition of this organ. The distribution of callosal fibers in nonhuman primates has been demonstrated several times (Seltzer and Pandya, 1983; Gould et al., 1986; O'Kusky et al., 1988; LaMantia and Rakic, 1990a,b; Beck and Kaas, 1994). Although LaMantia and Rakic (1990) approached gender differences in the course of their study, differences between the sexes with regard to fiber composition have yet to be sufficiently and specifically addressed.

De Lacoste and Woodward (1988) examined the midsagittal area of the corpus callosum in pongids, cercopithecoids, cebids, and strepsirhines. They found sex

differences in the size of the corpus callosum and the width of the splenium relative to brain size in pongids. They also found sex differences in the size of the corpus callosum relative to brain size in strepsirhines. While these results would suggest that sex differences in the corpus callosum exist in certain primate groups, it should be noted that the four primate groups used in the above study (pongids, cercopithecoids, ceboids, and strepsirhines) are comprised of thirty-four species. Thus, their results are merely suggestive of sex differences in primate groups and not specific species. Other primate studies in which species were not combined show less sexual dimorphism in the corpus callosum than was previously suspected. Holloway and Heilbroner (1992) report that there are no sex differences in the corpus callosum or its subsections relative to brain size in *Macaca mulatta*, *Macaca fascicularis*, *Collithrix jacchus*, and *Saguinus oedipus*. Only *M. mulatta* demonstrated a slight sexual difference in the width of the splenium, with males being larger than females. Recently Franklin et al. (2000) suggested that the total area of the corpus callosum is larger in *M. mulatta* males than females. They also showed that females possess a larger splenium. While these results are contrary to those of Holloway and Heilbroner (1992), it should be noted that the results of Franklin et al., (2000) are based on raw data and not relative measurements. Thus, these results merely serve to complicate the issue of sex differences in the corpus callosum.

While the above studies have sought to determine sex differences in the corpus callosum of nonhuman primates based on total callosal area or subsectional areas, few studies have attempted to address the question of fiber differences in this important structure. Seltzer and Pandya (1983), Gould et al. (1986), O'Kusky et al. (1988), and Beck and Kaas (1994) have examined the topography of the nonhuman primate corpus

callosum; however, these studies did not address the issue of sex differences. LaMantia and Rakic (1990a) also examined the development and topography of the nonhuman primate corpus callosum. In addition, to their primary data, they also include anecdotal data on sex differences in the fiber composition of the corpus callosum in *M. mulatta*. In a comparison of two age- and brain weight-matched individuals, the male possessed 10 million more axons than the female, although the female's corpus callosum was larger. While this difference appears large, they suggest that the disparity could quickly disappear with a larger sample, since the corpus callosum normally contains fifty to sixty million axons in *M. mulatta*.

In general there is a paucity of data on sex differences in the corpus callosum of nonhuman primates. While the above studies have provided intriguing clues to the lack of definitive sex differences in this structure, the disparity of their results mandates the need for additional data, especially in species such as *Pan*. This includes information on the relative size of the corpus callosum in individual species as well as supplementary data on the fiber composition of this structure. Such data are important to understanding the function, development and evolution of human and nonhuman primate brains. Information of this nature is one of the focuses of this dissertation, and will be discussed in later chapters.

CHAPTER 2

MATERIALS, SPECIMEN ACQUISITION, SPECIMEN PREPARATION AND ANATOMY OF THE CORPUS CALLOSUM IN *MACACA FASCICULARIS* AND *PAN TROGLODYTES*

2.1 CHAPTER OVERVIEW

The materials and methods, involving anatomical methods and anatomy of the corpus callosum are discussed here. This research used brains from the species *Macaca fascicularis* and *Pan troglodytes*. Section 2.2 discusses specimen acquisition and preparation of brains for quantitative analyses. Procurement of brains, issues of fixation and preparation, and the coding system used to identify each specimen for this study are also discussed within Section 2.2. Section 2.3 defines and describes the midsagittal morphology of the corpus callosum as it pertains to this study.

2.2 ANATOMICAL METHODS

2.2.1 Background to choice of specimens

Considerable controversy surrounds the existence of identifiable sexual differences in the nonhuman primate and human brain. An area that has come under increasingly greater focus is the corpus callosum. For example, many recent studies on humans have demonstrated morphological differences between the sexes in callosal measures (e.g., de Lacoste-Utamsing and Holloway, 1982; Wium, 1984; de Lacoste et al., 1986; Holloway

and de Lacoste, 1986; Holloway, 1990; Holloway et al., 1993) as well as fiber composition (Tomasch, 1954; Aboitiz et al., 1992a,b,c). Comparable data from nonhuman primates has, however, been generally lacking (e.g., LeMay, 1976; de Lacoste and Woodward, 1988; LaMantia and Rakic, 1990; Holloway and Heilbroner, 1992; Franklin et al., 2000). In addition, the few studies that have been performed on nonhuman primates have generally lacked an approach that takes into account the sexual dimorphism present in most primate groups.

2.2.1.1 Macaca fascicularis

A central component of this dissertation is the corpus callosum of *Macaca fascicularis*. This is the animal of choice for a number of reasons, each of which is discussed in more detail below. First, it is frequently chosen for investigations of the brain (e.g., Seltzer and Pandya, 1983; Gannon, 1995), meaning there is an understanding of its neurological architecture. Second, it is a cercopithecoid (Subfamily - Cercopithecinae), meaning it is more closely related to humans than strepsirhines or platyrrhines (Fleagle, 1999). Third, it is a nonendangered species that is readily available from laboratory breeding colonies. Acquisition of specimens does not require special permits or circumstances. Finally, the brain can be obtained from perfused animals, allowing for immediate preservation of tissue with minimal damage (Gannon, 1995). This provides a highly reliable data source compared to immersion fixation which requires passive penetration of fixative into the tissue, thus allowing for greater possibility of artifact or distortion (Bolam, 1992).

Much of our understanding of the nonhuman primate brain has come from studies on *Macaca mulatta* (a.k.a. rhesus monkey) and *Macaca fascicularis* (a.k.a. crab-eating macaque, long-tailed macaque, cynomolgus monkey). While two previous studies on the corpus callosum in nonhuman primates were performed on rhesus monkeys (LaMantia and Rakic, 1990b; Franklin, 2000), other studies have established similarities of the brain between *Macaca fascicularis*, *Macaca mulatta*, and humans (Galaburda, 1980; Galaburda and Pandya, 1982; Deacon, 1984, 1992; Holloway and Heilbroner, 1992). Most notably, however, Brodmann (1909, 1912) can be credited with establishing the similarities between human and nonhuman primate brains. His early studies on the brain demonstrated that areas within the brain could be defined histologically. In this way, cytoarchitectonic charts of the cortex could be formed and the degree and extent of the differentiation of the brain in extant primate species compared. The maps created from these studies first demonstrated the conservative nature of the primate brain, and subsequently enabled future research on the human brain using nonhuman primates as subjects (e.g., Hubel and Wiesel, 1977; Rakic, 1977, 1981, 1988), albeit *Macaca* lacks certain Brodmann's areas possessed by humans.

Macaca fascicularis can be found in most of Southeast Asia from southern Myanmar and Thailand where it is sympatric with *Macaca mulatta* to the island of Timor in Indonesia. Living in multimale, multifemale groups of between two to just under a hundred animals the social dynamics of *Macaca fascicularis* are interesting from an evolutionary prospective. As an "edge " species, it has shown exceptional adaptability at living in a variety of habitats – primary, riverine, coastal forest – provided they are close to water. This is similar to its larger cousin *Macaca mulatta* that also has demonstrated

success at occupying a variety of habitats from northern India to southern China and Southeast Asia. An average group consists of 30 individuals with a sex ratio of 1:2, adult males:adult females (Fooden, 1980; Burton, 1995). This is a sexually dimorphic species with an average adult male weight of 5.4kg and an average adult female weight of 3.6kg (Fleagle, 1999). Unlike *Macaca mulatta*, however, which lives in large groups of at least 30 individuals, *Macaca fascicularis* possesses stable group dynamics. In both species dominance hierarchies occur in each sex, and are usually based on age, kinship, and coalitions (Angst, 1975). Also, these are female-focal groups, where matrilines are stable and in which rank of the mother is conferred upon the daughters, with the youngest daughter second only to the mother. Females remain in their natal groups, while males emigrate at some point. In general, males are dominant over females with the alpha male having preferred reproductive access to females (Fooden, 1980; see also Lindberg, 1980). In *Macaca mulatta* evidence shows that while the alpha male has preferential access to females in estrous, females will often mate with other males, and more often than not offspring are sired by males other than the alpha male. *Macaca fascicularis*, however, does not follow this particular *Macaca* pattern. While females will mate with a number of different males when they are in estrous, the alpha male tends to father the majority of offspring (Tutin, 1979; Goodall, 1986; McMillan, 1989; Sprague, 1998). Thus, while *Macaca mulatta* and *Macaca fascicularis* share a number of behavioral traits, they possess enough unique social characters to maintain their status as separate species, despite their sympatry in certain areas (Fooden, 1964; Fooden, 1980).

2.2.1.2 Pan troglodytes

As the closest living relative of modern humans (Sibley and Alquist, 1987), the common chimpanzee (*Pan troglodytes*) represents an integral component of this study examining the presence of sexual dimorphism in the corpus callosum and the evolution of sex differences in the modern human brain. For a number of reasons, the study of *Pan troglodytes* here and elsewhere is important for an understanding of human brain evolution. First, the genus *Pan* represents the closest living nonhuman primate relative to modern humans. Second, *Pan* possesses a brain that approximates the size and possible complexity of the brains of the early ancestors of modern humans. Third, *Pan* is known to be a sexually dimorphic species with males and females exhibiting complex social interactions. Fourth, there is little information about the brain of *Pan*. This means that the use of *Pan* in this study provides not only information concerning the evolution of the modern human brain, but also novel information about the neuroanatomy of *Pan*. Finally, albeit *Pan troglodytes* is a protected species (CITES, 1973), its numbers compared to that of the other species in this genus, *Pan paniscus*, are more numerous in United States biomedical laboratories and museum collections.

The systematic position of *Pan* has been debated for many years (see reviews by Ciochon and Corruccini, 1983; Pilbeam, 1986; Boyd and Silk, 2000). While some individuals place *Pan* closer to gorillas based on morphologic evidence, others cite genetic data suggesting *Pan* may be more closely related to hominins than to *Gorilla*. The morphologic evidence comes from the extensive similarities between *Pan* and *Gorilla*. Both possess large, sexually dimorphic canines; shoulder joints that are suited for suspensory behavior; and are adapted for a unique form of terrestrial quadrupedalism,

knuckle-walking. Proponents of the idea that chimpanzees and gorillas arose from a common ancestor emphasize that it would be unlikely for knuckle-walking to evolve separately through convergent evolution had *Pan* arose from a hominin ancestor. A recent paper, however, suggests that the hominin lineage arose from a knuckle-walking ancestor. Based on a comparison between distal wrist bones of *Australopithecus* and modern apes, Richmond and Strait (2000) suggest that the ancestor of the hominin clade was a knuckle-walker. This evidence lends support to the hypothesis that *Pan* is more closely related to *Homo* than either is to *Gorilla*. It should be noted, however, that many morphologists review the anatomical data as favoring a *Pan-Gorilla* clade separate from the hominin clade. In addition, the findings of Richmond and Strait (2000) are open to controversy.

The genetic evidence is more supportive of placing *Pan* closer to *Homo* than to *Gorilla*. Numerous genetic markers have been analyzed in *Pan*, *Gorilla*, *Homo* and other nonhuman primates. Many of these studies indicate that *Pan* is more closely related to *Homo* than either is to *Gorilla*. While there have not been any genetic studies examining neurological markers in *Pan*, the genetic studies that have been performed, nevertheless, establish a strong relationship between modern humans and *Pan*. Despite the controversy concerning the taxonomic position of *Pan* within the hominidae, this data support the notion that examining the brains of this group may give significant insight into the evolution of the human brain, in particular the evolution of sexual dimorphism in the human brain.

Pan like many other nonhuman primates is characterized by marked sexual dimorphism. Of the two species of *Pan* (*Pan paniscus* and *Pan troglodytes*), *Pan*

troglodytes displays the greatest degree of sexual dimorphism. Males have larger bodies, canines, and brains on average compared to females. Not only is there morphologic sexual dimorphism in this species, but socially there is as well. The common chimpanzee (*Pan troglodytes*) lives in polygynous groups with a single dominant male, several mature males, and several mature females and their offspring. The social interactions between members of this species include vocal, physical, and gestural-visual behaviors. Based on work on the evolution of the human vocal apparatus (Laitman et al., 1979; Laitman, 1983, 1984, 1985; Laitman and Reidenberg, 1987; Lieberman, 1991) and brain (Holloway, 1995) and paleoarcheological evidence (Isaac, 1986; Blumenschine, 1987), it is likely the social interactions of early hominins were not much more complex than those exhibited by extant apes. If true this makes *Pan* a compelling candidate for understanding how early hominins may have behaved.

The application of *Pan* to understanding early human behavior and anatomy is based on the anatomic similarities between the two groups. These similarities such as brain size [375-410cc for *Pan* (Harvey et al., 1987; Falk, 2000) vs. 375-440 for *Australopithecus* (Holloway, 1995; Tobias 1995)] and postcranial skeletal morphology (Richmond and Strait, 2000; Stern, 2000) make *Pan* a better candidate for understanding early human evolution than either *Gorilla* or *Pongo*. Direct comparison of extant nonhuman primate social systems and early hominins is difficult not only because morphology is the only evidence from early human evolution, but also because the concepts of primate social systems, especially their origins, has been a hotly debated topic for decades (e.g., Crook and Gartlan, 1966; Clutton-Brock and Harvey, 1977; Wrangham, 1980; Terborgh and

Janson, 1986). The study of primate social systems, nevertheless, is important in understanding the social and neurological evolution of early hominin groups.

There is little disagreement about the complexity of chimpanzee social systems. They are the only group of primates to exhibit a collection of complex behaviors that mirror many seen in modern humans. These include infanticide (Nishida, 1979), cannibalism (Goodall, 1977), carnivory on other primates (Teleki, 1973a,b), homicide within and between groups (Lancaster, 1978), and political alliances (de Waal, 1998, 2000), albeit certain behaviors are exclusive to particular populations. In addition, some researchers (Whiten et al., 1999) have suggested that *Pan troglodytes,* as a species, possesses definable culture. While the origin, function, and application of these behaviors is debatable, they emphasize the importance of the use of *Pan* in understanding human evolution.

The use of *Pan* in understanding early human evolution receives compelling support from the above studies documenting complex behaviors in this group. The information available, however, for the use of sexually dimorphic morphology and behavior in *Pan* for investigating the origins of human sexual dimorphism in the brain is just as compelling. Lifelong studies of *Pan troglodytes* by researchers such Wrangham (1977, 1979, 1980) and Goodall (1977, 1990) have provided considerable data, yet little information on specific sexually dimorphic behavioral differences. While there is argument about the origins and selective pressures for these behaviors (see Wrangham, 1977, 1979, 1980 and Terborgh and Janson, 1986), there is agreement about the basic behaviors of each sex.

Chimpanzees are characterized by a multimale social system where a group of unrelated males overlap the ranges of females, which live in smaller groups. Although males tend to have frequent contact with each other and forage together, females are seen to interact less frequently and forage alone or often in small groups. Wrangham (1980) suggests that females distribute themselves in order to increase their efficiency with which they exploit food resources, while males are distributed in a manner that appears to optimize their access to females. In addition, it is possible that female *Pan* social structure is based on the need to optimize inclusive fitness. In a social system in which confrontation between males for rank is not uncommon (Goodall, 1977, 1986) and aggression towards the energetic costs of females in terms of infanticide is present (Nishida, 1979), it is likely that females have enhanced their inclusive fitness by living in smaller cohesive units. This is also supported by the idea that sexual swellings in females and their potential to mate with many members enhances their inclusive fitness by reducing aggression towards them and their offspring. The examples of complex social behaviors and the possible selective pressures for them emphasize the behavioral sexual dimorphism in this species.

In contrast to chimpanzees, gorillas are described as living in loosely structured groups (Wrangham, 1979) in which both males and females emigrate (Harcourt, 1978). The fact that the group is basically made up of unrelated individuals does account for the need for frequent male-female associations, necessary in order to increase inclusive fitness. Although this does increase energetic costs in terms of maintaining "relationships", it does act to reduce the overall energetic costs of the individual by reducing the need to actively gain access to mates (Wrangham, 1979). Thus, while gorillas may be similar to

chimpanzees in anatomy, they lack many of the complex social behaviors exhibited in *Pan*.

Their endemic location, morphology, behavior and genetic distance confound the use of orang-utans for understanding hominin evolution. Orang-utans represent the arboreal component of the great apes. Orang-utans tend to live a solitary life with the range of a male overlapping the range of several females (MacKinnon, 1974). Outside of territorial maintenance and rape by males against females (Galdikas, 1979, 1985) aggression is low in orang-utans, possibly to minimize feeding competition and, thus, increase inclusive fitness (Wrangham, 1979). Albeit morphologic sexual dimorphism is obvious within *Pongo*, the lack of complex social group dynamics makes this genera a poor choice for understanding human and early hominin behavior.

The morphology and behavior exhibited by extant great apes is varied. While it is difficult to decipher the origin and context of these behaviors in each species, they do provide important information about primate evolution in general. Among these species, *Pan* exhibits a morphology and behavioral suite that can best be applied to hominin evolution. In addition the genetic evidence supports a hominin-*Pan* ancestry. Thus, *Pan* not only stands as a provocative candidate for understanding early hominin evolution and behavior, but also as an example of when sexual dimorphism began in the hominidae, and specifically in hominin brains.

It has been demonstrated that the structure of the corpus callosum is related to hand and hemispheric speech dominance in humans (O'Kusky et al., 1988). It has also been shown that handedness and other features indicating lateralization of neural functions exist in nonhuman primates (Bradshaw, 1991; Hopkins and Pearson, 2000; Hopkins and

Rilling, 2000). Among nonhuman primates the condition appears to be most accentuated in great apes (Bresard and Bresson, 1987; Diamond and Harries, 1984; Fischer et al., 1982; Hopkins and Morris, 1993; Hopkins and Pearson, 2000). Due to this analogous condition seen in great apes when compared to humans, it is obvious that a study involving *Pan* must be undertaken. From such research it is possible to suggest underlying evolutionary mechanisms that resulted in the asymmetries witnessed in the brains of modern humans, especially when applied to sex related differences.

While it might be possible to draw parallels between each species in the hominidae and *Homo*, *Pan* stands as an obvious choice for human-ape comparisons, as well as explanations of evolutionary history. An area of interest in this study is the origins of sexual dimorphism in the human corpus callosum. Because of the evolutionary relationship of *Pan* and *Homo*, *Pan troglodytes* is a logical choice of species for investigating this question.

In addition to understanding the evolutionary origins of sexual dimorphism in the human corpus callosum another question of this study is the presence or absence of sexual dimorphism of the corpus callosum in *Pan*. While there have been many studies investigating the morphology of *Pan troglodytes*, there have been few studies specifically aimed at the anatomy, function, and sexual dimorphism of its brain. There have been several studies comparing the volume of certain structures in the brain of *Pan* with other primates and modern humans, however, there have been few that have simply noted the neuroanatomy and sexual dimorphism of these structures in this species. *Pan troglodytes*, thus, is the species of choice for this study not only to understand the origins

of sexual dimorphism in the human corpus callosum, but also to note the midsagittal morphology and axonal composition of this structure for the first time in this species.

2.2.2 Procurement of brains

The brains of *Macaca fascicularis* used in this study were obtained from the laboratory of Dr. Patrick Gannon, Department of Otolaryngology, The Mount Sinai School of Medicine and the laboratory of Dr. Ralph Holloway, Department of Anthropology, Columbia University. A total of 20 (10 male, 10 female) brains were graciously loaned by Dr. Gannon for use in this study. These brains were obtained by him and Dr. Gay Holstein, Department of Neurobiology, The Mount Sinai School of Medicine for use in Dr. Gannon's dissertation (Gannon, 1995). Either Dr. Gannon or Dr. Holstein obtained all of the brains from Dr. Gannon's laboratory used in this study from live animals by perfusion fixation (see Gannon, 1995 for explanation of procedures and acquisition of these brains). The brains from Dr. Holloway's collection (n = 20; 10 males, 10 females) were obtained by Dr. Peter Heilbroner for use in his dissertation (Heilbroner, 1987). These brains were immersion-fixed (see Heilbroner, 1987 for explanation of brain acquisition for these specimens).

Brains of *Pan troglodytes* for this study were obtained from Yerkes Regional Primate Center, Emory University (n = 7 brain tissue, n = 6 MRI), the Department of Mammals at the National Museum of Natural History, Smithsonian Institution (n = 6), and the collection of Dr. Ralph Holloway (n = 4). Because of the endangered status of *Pan troglodytes* in the wild (CITES, 1973), the use of this species in biomedical research is restricted (National Research Council, 1996). As it relates to this study, these restrictions prevent the euthanasia of *Pan troglodytes* for research purposes. Thus, brains from this species were obtained from animals who had either been collected prior to these restrictions, such as the brains obtained from the Smithsonian Institution, or from animals

that had died of natural causes or were euthanized due to terminal illness. All of the brains from *Pan troglodytes* used in this study were preserved using immersion fixation techniques. As these brains cannot be obtained as readily as those of *Macaca fascicularis*, acquisition of specimens was dependent on the availability of tissue, as opposed to availability of individual animals. Thus, the sample size of actual brain tissue was limited to a total of 17 brains (8 males, 9 females). Since few brains were available for use, MRI's obtained from live animals supplemented the number of males and females examined in order to raise the sample size to at least n = 10 for each sex. The MRIs (3 males, 3 females) were conducted by Dr. James Rilling, Yerkes Regional Primate Center for use in his dissertation (refer to Rilling, 1998 for an explanation of procedures used in acquiring these scans). Thus, the majority of brains (n = 17) used in this study were from actual tissue while midsagittal MRIs of 6 individuals were used to obtain a sample size of n = 23 (11 males, 12 females).

2.2.3 Coding of specimens

This study is concerned with the presence or absence of sex differences in the corpus callosum of *Macaca fascicularis* and *Pan troglodytes*. In such a study the potential for bias towards a particular sex, species, or individual exists. In order to prevent investigator bias all specimens were coded without designation to sex. Specimens used from Dr. Holloway's collection of both *Macaca fascicularis* and *Pan troglodytes* had previously been coded, and the same codes were maintained for this study. This was also the case for *Macaca fascicularis* specimens from Dr. Gannon's collection. Specimens from Yerkes Regional Primate Center were assigned a necropsy code at the time of necropsy. This code represents the animal's final designation. All tissues from the animal are assigned the same necropsy code. This code was maintained for this study as it is assigned randomly without designation to either sex or species. MRI's of *Pan troglodytes* received from Dr. Rilling (Yerkes Regional Primate Center) were not

received with the code that is assigned to each animal at the Center. Instead these images were identified by the animal's name, which generally indicated the animal's sex. These animals were assigned a code prior to examination of the MRI files, as to avoid associating an image with a particular individual. The specimens examined at the Smithsonian Institution had already been assigned a numerical code that gave no designation of species or sex. These codes, therefore, were maintained. The original animal designation and the code assigned to the animal for this study can be found in Table 2 - 1 for *Pan troglodytes* and Table 2 - 2 for *Macaca fascicularis*. No specimen was decoded until the final analysis of the data.

Table 2 - 1

Code designation for *Pan troglodytes* specimens used

Original assignment	Assignment this study	Location of specimen[1]
YN70-119	YN70-119	RLH
YN94-67	YN94-67	RLH/YRPC
YN95-60	YN95-60	RLH/YRPC
YN80-7	YN80-7	RLH
YN73-74	YN73-74	RLH
YN60-7	YN60-7	RLH
YN95-6	YN95-6L	RLH
YN88-256	YN88-256	RLH/YRPC
YN77-111	YN77-111	RLH/YRPC
YN97-139	YN97-139	RLH/YRPC
YN92-115	YN92-115	RLH/YRPC
Bodian 25a	Bod25J	RLH
Bodian 25b	YN25J	RLH
USNM 292178	292178	USNM
USNM 292177	292177	USNM
USNM 229100	229100	USNM
USNM 225776	225776	USNM
Jimmy	JKR 1	RLH/YRPC-MRI
Kengee	JKR 2	RLH/YRPC-MRI
Laz	JKR 3	RLH/YRPC-MRI
Lulu	JKR 4	RLH/YRPC-MRI
Mary	JKR 5	RLH/YRPC-MRI
Merv	JKR 6	RLH/YRPC-MRI

[1]RLH – Collection of Dr. Ralph L. Holloway, Department of Anthropology, Columbia University. RLH/YRPC – Specimen received from Yerkes Regional Primate Center and resides in the collection of Dr. Ralph L. Holloway. USNM – Specimen located in the Division of Mammals, NMNH, Smithsonian Institution. RLH/YRPC-MRI – MRI image received from Dr. James K. Rilling, Yerkes Regional Primate Center.

Table 2 - 2

Code designation for *Macaca fascicularis* specimens used

Assigned code	Location of specimen[1]
81A69	RLH
81A70	RLH
81A73	RLH
81A75	RLH
81A82	RLH
83A124	RLH
83A31	RLH
83A39	RLH
83A54	RLH
83A80	RLH
83A81	RLH
84C40	RLH
84C56	RLH
84N6	RLH
85C13	RLH
85C16	RLH
85C2	RLH
85N38	RLH
85C7	RLH
85N6	RLH
PGM50	PGM
C6-Q35	PGM
PGM56	PGM
PGM41	PGM
29Q	PGM
PGM48	PGM
PGM40	PGM
PGM54	PGM
2319Q	PGM
PGM47	PGM
PGM39	PGM
PGM53	PGM
PGM46	PGM
Z3	PGM
PGM38	PGM
PGM52	PGM
Z3-Q43	PGM
PGM45	PGM
PGM57	PGM
PGM43	PGM

[1]RLH – Collection of Dr. Ralph L. Holloway, Department of Anthropology, Columbia University. PGM – Collection of Dr. Patrick G. Gannon, Department of Otolaryngology, Mount Sinai School of Medicine.

2.2.4 Potential for fixation induced artifact

The effects of fixation on the brain include preservation of cell structure and integrity of myelin sheaths. An artifact of fixation, however, is a reduction in the pre-fixation size of the brain. For example, an examination of several whole chimpanzee brains from Yerkes Regional Primate Center used in this study showed an average brain weight reduction of 10% from the original post-mortem weight (Table 2 - 3). While this does not make measurements from fixed tissue suspect, it does, however, call for attention to the method of fixation used.

Table 2 - 3

Specimen	Post-mortem Brain Wt. (g)	Brain Wt (g) this study
YN88-256	360.14	315.35
YN77-111	370.7	338.96
YN97-139	408.6	374.67
YN92-115	409.0	364.80

Brain tissue can be fixed by either perfusion or immersion, but perfusion-fixation is the method of choice, since immersion fixation of nervous tissue may produce artifacts seen as vacuolar retraction spaces around neurons (Garman, 1990; Bolam, 1992). This is especially the case when experimental manipulations have been performed. Perfusion fixation is performed on a deeply anaesthetized animal, and the fixative is administered by means of a cannula inserted through the aorta. The fixative is then pumped through the vasculature by means of a peristaltic pump, forcing the vascular contents out of the body. The choice of this method over immersion fixation is that perfusion makes it possible to remove blood, and the fixation is more rapid. In addition, fixative can be forced into deeper tissue, allowing for more even fixation, seemingly to prevent the differential shrinkage of tissue. Perfusion fixation, however, is only applicable to non-endangered animals, and it does not produce better results in all cases (Molck et al., 1998).

Macaca fascicularis is a species of primate, as mentioned above, which can be perfused. The brain and tissue of *Pan troglodytes*, however, cannot be obtained through perfusion under most circumstances. Instead brains from this species are usually fixed by immersion. In this case the brain is removed upon the death of the animal and placed into a vat of cold fixative such as paraformaldehyde or formalin. The brain is left in this solution for a period of a week or more to allow the fixative sufficient time to infiltrate the deep tissues. In addition, the specimens should remain cold during this time as to slow tissue degeneration. Drawbacks of this method include differential shrinkage of tissue, and the possibility of myelin sheaths deep in the brain unraveling before they are fixed. However, if the dural and arachnoid meninges are removed along with some of the vasculature prior to immersion fixation, the midsagittal portion of the corpus callosum should become fixed at a rate equivalent to the outer cortex.

One concern of this study was an examination of the midsagittal axonal composition of the corpus callosum in a limited number of specimens (see Chapter 4). For this reason the integrity of the tissue was important, albeit morphological measurements did not depend on a strict mode of fixation. The brains of *Macaca fascicularis* may be perfused, providing a reliable sample for histological study, and only such brains were used for this aspect of the research. The study of *Pan troglodytes*, however, is important for a comparison to modern humans. These brains, though, are not perfusion-fixed. Nevertheless, since perfusion fixation is not fundamentally better than immersion for morphological studies (Molck et al, 1998), the use of immersion-fixed *Pan troglodytes* brains for intraspecies comparisons is warranted, provided the possible presence of fixation artifact at the cellular level in all specimens is considered. Since all of the brains of *Pan troglodytes* used here were immersion-fixed, the brains from animals that exhibited the highest degree of myelin integrity were chosen for histology, while all brains that exhibited general tissue integrity were used to examine callosal morphology.

2.2.5 Brain weight measurements

Brains of both *Macaca fascicularis* and *Pan troglodytes* were originally weighed by Dr. Gannon, Dr. Holloway, or individuals at the Smithsonian. These brains, however, were re-weighed for use in this study, since as mentioned above tissue may shrink over time. No differences were found for the weights of the brains from Dr. Gannon's and Dr. Holloway's collections. There were, however, differences in the weights of the Smithsonian brains. The last recorded weights for these brains are their original collection weights, which were taken at the beginning of the twentieth century. There was an approximate twenty-five percent difference between the original weights and the weights obtained for this study.

Brains were prepared for weighing by removing any arachnoid mater remaining, and rinsing each brain of fixative. The brain was then patted dry to remove excess fluid on its surface after which the brain was weighed using a Sartorius digital scale to the nearest 0.1 gram. Prior to placing a brain on the scale, the scale was calibrated until it held a steady zero. The brain was then left on the scale for several seconds until the weight stabilized. Some brains had been hemisected prior to weighing. In these cases each hemisphere was weighed separately. The weight from each hemisphere was subsequently added together to obtain the overall brain weight of the specimen.

2.2.6 Sectioning of whole brains

The corpus callosum is the largest interhemispheric pathway in the brain. Its tracks cover a large area of the cerebral cortex, making assessment of the entire structure difficult. Other studies have assessed sex differences in the corpus callosum using a

midsagittal view of this structure (e.g. de LaCoste and Holloway, 1982; Aboitiz, 1992a,b; Juraska and Kopcik, 1988). As mentioned above the midsagittal section of the corpus callosum affords a view of this structure at its narrowest, most definable point. It was determined, therefore, that this study would examine the corpus callosum along its midsagittal area.

Individuals other than the author had sectioned many of the brains used in this study prior to examination. No further modification, therefore, was made to these brains when examined. Other brains, though, were received whole and unsectioned. While some of these brains could be sectioned midsagittally through the entire brain, others could not. Brains of *Pan troglodytes* from Yerkes Regional Primate Center that were received whole could be sectioned midsagittally. To reveal the midsagittal portion of the corpus callosum the midsagittal line of these brains was determined using the longitudinal fissure of the cerebrum and the midline of the pons, optic chiasma and infundibulum. The use of these landmarks yields a midsagittal view of the corpus callosum as well as other midline cerebral, cerebellar, and midbrain structures. After a brain was weighed the midline of the above structures was found, and the brain was sectioned midsagittally along the midpoint of these structures, using a brain knife. The brain was then photographed using the techniques outlined below.

The brains of *Macaca fascicularis* from Dr. Holloway's collection had been sectioned previously by Dr. Heilbroner to reveal the midsagittal area of the corpus callosum for a separate corpus callosum study. The brains from Dr. Gannon's lab, however, were whole, requiring sectioning. These brains were to be used for a separate study on the midbrain. Thus, it was not feasible to examine the midsagittal section of the corpus

callosum by sectioning the entire brain. Instead the corpus callosum was excised from these brains. To remove the callosum from these the midline of the callosum was first approximated using the midline structures mentioned above and the distance between the two cerebral hemispheres as measured along the dorsal surface of the corpus callosum. When the midline of the corpus callosum was determined, an incomplete cut was made along this line using a 15 gauge scalpel. Next the cerebral hemispheres were spread laterally to reveal the corpus callosum, and the corpus callosum was severed from the right cerebral hemisphere by way of a parasagittal cut. The distance between the midline and this parasagittal cut was variable for each specimen, since the total distance was based on how far the cerebral hemispheres could be spread apart. In addition, the parasagittal face of the corpus callosum was demarcated by using a series of incomplete cuts to complete the excision. This produced a jagged surface identifying the lateral portion of the corpus callosum. Once the corpus callosum had been severed parasagittally, the initial midsagittal cut was completed using a continuous, smooth, anterior to posterior cut, and the corpus callosum was removed. Using this technique, a consistent midsagittal surface could be viewed between all specimens. In addition, it left the entire brain intact, minus minor tearing of the fornix and septum pellucidum. The left midsagittal portion of the corpus callosum was not removed because the cerebral hemispheres could not be spread far enough apart to permit removal of this portion without possible damage to the midsagittal area of the corpus callosum or the left cerebral hemisphere.

2.2.7 Photographic and digital imaging of brains

Digital images of the midsagittal area of the corpus callosum are useful for the production of a permanent record of the midsagittal morphology of the corpus callosum of individual specimens. In addition, digital images provide the only means of measurements using the particular measurement software used in this study. As such photographs were taken of each specimen in order to provide images for analysis and to create a permanent record of the midsagittal morphology of the corpus callosum.

Photographs were taken of each *Pan troglodytes* and *Macaca fascicularis* specimen from Dr. Holloway's collection as well as each *Pan troglodytes* specimen from the Smithsonian Institution used in this study (Appendix 1). After each hemisphere or whole brain was weighed and sectioned a photographic slide was taken of the midsagittal and lateral surface of each brain. Each brain was photographed with a centimeter scale, which was placed in the same vertical plane as the corpus callosum. The same camera, lens and scale were used for all images. Each slide was taken using Kodak Ektachrome T160 slide film on a photographic stand with tungsten lamps. When available the midsagittal face of both hemispheres was photographed. The brain of individual specimens was rinsed of preservative and patted dry to remove fluid from the midsagittal surface that would produce glare under the tungsten lamps. The brain was then placed on the photographic stand and oriented so that the midsagittal surface was parallel to the lens of the camera. This was done by first leveling the camera on the stand, so that the lens would be parallel to the plane of the stand base, and then fixing its position. The brain was then placed on the base of the stand and oriented relative to the plane of the lens and stand base. The same camera stand was used for the specimens in Dr. Holloway's

collection, while specimens from the Smithsonian were photographed on site using a similar camera stand at the Smithsonian Institution.

The corpus callosa removed from the *Macaca fascicularis* specimens in Dr. Gannon's lab were not photographed. Instead these specimens were scanned directly into digital files[1]. To create an adequate image from a flatbed scanner, the area surrounding the object of interest should be devoid of data and "whited-out". Because of the size of the intact cerebral hemispheres in the specimens where the corpus callosum was not excised, it would not have been possible to close the lid of the scanner sufficiently without damaging and distorting the tissue. For this reason photographs were used of these specimens. The excised corpus callosum of the specimens from Dr. Gannon's laboratory (PGM), however, did not present this problem. Since the individual corpus callosum had a depth of only a few millimeters, it was possible to scan them without distorting or damaging the tissue.

After the corpus callosum was removed from the PGM specimens, the corpus callosa were laid on an AGFA StudioStar flatbed scanner in groups of ten. The individual callosa were placed with the midsagittal side face down on the bed surface. In addition, the callosa were arranged into rows of three with the ventral surface of each callosa parallel to the front edge of the scanner bed. The same centimeter scale that was used for the photographic slides was scanned with each set of PGM corpus callosa. The lid of the scanner was closed to within a few millimeters of the callosal tissue by placing separators between the scanner bed and the lid. Each set of callosa was scanned into a gray-scale

[1] Since the corpus callosum was not removed from specimens in the Smithsonian or Dr. Holloway's collection, it was not feasible to scan each directly into a digital file.

image file at a resolution of 800 dots per inch. These image files could then be directly analyzed.

MRI midsagittal images of *Pan troglodytes* obtained from Dr. James Rilling were sent via diskette as TIFF files. These images were obtained by Dr. Rilling from adult individuals housed at the Yerkes Regional Primate Center in Atlanta, GA. Since the images were stored originally as TIFF files during MR scans of the individual, no scanning or alternate storage of the images was necessary. In addition, each scan included a scale by which the individual callosum could be measured.

2.3 ANATOMY OF THE CORPUS CALLOSUM

2.3.1 General gross anatomy of the corpus callosum from a midsagittal perspective

Eutherian mammals possess three major cerebral interhemispheric pathways, the anterior commissure, the hippocampal commissure, and the corpus callosum. Of these the corpus callosum is by far the largest fiber bundle in the brain, containing more than 300 million axons in humans (Nolte, 1993) and 60 million in *Macaca* (LaMantia and Rakic, 1990). Nearly all cortical areas receive commissural fibers, and as such the corpus callosum has an obvious midsagittal appearance (Myers, 1965; LaMantia and Rakic, 1990a,b) (Fig. 2.1). In this view the corpus callosum forms an arch with the anterior end recurving inferiorly in front of the septum pellucidum and lamina terminalis. The trunk of the corpus callosum arches back and is convex above ending in a posteriorly expanded section. The indusium griseum, a poorly differentiated film of grey matter, and the cingulate gyrus of the limbic system bound it anteriorly and superiorly. This gyrus

continues around the posterior border of the corpus callosum as the isthmus of the cingulate gyrus. Continuing around the bulbous posterior portion of the corpus callosum, the inferior portion of the posterior fifth serves as the roof of the cistern of the great cerebral vein. Anteriorly the corpus callosum contacts the crura of the forming fornix, the efferent portion of the hippocampal formation. The fornix travels a short distance along the inferior edge of the corpus callosum between the posterior fifth and posterior third until it descends away from the corpus callosum towards the mammillary bodies. As the fornix descends, the septum pellucidum, a paired membrane dividing the two lateral ventricles, appears. This septum continues along the concave inferior border of the anterior two thirds of the corpus callosum where ends at the lamina terminalis. This anterior edge of the lateral ventricles extends from the recurved anterior edge of the corpus callosum and continues inferiorly to the anterior commissure.

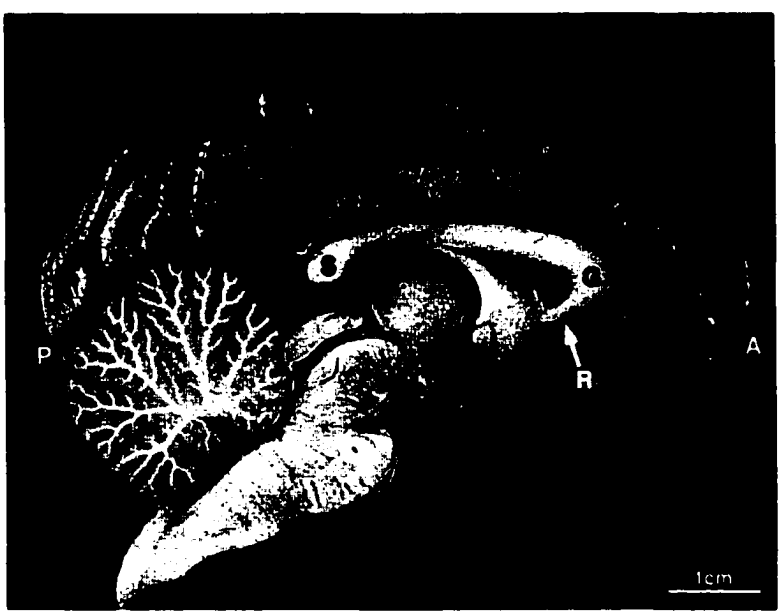

Fig. 2.1. Midsagittal view of the brain of *Macaca fascicularis*. A: anterior, P: posterior, S: splenium, G: genu

The corpus callosum has been traditionally parcelled into five regions. Although there are no anatomical or histological landmarks defining each region, they can be defined according to a straight rostrocaudal length, dividing the corpus callosum into thirds and fifths to delineate each region (Mall, 1909; de Lacoste and Holloway, 1982; Witelson, 1989; Aboitiz et al., 1992a,b). The different callosal regions defined by this method are (i) rostrum (anterior one-third); (ii) genu (area between the anterior one-fifth and anterior one-third); (iii) midbody (middle one-third); (iv) isthmus (area between the posterior one-third and posterior one-fifth); (v) splenium (posterior one-fifth) (Fig. 2.2). Further, some researchers (Aboitiz et al., 1992a) divide the midbody into an anterior midbody (area between the anterior one-third and one-half) and posterior midbody (area between the posterior one-half and one-third). Each of these areas minus the separate divisions of the midbody is described below.

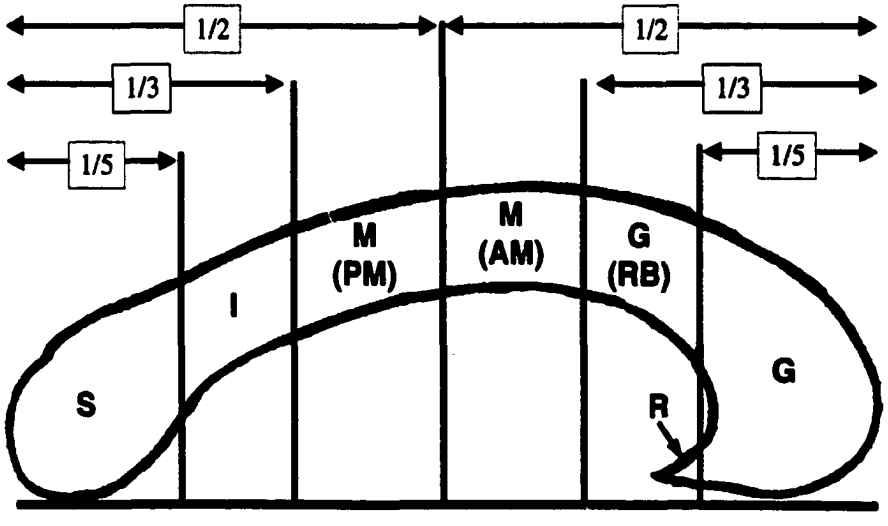

Fig. 2.2. Diagram of the midsagittal view of the corpus callosum of an adult human, showing the regional subdivisions. S: splenium, I: isthmus, M (PM): midbody, posterior midbody, M (AM): midbody, anterior midbody, G (RB): genu, rostral body, G: genu, R: rostrum. (after Witelson, 1989)

2.3.2 Regional anatomy of the corpus callosum

2.3.2.1 Rostrum

The rostrum represents the anterior-most fifth of the corpus callosum (Fig. 2.2, section R). This recurved section connects postero-inferioly with the lamina terminalis and antero-superiorly with the genu of the corpus callosum. It is attached superiorly with the septum pellucidum and forms a portion of the narrow floor of the anterior cornu of the lateral ventricle. Inferiorly and anteriorly this callosal division is bound by the cingulate gyrus.

2.3.2.2 Genu

The area between the rostrum and midbody (between the anterior third and anterior fifth) of the corpus callosum is the genu or knee (Fig. 2.2, section G). It represents the callosal portion closest to the frontal poles, and is bound anteriorly and superiorly by the indusium griseum and the cingulate gyrus. Inferiorly it is continuous with the septum pellucidum.

2.3.2.3 Midbody

The middle third of the corpus callosum is represented by the midbody (Fig. 2.2, section M). This sometimes narrow area is convex superiorly. As the floor of the longitudinal fissure, the midbody has cerebral vessels and the inferior border of the falx cerebri running over it. In addition the cingulate gyrus runs superiorly to it just lateral of the vessels and falx. Along its concave inferior surface the midbody is connected to the septum pellucidum, and occasionally it fuses posteriorly with the fornix.

2.3.2.4 Isthmus

Originally define by Bean (1906), the area between the posterior fifth and third of the corpus callosum is occupied by the isthmus (Fig. 2.2, section I). This narrowing between the midbody and the splenium is bound superiorly by cerebral vessels and falx cerebri. Just lateral, the isthmus is bound by the cingulate gyrus. Inferiorly it is connected to the crus of the fornix and fornix proper. Posterior to this connection the isthmus is covered by indusium griseum and forms part of the superior surface of the cistern of the great cerebral vein.

2.3.2.5 Splenium

The posterior fifth of the corpus callosum is the splenium (Fig. 2.2, section S). This section is covered by indusium griseum and sits over the cistern of the great cerebral vein. Along its inferior border it is attached anteriorly to the crus of the fornix. Superiorly cerebral vessels and the falx cerebri cover the splenium. In a dissected, midsagittally sectioned view the splenium is bound posteriorly and superiorly by the cingulate gyrus.

CHAPTER 3

METHODS, MEASUREMENTS AND RESULTS FOR TOTAL AND REGIONAL AREA OF THE CORPUS CALLOSUM IN *MACACA FASCICULARIS* AND *PAN TROGLODYTES*

3.1 CHAPTER OVERVIEW

The methods and results of the morphological midsagittal areas of the entire corpus callosum and its regions are discussed here. Section 3.2 discusses the means by which digital images were created, the justification for the use of image analysis software and the specific methodology used in measuring the corpus callosum in the sample as well as the need for standardizing the measurement results. Section 3.3 discusses the results of the measurements of total callosal area in each species and sex. Finally, section 3.4 presents the results of the measurements of the callosal regions (splenium, isthmus, midbody, genu and rostrum). In addition, section 3.4 also describes two methods used to divide the corpus callosum into regions.

3.2 DISCUSSION OF DIGITAL IMAGE ACQUISITION, SOFTWARE APPLICATION ANALYSIS AND STANDARDIZATION OF RESULTS

This section discusses the means by which digital images were created for software analysis. In section 3.2.2 the justification for the use of an image analysis software application is discussed, as well as an explanation for the particular software package chosen for the morphological aspect of this project. Finally, section 3.2.3 discusses the

justification and requirement for the standardization of measurements obtained from callosal areas to brain weight.

3.2.1 Photography and scanning of midsagittal view of the brain

Midsagittally sectioned brains of *Macaca fascicularis* and *Pan troglodytes* from Dr. Holloway's lab and the Smithsonian Institution were photographed as detailed in section 2.2.7. These photographic slides were scanned as gray-scale images on an AGFA Arcus II flatbed scanner at 800 dots per inch. The images were then saved as TIFF files to be analyzed using SigmaScan Pro software. Callosa of *Macaca fascicularis* from Dr. Gannon's collection were scanned directly on an AGFA Studio Star flatbed scanner as gray-scale images at 800 dots per inch. The methodology of this technique is described in section 2.2.7. MR images of the midsagittal area of the corpus callosum were stored as TIFF files. Measurements were made on these original files.

3.2.2 Overview of measurement software

SigmaScan Pro4.0 by SPSS Science is an image analysis software application designed to measure digital images. This particular software package was chosen because image analysis software offers advantages over traditional manual measurements. With traditional measurements the standard of error may be >2mm, depending on the angle at which the specimen is measured and/or the type and accuracy of the calipers used. With digital measurements the error is significantly reduced (i.e., <0.5mm). The reason for this variation is that while a hard tissue physical specimen has unalterable dimensions, the dimensions of a soft tissue specimen can suffer alteration

when measured with rigid calipers. Moreover, a digital image can be enlarged or reduced as desired to either examine features more closely or to examine the image from a different range. Also, while the accuracy of the calipers is determined by the manufacturer and the ability of the calipers to maintain that accuracy over time, the accuracy of the SigmaScan Pro software is constant. This is because the software performs its measurements according to pixels. Thus, the more pixels in an image the more accurate the measurements on that image will be. Finally, the software measures an image whose consistency remains unchanged. These points will be discussed in more detail below.

SigmaScan Pro uses the pixel dimensions of an object for measurements. While the raw data of a measurement is in the form of binary pixels, the final linear distance, area, or volume of an object can be presented in a variety of formats. Since previous studies on the corpus callosum have presented areas in centimeters (e.g., de Lacoste and Holloway, 1982; Holloway et al., 1993), this unit of measurement was chosen for this study. The Standardize Toolbar feature in SigmaScan Pro was used to determine how many pixels are in a centimeter for each image. For example, with most of the images analyzed in this study 1cm was equivalent to approximately 100 pixels. Another advantage of the pixel measurement feature of the software is that with the raw data stored as pixels, it is possible to return to the data and perform future analyses regarding area dimensions without discarding the original data or re-measuring the corpus callosum. In addition, the measurement accuracy of SigmaScan Pro affirms the highly reproducible nature of the data.

Measurements on the corpus callosum present a special problem not associated with some other morphological measurements. Until recently with refinements in the field of morphometrics (e.g., Bookstein, 1997; Thompson et al., 2000), researchers relied on linear measurements to obtain comparative data. Although these measurements still provide valuable comparative morphological data (e.g., Broadfield et al., 1999), these linear measurements are not useful for all aspects of morphology. For example, Bookstein et al. (1999) and Prossinger et al. (2000) demonstrated that curvilinear relationships utilizing Cartesian coordinates and Procrustes analysis provide important data unobtainable through traditional linear measurements. While comparing the area of the corpus callosum between sexes need not involve such complex analyses, morphometric analyses on complex curvilinear structures like the skull indicate that mere linear measurements of length and height do not provide sufficient data to suggest the presence or lack of sex differences in an equally dynamic structure, the corpus callosum.

The midsagittal profile of the corpus callosum presents a continuous structure with a complex shape, making anatomical dissection into regions difficult (see section 2.3). The rostrum often recurves under the genu in human and nonhuman primates, making strict linear measurements via rigid calipers impracticable. Since area data on the corpus callosum must be compared not only in terms of total area but also in terms of regional areas, area measurements derived from linear measurements would not be as reliable as data derived from SigmaScan Pro. This is because SigmaScan Pro can measure adjacent regions of the corpus callosum without overlap. The tip of high quality needle calipers (if used to measure an 800dpi digital image) would occupy approximately 10 pixels, leaving a possible error of 20 pixels or more for any given measurement. If this error persists

across the regional measurements of the corpus callosum, a 100 pixel error could result. SigmaScan Pro on the other hand offers the ability of automated measurements. If the edge of the structure to be measured is well defined, SigmaScan Pro can perform an automated area measurement with virtually no error in the number of pixels measured away from the true edge of the object. Moreover, the measurements can be obtained on a soft-tissue structure like the corpus callosum without the damage that occurs in soft-tissue specimens measured with calipers. While the error discussed above may be acceptable for a large object such as a modern human skull, it is unacceptably significant for a small object such as the corpus callosum, which in *Macaca fascicularis* may cover an area of less than $1.0cm^2$.

In the auto-tracing mode SigmaScan Pro relies on a distinct difference between the pixel intensity of the object to be traced and the surrounding tissue. While measurements made with SigmaScan Pro are more accurate than those made with calipers due to the auto-tracing feature of the program, the auto-tracing technique was not without limitations. Occasionally, SigmaScan Pro would encounter trouble when tracing the edge of the corpus callosum. In these situations, which occurred only with the images derived from the *Macaca* and *Pan* samples located in Dr. Holloway's and the Smithsonian collection, SigmaScan Pro would be unable to locate reliably the edge of the corpus callosum. In such a case the program would cease auto-tracing at the pixel where it last identified an edge point. This situation occurred in instances where subtle differences in gray-scale color occur between the edge of the corpus callosum and the surrounding tissue, or a line was drawn through the corpus callosum to demarcate a particular region. When this scenario occurs, the edge tracing is continued manually until a point is reached

along the edge of the corpus callosum where SigmaScan Pro can differentiate between the edge of the structure and the surrounding tissue. To continue the tracing manually the image was enlarged to 300 percent, so that the next pixels corresponding to the edge could be located and the tracing line continued along them. At no time was it difficult to distinguish visually between the edge of the corpus callosum and the surrounding tissue.

The choice of SigmaScan Pro for the total area and regional area measurements of the corpus callosum in the specimens sampled was made so that reliable and accurate measurements could be obtained on this complex soft tissue structure. While another software program was available at the time this project was undertaken (e.g., NIH Image), the version available lacked the features and ease-of-use available in SigmaScan Pro. SigmaScan Pro was chosen because i) it is specifically designed to perform morphological measurements; ii) it is user-friendly; iii) measurements can be layered and stored as separate layers; and iv) it produces data that can be read by SPSS, SigmaStat, and Excel. In addition, the choice of using a software package like SigmaScan Pro was made in order to have a permanent record of those callosa sectioned for histology, so that they could be available for future data collection and analysis.

3.2.3 Standardization of area measurements to brain weight

As discussed in section 2.2.1 on the choice of species, *Macaca fascicularis* and *Pan troglodytes* were chosen for this study because both are sexually dimorphic species of primates. A problem, however, in performing sex comparisons of sexually dimorphic species is taking into account the dimorphism displayed. For example, a comparison of brain size between males and females of a given species in which males are much larger

than females (e.g., *Macaca* sp.) would demonstrate that the brains of males are larger than females. The results of such a comparison would be confounded by the lack of consideration for allometry.

Jerison (1973) suggested that brain size increases with body size when individual specimens from different taxonomic orders are compared. Jerison suggested that the slope of the log/log plot of brain weight and body weight is .666. This slope, however, is derived from a sampling of mammalian taxa. Due to this broad derivation of Jerison's slope, others have attempted to refine his slope for finite groups, such as primates or hominins. For example, Bauchot and Stephan (1969) suggested a slope of .657 for strepsirhines and .58 for pongids, while Martin (1982) proposed a slope of .75 for mammals based on body weight. A slope of .65 was proposed by Holloway and Post (1982) for modern humans. However, their slope was only used to provide encephalization quotients in hominins and not as an allometric approach for all primates. Despite the mere coincidental nature for the causes of his surface dependent exponent (Gould, 1975; Holloway and Post, 1982; Martin, 1982), Jerison's (1973, 1982) original slope of .666 has generally been used to account for allometric differences between taxa, groups, or sexes within a species when comparing brain size. Moreover, while Holloway and Post (1982) and Martin (1982) among others disagree with in Jerison's slope, they nevertheless recognize its usefulness as a standard measurement for scaling of brain size measurement transformations.

With the general acceptance of Jerison's allometric slope (.666) for brain size comparisons, it is remarkable that many investigators would ignore the need for scaling or standardizing measurements on the brain when comparing sex, groups, or taxa. In an

examination of the presence or absence of sex differences in the corpus callosum in rats, Fitch and Denenberg (1998) declared that there is no need to standardize their results to relative brain size. The logic in their argument echoes the opinion taken by others investigating sex differences in the corpus callosum (e.g., Witleson, 1985; Weber and Weis, 1986; Yoshii et al., 1986; Kertesz et al., 1987; Oppenheim et al., 1987; Byne et al., 1988; Demeter et al., 1988; Elster, 1990; Going and Dixson, 1990; Steinmetz et al., 1992; Bishop and Wahlsten, 1997). Fitch and Denenberg (1998) contend that since there is not a statistically significant correlation between brain size and the size of the corpus callosum and their factor analysis failed to load brain weight and callosal area on a combined factor, there is no need to standardize or "correct" callosal measures by some measure of brain size in males and females. In addition, they note that most other studies have failed to report a correlation between corpus callosum size and any measure of brain size[1].

The argument for not using relative measures of the corpus callosum as demonstrated by Fitch and Denenberg (1998) among others is essentially flawed (see reply by Holloway, 1998). The basic tenet of standardizing measurements of the brain or its constituents is that one must account for allometric differences. While investigators such as Jerison (1973), Martin (1982), Holloway and Post (1982) and Peters et al. (1998) may disagree about the constant used to standardize brain measurements, all agree that some relative measurement is necessary. In essence, once the presence of allometric differences are acknowledged, then it is not possible to discard the use of relative

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[1] Although it was not a necessary criterion for choosing to standardize the measurements of the corpus callosum in this study, the correlation for both males and females between total callosal area and brain weight for *Macaca fascicularis* is .511 ($p \leq 0.05$), and .713 ($p \leq 0.01$) for *Pan troglodytes*.

measures. Fitch and Denenberg (1998) argue that IQ and spatial ability are not correlated to brain size. While this may be true it ignores the fact that the corpus callosum, unlike these cognitive measurements, contributes to brain size. Due to this relationship between brain size and corpus callosum size, relative measures of the corpus callosum for comparison between males and females were used in this study.

Absolute measurements on the total callosal area and the areas of its regions were recorded. In addition, statistical calculations were performed using absolute measurements. The final analyses of the areas calculated on the corpus callosum and the conclusions drawn from these analyses were, however, conducted using only standardized measurements. Measurements on the corpus callosum were standardized according to the following formula:

$$(\text{CC measure})/(\text{Brain Weight})^{2/3} \qquad (1)$$

3.3 MEASURES OF TOTAL AREA OF THE CORPUS CALLOSUM IN *MACACA FASCILUARIS* AND *PAN TROGLODYTES*

This section presents the results obtained for the total area of the corpus callosum as well as the results of the regional areas of the corpus callosum for both *Macaca fascicularis* and *Pan troglodytes*. Measurements on callosal regional areas were performed using two separate methods, the straight-line method and the radial-line method. These methods are discussed in section 3.4.1.

3.3.1 Results and analysis of the total midsagittal area of the corpus callosum in *Macaca fascicularis*

This section presents the results for the measurements of total midsagittal area of the corpus callosum for *Macaca fascicularis*. Section 3.3.1.1 presents the absolute area values obtained. Section 3.3.1.2 presents the standardized values for the total area measurements given in the preceding section. Finally, section 3.3.1.3 presents the statistical results for the standardized values.

3.3.1.1 Total area of the corpus callosum

The total area of the midsagittal profile of the corpus callosum was measured in *Macaca fascicularis* (n = 40; 20 females, 20 males) using SigmaScan Pro (Fig. 3.1). Using the methods described above in section 3.2.2, the edge of the corpus callosum was located on digital images. Once the edge of the corpus callosum was defined within the software, SigmaScan Pro could then outline the entire corpus callosum automatically. From this outline an area measurement (cm^2) of the structure was made. Below (Table 3

- 1) are the absolute values for the total area of the corpus callosum for each individual measured within *Macaca fascicularis*.

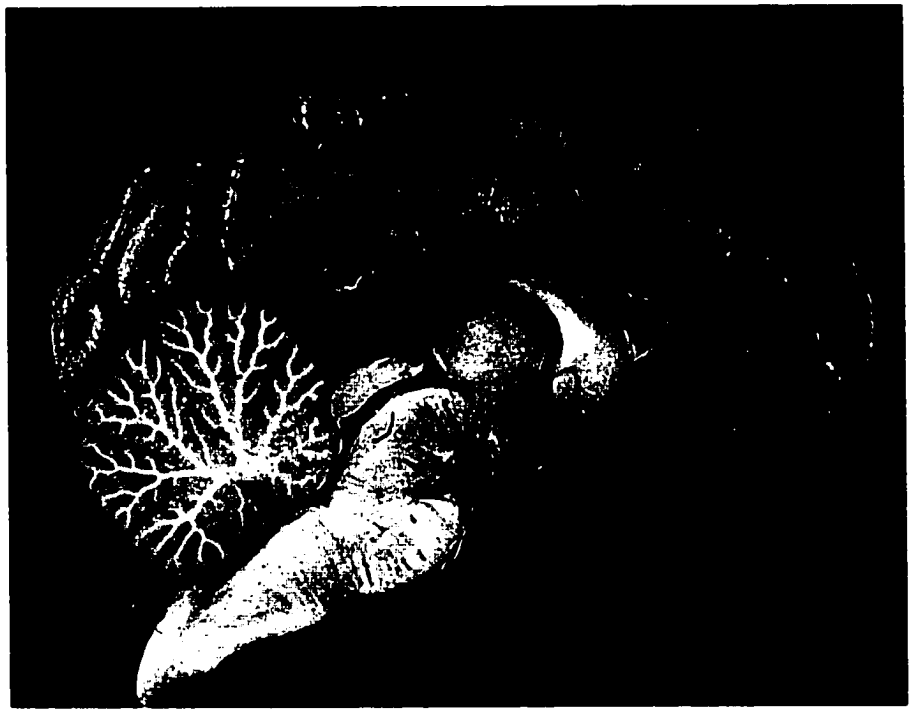

Fig. 3.1. Midsagittal view of the brain of *Macaca fascicularis*. Highlighted area represents the total area of the corpus callosum as measured with SigmaScan Pro.

For total callosal area the mean size of the corpus callosum for males is 0.793cm^2 ± 0.273(S.D.) (Coefficient of variation, c.v., 34.4) and 0.76 ± 0.204cm^2 (c.v. 26.8) for females. In males the size of the corpus callosum ranged in size from 0.479cm^2 to 1.55cm^2, while in females the range was from 0.462cm^2 to 1.17cm^2. In general the midsagittal area of the corpus callosum is larger in males than females.

Table 3 – 1. Absolute values for total area of the corpus callosum in *Macaca fascicularis* (cm^2)

Female	Male
.46173013	.47910062
.51504199	.49625927
.52987046	.50805143
.55034786	.52711660
.58438272	.56432898
.60549563	.56482327
.63938926	.61841757
.66007850	.64433208
.67653103	.68542811
.67766082	.70534062
.73149412	.75972941
.74464269	.83650734
.83516421	.88894140
.91752538	.89081123
.94311339	.89710884
.94328799	1.02465986
.96306479	1.05479827
1.01760000	1.07492548
1.02504726	1.09348121
1.17668055	1.55108931
Avg. = 0.76 ±0.2	Avg. = 0.79 ±0.3

3.3.1.2 Relative area measurements

In order to identify the presence of sex differences in the corpus callosum, it is necessary to first standardize the absolute values of the obtained measurements as described in section 3.2.3. Individual measurements of total callosal area were standardized using equation (1). Below (Table 3 - 2) are the standardized values of the total area of the corpus callosum for each individual measured within *Macaca fascicularis*.

As noted above in section 3.3.1.1 the corpus callosum is larger absolutely in males than females. However, when the measurements are standardized to yield the relative size of the corpus callosum for each specimen, females are seen to have relatively larger callosa. For females the relative size of the corpus callosum is $0.0477 \pm 0.0077 cm^2$ (c.v. 16.2), and in males the relative size of the corpus callosum is $0.0459 \pm 0.01 cm^2$ (c.v. 22.2). Thus, it would appear that while females have smaller brains on average compared to males, the corpus callosum in females is closer in relative size to males rather than smaller.

Table 3 – 2. Relative values for total area of the corpus callosum in *Macaca fascicularis*

Female	Male
.03440701	.03243246
.03972747	.03384753
.04033441	.03552340
.04108193	.03671117
.04116706	.03807058
.04387734	.04097091
.04396500	.04121633
.04464418	.04239051
.04473937	.04291120
.04533366	.04424706
.04664984	.04572146
.04686371	.04580840
.04905960	.04645383
.05029308	.04684863
.05035828	.04875888
.05074195	.05188078
.05555857	.05386838
.05765491	.05674617
.06056958	.05698836
.06631083	.07687000
Avg. = 0.048 ±0.008	Avg. = 0.046 ±0.01

3.3.1.3 Statistical analysis of sex versus relative callosal area

The *Macaca* sample was analyzed statistically using a Student's t-test for significant differences of the mean in order to determine the presence or lack of sex differences in the total area of the corpus callosum. The sample did not show statistically significant sex differences with regard to the absolute values of total callosal area or relative callosal area (Table 3 – 3). For absolute callosal area $p = 0.664$ ($t = -0.438$) and for relative callosal area $p = 0.542$ ($t = 0.615$). With the values for p being so large for total callosal area, it can be assumed with certainty that there are no sex differences in the total area of the corpus callosum in *Macaca fascicularis*. However, despite this lack of sex difference, regional sex differences may occur in the corpus callosum in this species. In order to elucidate the presence or lack of sex differences in specific portions of this structure, regional areas were also taken. The results of the measurements are presented below in section 3.4.

Table 3 – 3. Statistical results of sex versus absolute and relative callosal area for *Macaca fascicularis*

Measurement	t	Sig.
Total Area (absolute) Corpus Callosum	-0.438	0.664
Total Area (relative) Corpus Callosum	0.615	0.542

n = 40 (20 females, 20 males)
The correlation between the absolute area of the corpus callosum and brain size is $R^2 = 0.874$ ($p \leq 0.01$).

3.3.2 Results and analysis of the total midsagittal area of the corpus callosum in *Pan troglodytes*

This section presents the results for the measurements of total midsagittal area of the corpus callosum for *Pan troglodytes*. Section 3.3.2.1 presents the absolute area values obtained. Section 3.3.2.2 presents the standardized values for the total area measurements given in the preceding section. Finally, section 3.3.2.3 presents the statistical results for the standardized values.

3.3.2.1 Total area of the corpus callosum

The total area of the midsagittal profile of the corpus callosum was measured in *Pan troglodytes* (n = 23; 12 females, 11 males) using SigmaScan Pro (Fig. 3.2). The area of the corpus callosum was obtained via SigmaScan Pro using the methodology outlined above. From the outline of the corpus callosum created within the software package an area measurement (cm^2) of the structure was made. Below (Table 3 - 4) are the absolute values for the total area of the corpus callosum for each individual measured within *Pan troglodytes*.

Fig. 3.2. Midsagittal view of the brain of *Pan troglodytes*. Highlighted area represents the total area of the corpus callosum as measured with SigmaScan Pro.

For total callosal area the mean size of the corpus callosum is 2.38cm^2 ± 0.526 (S.D.) (c.v. 22.1) for females and 2.29 ± 0.46cm^2 (c.v. 20.1) for males. In females the range ranged in size from 1.37cm^2 to 3.03cm^2, while in males the size was size from 1.35cm^2 to 2.93cm^2. The average brain weight for males was 317.78 ± 61g (c.v. 19.3), while females have slightly smaller brains averaging 312.49 ± 53g (c.v. 16.9). Thus, while males have slightly larger brains, females, in general, have slightly larger callosa than males.

Table 3 – 4. Absolute values for total area of the corpus callosum in *Pan troglodytes* (cm^2)

Female	Male
1.371251	1.356367
1.529582	1.700965
2.022592	2.037085
2.298983	2.218107
2.336420	2.235781
2.336504	2.414412
2.519342	2.420927
2.563100	2.479424
2.736626	2.660975
2.810452	2.765161
2.997737	2.927467
3.028125	
Avg. = 2.38 ±0.5	Avg. = 2.29 ±0.46

3.3.2.2 Relative area measurements of the corpus callosum

As with *Macaca* the measurements on the corpus callosum in *Pan* were standardized according to brain weight to produce relative measures as outlined in section 3.2.3. The mean relative size of the corpus callosum in *P. troglodytes* females is 0.0517cm^2 ± 0.0089(S.D.) (c.v. 17.2) and 0.0491 ± 0.005cm^2 (c.v. 10.1) for males (Table 3 – 5). These results indicate that females have an absolutely and relatively larger corpus callosum than males on average. These results differ from those obtained for *Macaca* where females exhibit relatively large callosa than males. The significance of this result is discussed below.

Table 3 – 5. Relative values for total area of the corpus callosum in *Pan troglodytes*

Female	Male
.036099	.040203
.039177	.041248
.046546	.046539
.047794	.048465
.048514	.048773
.050432	.049798
.054034	.051066
.054634	.051704
.054746	.052221
.058461	.054161
.063772	.056328
.065995	
Avg. = 0517±0.0089	Avg. = 0.0491±0.005

3.3.2.3 Statistical analysis of sex versus callosal area measurements

While female common chimpanzees may exhibit both absolutely and relatively larger callosa than males on average, a statistical Student's t-test indicates that the degree of difference is not statistically significant (Table 3 – 6). With regard to the absolute values of callosal area $p = 0.680$ ($t = 0.419$). For relative total callosal area $p = 0.413$ ($t = 0.836$). These statistical results indicate that despite the presence of sex differences in the values of the average size of the corpus callosum, both absolutely and relatively, there is not sufficient statistical evidence to suggest that sex differences in the corpus callosum exist within *Pan troglodytes*. However, the corpus callosum is a complex structure, making the presence of sex differences within certain regions of the corpus callosum

possible. As such regional areas of the corpus callosum were measured and compared. These results are described below.

Table 3 – 6. Statistical results of sex versus absolute and relative callosal area for *Pan troglodytes*

Measurement	t	Sig.
Total Area (absolute) Corpus Callosum	0.419	0.680
Total Area (relative) Corpus Callosum	0.836	0.413

n = 23 (12 females, 11 males)
The correlation between the absolute area of the corpus callosum and brain size is R^2 = 0.796 ($p \leq 0.01$).

3.4 MIDSAGITTAL REGIONAL AREA MEASUREMENTS OF THE CORPUS CALLOSUM IN *MACACA FASCICULARIS* AND *PAN TROGLODYTES*

This section presents the results of regional area measurements in the corpus callosum of *Macaca fascicularis* and *Pan troglodytes*. As explained above, the lack of sex differences in the total area of the corpus callosum does not dictate that sex differences may not exist in certain regions of this structure (e.g., Holloway et al., 1993). Because the corpus callosum lacks landmarks, which allow for it to be divided into discrete regions, the corpus callosum first had to be divided into sections which could then be measured. The techniques used to divide the corpus callosum are described in section 3.4.1. These techniques yielded regions of the corpus callosum (i.e., the rostrum, genu, midbody, isthmus and/or splenium). After each region was demarcated using one of the

two methods described in section 3.4.1, its area was subsequently measured. The regional area measurements for *Macaca fascicularis* (n = 40; 20 females, 20 males) are described in section 3.4.2 for the straight-line method and section 3.4.3 for the radial-line method. Regional area measurements for *Pan troglodytes* (n = 23; 12 females, 11 males) are described in section 3.4.4 for the straight-line method and section 3.4.5 for the radial-line method.

3.4.1 Methods for measurement of regional areas of the corpus callosum

As discussed in section 2.3.2, the corpus callosum can be divided into regions. These include the rostrum, genu, midbody, isthmus and splenium. The designation of these regions, however, is complicated by the lack of morphological or anatomical landmarks defining each region. In general, the regions of the corpus callosum have been identified by which portion of the structure they occupy. The basic model that has been used with humans is as follows: splenium (posterior 1/5), genu (the area between the anterior 1/3 and the anterior 1/5), rostrum (anterior 1/5), midbody (middle 1/3), and isthmus (the area between the posterior 1/3 and posterior 1/5) (Aboitiz et al., 1992a). While these basic divisions allow for the designation of regional areas of the corpus callosum, the method used for dividing the corpus callosum has not been agreed upon by all investigators.

While early and many current investigators have divided the corpus callosum using the proportions described above, other variations on this methodology have been used. Mall (1909) first described the methodology for dividing the corpus callosum into regions using equal divisions. This methodology was later refined by Witelson (1989) to depict the origins of the fibers through the corpus callosum through the use of perpendicular

divisions. In her design Witelson (1989) first identifies the most anterior (ACC) and posterior (PCC) points on the corpus callosum. From the distance derived from these points the midpoint of the corpus callosum can be defined. Once these three points have been established the corpus callosum may now be divided into separate regions. Under Witelson's (1989) methodology the regions of the corpus callosum may be defined according to perpendiculars dropped through the ACC – PCC axis (Fig. 3.3). In this case the splenium is defined as the posterior one-fifth, and the isthmus is the area between the posterior one-third and the posterior one-fifth. The midbody is divided into anterior and posterior portions with the posterior portion occupying the area between the perpendicular demarcating the midpoint of the corpus callosum and the posterior one-third. The anterior midbody is the area between the midpoint and the anterior one-third. In Witelson's (1989) design the dividing the anterior corpus callosum is more complicated in that the designations are somewhat arbitrary. For example, the area between the anterior one-third and the anterior one-fifth minus the rostrum is occupied by the rostral body, a region mentioned only by Witelson. The genu is represented as the area occupying the anterior one-fifth minus the rostrum, while the rostrum is defined as the inferior extension of the anterior portion of the corpus callosum.

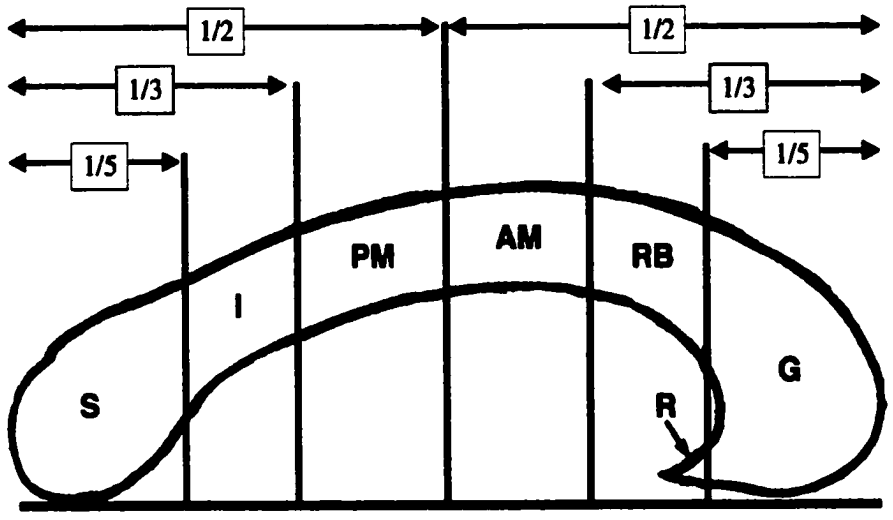

Fig. 3.3. Diagram of the midsagittal view of the corpus callosum of an adult human, showing the regional subdivisions. S: splenium, I: isthmus, PM: midbody, posterior midbody, AM: midbody, anterior midbody, RB: genu, rostral body, G: genu, R: rostrum. (after Witelson, 1989)

Unlike other callosal regions that were defined by proportional divisions along the ACC – PCC line, the areas occupied by the rostral body, genu, and rostrum are somewhat more arbitrary. The area of these regions is dependent upon the position of point G, which Witleson (1989) describes as the most anterior point of the inner convexity of the anterior corpus callosum. Through this point a line is drawn perpendicular to the ACC – PCC line. The posterior border of this line defines the anterior border of the rostral body along the main body of the corpus callosum, and the rostrum along the recurved portion of the callosum. The anterior border of this line is the posterior border of the genu.

Witelson (1989) proposed this scheme as a way to subdivide the corpus callosum relative to cortical regional connections (Table 3 - 7). As discussed in section 1.3.3, though, the cortical maps derived from callosal connections demonstrate considerable overlap of fiber pathways in the corpus callosum (de Lacoste, 1981). That Witelson

(1989) admits that such considerable overlap occurs, means that arbitrary division of the anterior corpus callosum may yield no better results than traditional proportional divisions. In addition, the application of Witelson's scheme to other species is complicated by the range of variation in callosal morphology seen in other mammals.

Table 3 – 7. Callosal regions and related cortical connections

Region	*Anatomical label*	*Cortical region*
1	Genu/rostrum	Prefrontal, inferior premotor
2	Rostral body	Premotor, supplementary motor
3	Anterior midbody	Motor
4	Posterior midbody	Somaesthetic, posterior parietal
5	Isthmus	Superior temporal, posterior parietal
6	Splenium	Occipital, inferior temporal

After Witelson. 1989

In humans the anterior portion of the corpus callosum often recurves under the structure. Such a morphology permits the division of the corpus callosum into regions as described by Witleson (1989). Other mammals including nonhuman primates often lack a distinctly recurved rostrum, or the presence of a recurved rostrum is variable within a given species. Thus, while it may be possible to apply Witelson's scheme to the species studied here, the results would be inconsistent. This is because a distinct recurved rostrum as seen in most modern humans is lacking in *Pan troglodytes* and *Macaca fascicularis*. Due to the variability in the shape of the anterior portion of the corpus callosum in these species, Witelson's scheme was abandoned for a methodology that divides the corpus callosum more simply and into consistent proportions.

Since there is overlap of fiber pathways through the corpus callosum, there is no method for subdividing this structure which would yield regions that correspond to specific fiber pathways. Instead the corpus callosum may be subdivided into regions which merely approximate certain fiber pathways. With the inapplicability of Witelson's (1989) scheme to the species examined here, the corpus callosum was divided using simple proportions. Since Witelson (1989) proposed her methodology for defining callosal regions, one other method (radial-line) for subdividing the corpus callosum has been proposed, as well as a simplified model of Witelson's scheme. Since investigators have used one, the other, or both of these schemes, both schemes were used here. In the first method the corpus callosum is subdivided using perpendicular lines drawn at certain positions along the ACC – PCC line. This will be called the straight-line method. In the second scheme the corpus callosum is subdivided using radial lines drawn at certain proportions. This is known as the radial-line method. Both methods area described below.

3.4.1.1 Straight-line method

The straight-line method here is similar to that used by Witelson (1989) with the exception that the anterior regions are not defined according to a perpendicular line drawn through the most anterior point of the inner convexity of the anterior corpus callosum. Instead for the method used here each region was defined by perpendiculars drawn at fifths through the corpus callosum (Fig. 3.4). The regions are specifically defined as follows. The perimeter of the corpus callosum was defined and the anteriormost (ACC) and posteriormost (PCC) points of the corpus callosum were identified. The ACC and PCC were connected by a line. From this line perpendiculars to the ACC – PCC line were drawn tangent to the ACC and PCC. After the anterior and posterior verticals were drawn, the ACC – PCC line was carried tangent to the ventral-most points of the corpus callosum (i.e., ventral to the splenium and rostrum), so that this line would not interfere with subsequent measurements. With the ACC – PCC line in its new position the distance between the ACC and PCC was calculated and the corpus callosum was divided into five regions by constructing four equidistant lines perpendicular to the horizontal line. The splenium (1) is indicated as the most posterior region. The isthmus (2) is defined as the region just anterior to the splenium. The midbody (3, 4) is divided into a posterior and anterior midbody where the posterior midbody (3) is that region just anterior to the isthmus. The anterior midbody (4) then is defined as that region anterior to the posterior midbody. Finally the most anterior region is defined here as the genu (5), although under this scheme it is comprised of both the genu and rostrum.

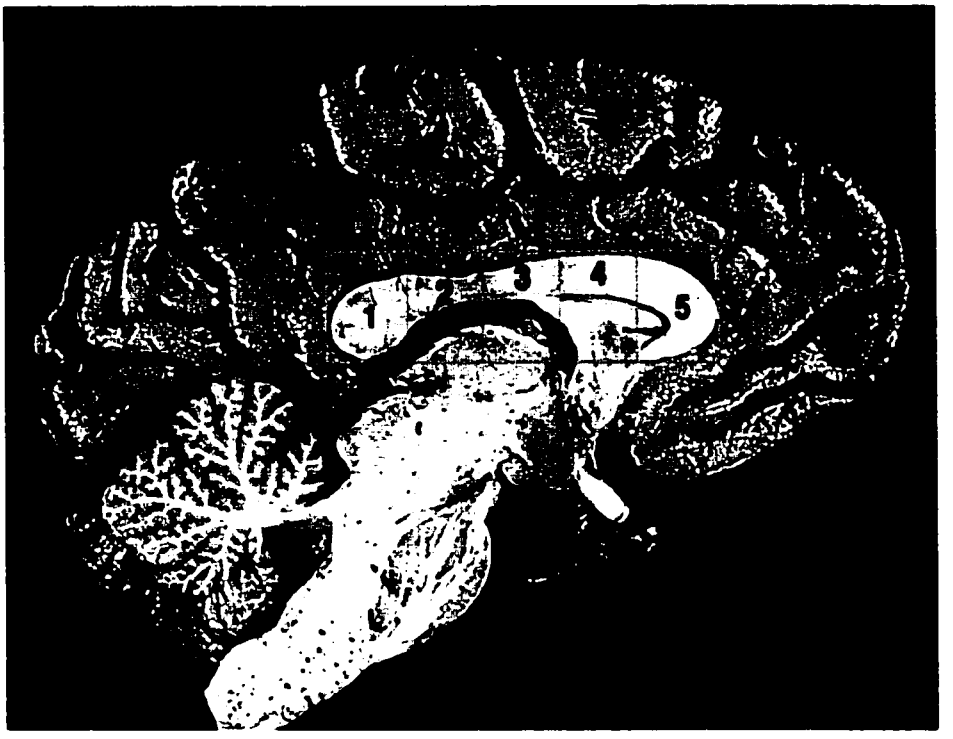

Fig. 3.4. Midsagittal view of the brain of *Pan troglodytes*, showing the straight-line method of callosal division. 1: splenium, 2: isthmus, 3: posterior midbody, 4: anterior midbody, 5: genu.

3.4.1.2 Radial-line method

A second method for defining regions of the corpus callosum is the radial-line method (O'Kusky et al., 1988; Hynd et al., 1991; Rajapakse et al., 1996). Under this method the corpus callosum is subdivided into 6 regions (Fig. 3.5). As with the straight-line method the first step in the radial-line method is to define the anteriormost (ACC) and posteriormost (PCC) ends of the corpus callosum. Once ACC and PCC are defined a line is drawn connecting the two points. This line is carried below the corpus callosum so that it is tangent to its ventral border of the splenium and rostrum. Next the distance of this line is measured and the midpoint of this line defined. From this midpoint six callosal regions are demarcated by rotating the ventral line every 30° from 0° through

180° using the midpoint of the ACC – PCC line as the origin of the rotation. From this the splenium (1) is defined as the posteriormost region, while the isthmus (2) is defined as the region just anterior to the splenium. The midbody is divided into an anterior and posterior midbody with the posterior midbody (3) being that region just anterior to the isthmus. The rostral body (5) sits just anterior to the anterior midbody (4), and the genu/rostrum (6) represents the anteriormost region of the corpus callosum. As noted above the genu as defined here includes the genu and the rostrum, since the lack of recurvature of the rostrum in *Macaca fascicularis* and *Pan troglodytes* prevents reasonable means of identifying it as a separate region.

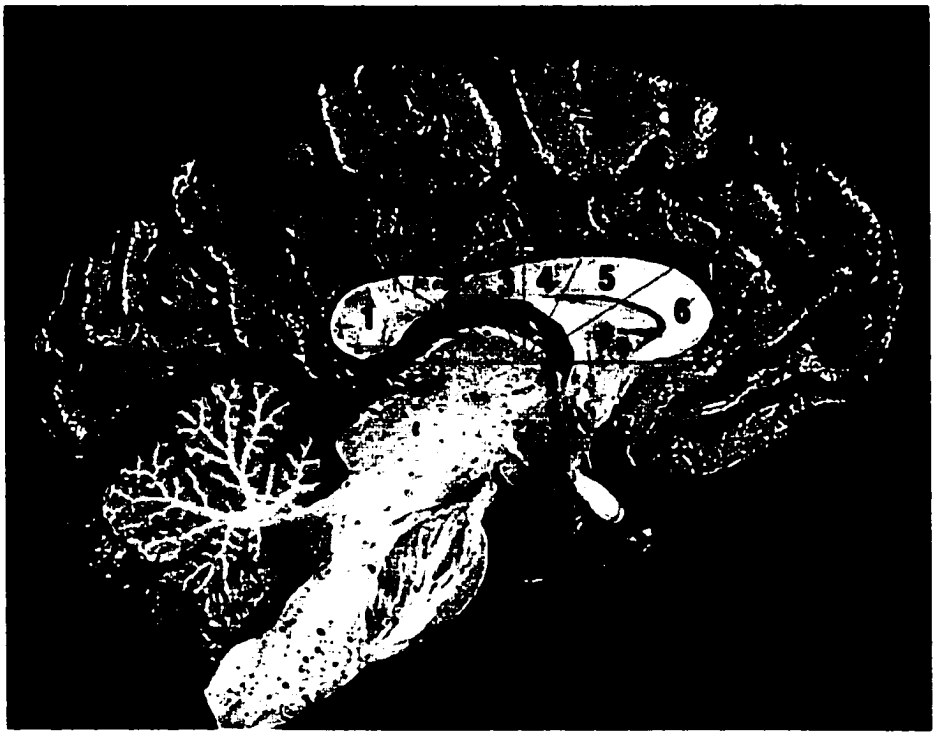

Fig. 3.5. Midsagittal view of the brain of *Pan troglodytes*, showing the radial-line method of callosal division. 1: splenium, 2: isthmus, 3: posterior midbody, 4: anterior midbody, 5: rostral body, 6: genu/rostrum.

3.4.2 Straight-line measures of regional areas of the corpus callosum in *Macaca fascicularis*

Once the regions of the corpus callosum had been defined using the straight-line method (Fig. 3.6) in each of the specimens used in this study, area measurements of each region were conducted, using SigmaScan Pro. These regions were then separately compared for the presence or lack of sex differences in *Macaca fascicularis*. Below are the results of these measurements for each region. The statistical results for the raw and standardized measurements of each area are discussed in section 3.4.2.6.

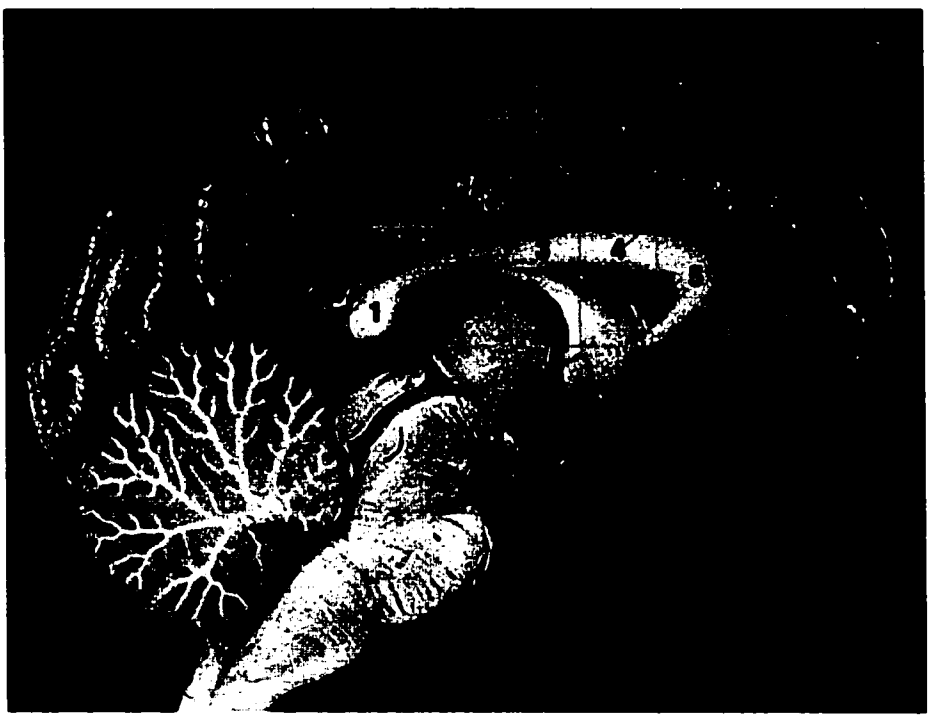

Fig. 3.6. Midsagittal view of the brain of *Macaca fascicularis*, showing the straight-line method of callosal division. 1: splenium, 2: isthmus, 3: posterior midbody, 4: anterior midbody, 5: genu.

3.4.2.1 Splenium

Using the straight-line measurement of dividing the corpus callosum the splenium is identified as the posteriormost region (Fig. 3.6 , section 1). Areas of the splenium measured using SigmaScan Pro yield a mean area for females of 0.167 ± 0.046cm^2 with a range between 0.1cm^2 and 0.26cm^2, and 0.186 ± 0.063cm^2 for males with a range between 0.098cm^2 and 0.28cm^2 (Table 3 - 8). In this case males possess absolutely larger splenia on average. However, when the splenial areas have been standardized for brain weight any sex difference in the area of the splenium virtually disappears. Standardized measurements show that the average size of the splenium in males is 0.0108 ± 0.002cm^2 and 0.0105 ± 0.0024cm^2 in females (Table 3 - 9).

Table 3 – 8. Splenial area using straight-line method for *Macaca fascicularis* (cm^2)

Female	Male
.10119342	.09843938
.10338253	.10084034
.11002048	.11955370
.12216651	.12273145
.13191159	.12654474
.13473625	.13699597
.14087988	.13967940
.14172728	.14278653
.14434009	.14455194
.15295530	.16806723
.16800403	.19908795
.17257534	.21195652
.18966033	.21503123
.19268499	.22260669
.20352274	.24631519
.21357341	.25033939
.21644612	.26473923
.21800000	.26913989
.22169944	.26922514
.26293873	.28202807
Avg. = 0.167 ±0.046	Avg. = 0.186 ±0.063

Table 3 – 9. Relative splenial area using straight-line method for *Macaca fascicularis* (cm^2)

Female	Male
.00754069	.00687785
.00773321	.00729889
.00858827	.00798402
.00861602	.00872408
.00930093	.00891671
.00938421	.00901294
.00957809	.00913959
.00973371	.00933139
.01010196	.00990777
.01020422	.01073647
.01030277	.01091514
.01034655	.01107631
.01059530	.01142543
.01135350	.01204283
.01183562	.01240650
.01220794	.01298991
.01232091	.01338751
.01235139	.01391786
.01278970	.01448381
.01481769	.01469830
Avg. = 0.0105 ± 0.0024	Avg. = 0.0108 ± 0.002

3.4.2.2 Isthmus

The isthmus is the region just anterior to the splenium (Fig. 3.6, section 2). Area measurements on the isthmus as defined using the straight-line method yield an average area of 0.12 ± 0.038cm^2 (range 0.07cm^2 to 0.2cm^2) for females and 0.12 ± 0.04cm^2 (range: 0.06cm^2 and 0.18cm^2) for males (Table 3 - 10). Therefore, with regard to absolute measurements there is no apparent difference between males and females in this group. Once the areas are standardized, however, females display a relatively larger

isthmus on average. For females standardized measurements yield an average isthmus area of 0.0076 ± 0.002cm^2, while for males standardized measurements yield an average isthmus area of 0.0069 ± 0.0016cm^2 (Table 3 – 11). This would indicate that females have a relatively larger isthmus compared to males.

Table 3 – 10. Isthmus area using straight-line method for *Macaca fascicularis* (cm^2)

Female	Male
.06913354	.06150696
.07824306	.06376668
.07993786	.06757997
.08368053	.07676012
.08488101	.07690135
.10084034	.08481039
.10366500	.09476732
.10437116	.09603842
.10654307	.09914554
.10952616	.11870631
.11001948	.12584460
.11435655	.13949868
.11694089	.14035917
.13530303	.14188166
.15249433	.14455978
.15588621	.14597806
.16673172	.15929705
.16780000	.17107750
.17982042	.18018154
.20464010	.18469896
Avg. = 0.12 ± 0.038	Avg. = 0.12 ± 0.04

Table 3 – 11. Relative isthmus area using straight-line method for *Macaca fascicularis* (cm^2)

Female	Male
.00541404	.00419510
.00585273	.00439627
.00588272	.00472805
.00595678	.00490013
.00615268	.00505933
.00636402	.00612536
.00638766	.00619965
.00659932	.00678659
.00684399	.00687958
.00716813	.00691338
.00732201	.00691537
.00755763	.00733482
.00810057	.00741962
.00815379	.00770939
.00832213	.00793407
.00861511	.00794609
.00950717	.00825698
.00961864	.00837456
.01062551	.00962585
.01153232	.00969343
Avg. = 0.0076 ± 0.002	Avg. = 0.0069 ± 0.0016

3.4.2.3 Posterior midbody

While the midbody may be considered as a whole, it has been divided and analyzed here as separate anterior and posterior components, following the scheme of Witelson (1989), Aboitiz et al. (1992a,b,c) and Rajapakse et al., 1996 among others. This is due to the presence of fiber tracts connecting differing cortical areas in anterior and posterior midbody (Pandya et al., 1971; Seltzer and Pandya, 1983; Barbas and Pandya, 1984).

Just anterior to the isthmus is the posterior midbody (Fig. 3.6, section 3). Area measurements for this region in female *Macaca fascicularis* yield an average area of 0.12 ± 0.036cm^2 (range: 0.06cm^2 – 0.19cm^2) (Table 3 - 12). For males the average area of the posterior midbody is 0.12 ± 0.038cm^2 (range: 0.07cm^2 – 0.18cm^2). Again as with the isthmus there is little difference between the absolute area values for the posterior midbody. Also, standardized areas for the posterior midbody show little difference between male and female average areas. The relative area of the posterior midbody in females is 0.008 ± 0.0016cm^2, while for males it is 0.007 ± 0.0014cm^2 (Table 3- 13). Again there is virtually no difference between males and females for either the absolute or relative area of the posterior midbody.

Table 3 – 12. Posterior midbody area using straight-line method for *Macaca fascicularis* (cm^2)

Female	Male
.06447285	.07047525
.07421792	.07082833
.07683073	.07661888
.09060095	.07732505
.10091095	.08594026
.10218205	.09526163
.10281760	.09582657
.10444178	.09723890
.10599534	.10105219
.11574041	.10119342
.11975451	.10419878
.12631801	.13227694
.13487773	.14512472
.14102252	.14569161
.14104426	.14801999
.14666649	.15989249
.15664951	.16162571
.17414934	.16356242
.18560000	.17202354
.19036288	.18454631
Avg. = 0.12 ± 0.036	Avg. = 0.12 ± 0.038

Table 3 – 13. Relative posterior midbody area using straight-line method for *Macaca fascicularis* (cm^2)

Female	Male
.00480436	.00452147
.00556455	.00480679
.00601683	.00498427
.00622240	.00558474
.00699784	.00561926
.00712224	.00626725
.00717974	.00637213
.00721184	.00652331
.00734223	.00657201
.00741943	.00664928
.00754833	.00709573
.00757696	.00740818
.00758855	.00759722
.00782992	.00765929
.00787711	.00788769
.00903701	.00810594
.00910391	.00844615
.01029041	.00860192
.01051568	.00890704
.01072774	.00896526
Avg. = 0.008 ± 0.0016	Avg. = 0.007 ± 0.0014

3.4.2.4 Anterior midbody

The anterior midbody, which lies just anterior to the posterior midbody (Fig. 3.6, section 4), is thought to connect motor areas (Barbas and Pandya, 1984). Since there is little difference in the motor skills of male and female *Macaca*, one would not expect to find any sex difference in the callosal area connecting motor areas. Indeed this is the case. Average area measurements of the anterior midbody using the straight-line method are 0.144 ± 0.05cm^2 (range: 0.07cm^2 – 0.25cm^2) for females and 0.138 ± 0.04cm^2 (range:

0.07cm^2 – 0.21cm^2) for males (Table 3 – 14). For relative measurements females, though, possess a slightly larger anterior midbody on average than males. Females have and average relative anterior midbody area of 0.009 ± 0.002cm^2 while the average relative area for males is 0.008 ± 0.002cm^2 (Table 3 – 15).

Table 3 – 14. Anterior midbody area using straight-line method for *Macaca fascicularis* (cm^2)

Female	Male
.06969847	.07266436
.08226820	.07972601
.09180143	.08064402
.09554410	.09370807
.10260575	.09942801
.10507733	.10451239
.11630535	.10656027
.11898877	.11157404
.12123574	.12033049
.12181343	.12922816
.13565426	.14573924
.14946057	.14875040
.17373295	.15277778
.18135571	.16993464
.18158968	.17036862
.18197877	.17896424
.18967485	.18145009
.20274102	.20014178
.20880000	.20379819
.25312314	.20620190
Avg. = 0.144 ± 0.05	Avg. = 0.138 ± 0.04

Table 3 – 15. Relative anterior midbody area using straight-line method for *Macaca fascicularis* (cm^2)

Female	Male
.00519376	.00508947
.00616813	.00524813
.00666570	.00550035
.00721315	.00609597
.00727211	.00687585
.00748233	.00737219
.00804216	.00774198
.00817205	.00776780
.00826517	.00777593
.00869986	.00785947
.00896073	.00830365
.00939685	.00833077
.00969699	.00839273
.00970422	.00872200
.00977000	.00886924
.01012596	.00890303
.01049823	.00965975
.01183014	.00976167
.01197987	.01071407
.01426454	.01074651
Avg. = 0.009 ± 0.002	Avg. = 0.008 ± 0.002

3.4.2.5 Genu

As discussed above the genu as defined using the straight-line method includes the genu and the rostrum (Fig. 3.6, section 5). In females the genu occupies an area of 0.23 ± 0.07cm^2 (range: 0.14cm^2 – 0.34cm^2) on average (Table 3 - 16). Males with an average genu area of 0.23 ± 0.07cm^2 (range: 0.07cm^2 – 0.21cm^2) exhibit no difference from females in the average absolute area of this region. Relative values for the area of this

region (Table 3 – 17) yield no difference as well. Females have an average relative genu area of 0.015 ± 0.003cm^2, while males have and average area of 0.014 ± 0.003cm^2.

Table 3 – 16. Genu area using straight-line method for *Macaca fascicularis* (cm^2)

Female	Male
.14539934	.14441071
.15055434	.15436763
.15239037	.16291222
.16171174	.16474825
.16834969	.16488949
.16940894	.17534072
.18353224	.18099004
.18868724	.18127251
.19059388	.18324977
.20754184	.19645505
.24166188	.24053818
.24395681	.24141375
.26918378	.24763705
.27179011	.27124183
.28647817	.27947846
.30222117	.28746039
.31060000	.31439491
.31912144	.32703214
.33800661	.36279763
.34711481	.37244898
Avg. = 0.23 ± 0.07	Avg. = 0.23 ± 0.07

Table 3 – 17. Relative genu area using straight-line method for *Macaca fascicularis* (cm^2)

Female	Male
.01087614	.01052604
.01135576	.01070748
.01179034	.01071733
.01212447	.01126453
.01222386	.01153564
.01260485	.01176621
.01307048	.01177276
.01332537	.01182949
.01339869	.01255867
.01370197	.01294089
.01449565	.01301903
.01450976	.01339928
.01462614	.01347135
.01503582	.01392166
.01531784	.01498142
.01759789	.01518997
.01785811	.01578406
.01818563	.01691386
.01840990	.01797979
.01956136	.01958037
Avg. = 0.015 ± 0.003	Avg. = 0.014 ± 0.003

3.4.2.6 Statistical analysis of sex versus specific midsagittal regional areas of the corpus callosum

Some absolute values or regional areas of the corpus callosum of *Macaca fascicularis* indicate that sex differences may exist in this structure (e.g., splenium, anterior midbody). The degree of overlap seen in absolute values as well as relative values, however, indicates that any difference is not significant (Table 3 - 18). Indeed, statistical analyses of both absolute and relative areas demonstrate that no statistically significant sex differences exist in the different regions of the corpus callosum in *Macaca fascicularis*. Student's t-test results on the absolute regional areas are no more statistically significant than the $p < 0.3$ level. In addition, Student's t-tests on the relative areas also do not show any statistical significance. While the degree of significance for relative areas indicates that sex differences are not present in the corpus callosum as a whole or its regions in *M. fascicularis*, the degree of significance for relative areas of the anterior and posterior midbody ($p = .11$) indicate that there may be a trend towards females having larger midbodies than males. However, since the degree of significance is well above the acceptable values of $p < .05$, to suggest sex differences may exist in these regions is merely conjecture. Moreover, if a Bonferroni correction (see Rice, 1989) was applied to these values, the degree of significance required to suggest sex differences between males and females would be $p < 0.005$. Thus, males and females are more similar with regard to regional callosal areas than the t-tests alone suggest.

Table 3 – 18. Statistical results of sex versus regional callosal areas for *Macaca fascicularis*

Measurement	t	Sig.
Splenium	-1.15	.27
RELSplenium	-.415	.68
Isthmus	.21	.84
RELIsthmus	1.4	.17
Posterior Midbody	.28	.78
RELPosterior Midbody	1.7	.11
Anterior Midbody	.43	.67
RELAnterior Midbody	1.6	.11
Genu	-.011	.99
RELGenu	1.2	.22

n = 40 (20 females, 20 males);
relative area (REL) = area (cm^2) of callosal region/(brain weight)$^{2/3}$

3.4.3 Radial-line measures of regional areas of the corpus callosum in *Macaca fascicularis*

The radial-line method (Fig. 3.7) divides the corpus callosum into regions similar to those created via the straight-line method with one exception. Like Witelson's (1989) scheme for modern humans the radial-line method as used here yield a region designated the rostral body. In addition, the genu is slightly smaller when the corpus callosum is divided using the radial-line method instead of the straight-line method. Below are the results of absolute and relative area measurements of the regions created by the radial-line method. The statistical analyses of these results are discussed in section 3.4.3.7.

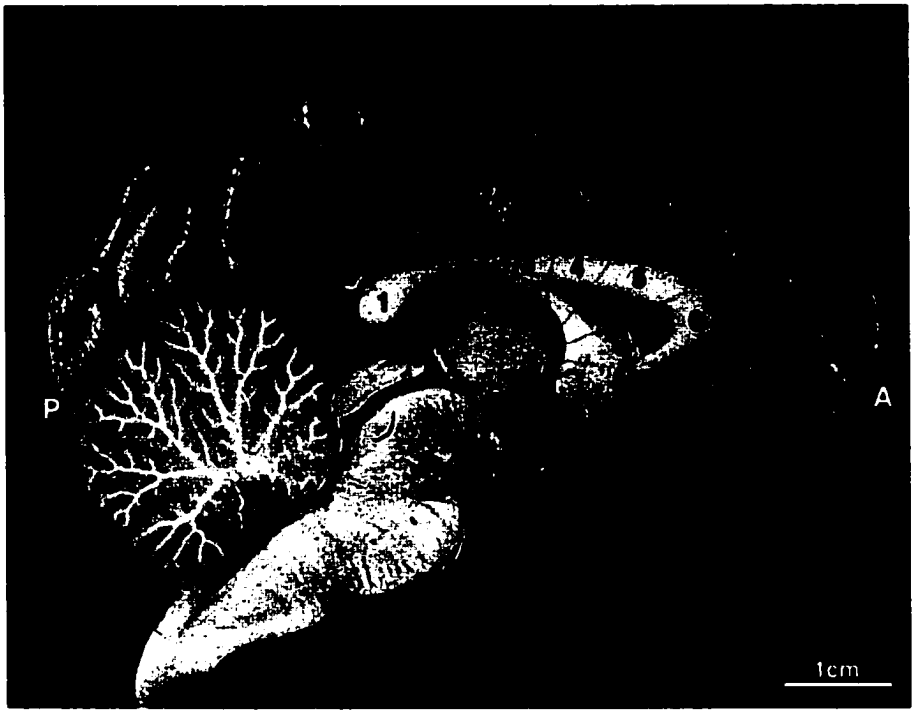

Fig. 3.7. Midsagittal view of the brain of *Macaca fascicularis*, showing the radial-line method of callosal division. 1: splenium, 2: isthmus, 3: posterior midbody, 4: anterior midbody, 5: rostral body, 6: genu.

3.4.3.1 Splenium

The splenium, the most posterior region of the corpus callosum (Fig. 3.7, section 1), is slightly larger on average in each sex for this regional scheme. In females the area of the splenium (range: $0.12cm^2 - 0.3cm^2$) is $0.19 \pm 0.044cm^2$ on average. In males the average area of the splenium is $0.2 \pm 0.06cm^2$ (range: $0.12cm^2 - 0.32cm^2$) (Table 3 – 19). With regard to relative areas, females have an average relative area of $0.012 \pm 0.002cm^2$ and males have an average relative area of $0.012 \pm 0.002cm^2$ (Table 3 – 20). Thus, for both absolute and relative area there appears to be no difference between males and females.

Table 3 – 19. Splenial area using radial-line method for

***Macaca fascicularis* (cm^2)**

Female	Male
.12266083	.11510487
.12936939	.13678413
.13233529	.14426947
.14991879	.14977756
.16022880	.15394393
.16347716	.15683921
.16990326	.16185298
.17969291	.16460702
.18113128	.16679613
.18723130	.17195113
.18995834	.20450827
.19664952	.22166084
.20311142	.22702807
.20771095	.23157596
.21703802	.24267486
.22830309	.25874291
.23529255	.27928634
.23936673	.28007328
.24760000	.28373019
.29654967	.31801689
Avg. = 0.19 ± 0.044	Avg. = 0.2 ± 0.06

Table 3 – 20. Relative splenial area using radial -line method for

***Macaca fascicularis* (cm^2)**

Female	Male
.00930313	.00853457
.00960592	.00889819
.00964029	.00985384
.01045819	.01001214
.01051475	.01049980
.01077328	.01052764
.01092792	.01076734
.01121421	.01116738
.01121706	.01123077
.01158677	.01145350
.01187007	.01165236
.01198271	.01188861
.01201329	.01195379
.01304618	.01248811
.01402846	.01258642
.01411360	.01268158
.01414407	.01388007
.01424742	.01491625
.01581469	.01502508
.01671180	.01657391
Avg. = 0.012 ± 0.002	Avg. = 0.012 ± 0.002

3.4.3.2 Isthmus

The area of the isthmus in either sex is slightly smaller using the radial-line method (Fig. 3.7, section 2) of callosal division than is found using the straight-line method. Regardless, of the method for callosal division used, though, there appears to be no difference in the area occupied by the isthmus in males and females. For females the average area of the isthmus using the radial-line method is 0.1 ± 0.03cm^2 (range: 0.06cm^2 – 0.16cm^2). For males the average area of this region is 0.1 ± 0.04cm^2 (range: 0.03cm^2 –

0.15cm^2) (Table 3 – 21). Relative area values show that there again is no sex difference in the area of this region. Females have an average relative area of 0.006 ± 0.001cm^2, while males have an average relative area of 0.006 ± 0.002cm^2 (Table 3 – 22).

Table 3 – 21. Isthmus area using radial-line method for *Macaca fascicularis* (cm^2)

Female	Male
.05564579	.02704611
.05635195	.04858414
.05967093	.05974154
.06419038	.06193065
.06510840	.06539086
.06666196	.06539086
.07273498	.08029094
.07308806	.08106772
.08233882	.08714074
.08298986	.09921616
.08798814	.10083797
.10739619	.10867920
.11482701	.10916824
.11817741	.11495579
.12354877	.12970349
.12411783	.13374291
.12740000	.14115646
.13500926	.14214577
.14248582	.14625850
.15645449	.15271903
Avg. = 0.1 ± 0.03	Avg. = 0.1 ± 0.04

Table 3 – 22. Relative isthmus area using radial-line method for *Macaca fascicularis* (cm^2)

Female	Male
.00416241	.00184469
.00423787	.00360232
.00441308	.00381372
.00444653	.00407441
.00451010	.00425386
.00497800	.00472280
.00499804	.00538600
.00513807	.00543802
.00528128	.00557129
.00565496	.00562528
.00635825	.00570486
.00641390	.00590018
.00643881	.00591568
.00660609	.00644360
.00692097	.00645504
.00716028	.00726405
.00720759	.00740813
.00721820	.00742087
.00841942	.00794932
.00881686	.00821600
Avg. = 0.006 ± 0.001	Avg. = 0.006 ± 0.002

3.4.3.3 Posterior midbody

The posterior midbody (Fig. 3.7, section 3) in females yields an average area of 0.07 ± 0.03cm^2 (range: 0.03cm^2 – 0.11cm^2). For males the average area is 0.07 ± 0.03cm^2 (range: 0.02cm^2 – 0.11cm^2) (Table 3 – 23). Relative area values for this region show a similar range of difference between males and females as well. For females the average relative area is 0.004 ± 0.001cm^2, and for males it is 0.004 ± 0.001cm^2 (Table 3 – 24).

From these averages it is apparent that there is no sex difference with regard to absolute or relative measurements of the posterior midbody.

Table 3 – 23. Posterior midbody area using radial-line method for *Macaca fascicularis* (cm^2)

Female	Male
.02930387	.02125413
.03523526	.03580015
.03594137	.03650627
.03721238	.03827156
.03862462	.04194337
.04307316	.05486532
.04907516	.05556315
.05465348	.05592449
.05846652	.05931386
.05860774	.06404484
.06030265	.07655955
.06765295	.07979126
.08276644	.08227605
.08757488	.08272661
.09033238	.08867554
.09080657	.09186786
.09368520	.09410431
.10288009	.09807256
.10350982	.10798677
.10751418	.11079524
Avg. = 0.07 ± 0.03	Avg. = 0.07 ± 0.03

Table 3 – 24. Relative posterior midbody area using radial-line method for *Macaca fascicularis* (cm^2)

Female	Male
.00218365	.00144965
.00244077	.00232890
.00279003	.00233045
.00281467	.00283768
.00288919	.00299643
.00337044	.00302933
.00338805	.00354177
.00360824	.00390225
.00361714	.00400081
.00405605	.00408093
.00412011	.00409982
.00424525	.00422287
.00442548	.00444399
.00467526	.00478783
.00486012	.00494725
.00507219	.00496628
.00540464	.00518205
.00582895	.00521193
.00583321	.00533036
.00635296	.00596058
Avg. = 0.004 ± 0.001	Avg. = 0.004 ± 0.001

3.4.3.4 Anterior midbody

In females the anterior midbody (Fig. 3.7, section 4) has an average area of 0.08 ± 0.03cm^2 (range: 0.03cm^2 – 0.13cm^2), and in males this region has an average absolute area of 0.08 ± 0.03cm^2 (range: 0.03cm^2 – 0.12cm^2) (Table 3 – 25). For relative area of the anterior midbody, females have and average area of 0.005 ± 0.001cm^2, and males

have an average relative area of 0.0045 ± 0.001cm^2 (Table 3 – 26). Like the area measurements mentioned above, there is no sex difference between the absolute and relative area measurements of the anterior midbody.

Table 3 – 25. Anterior midbody area using radial-line method for *Macaca fascicularis* (cm^2)

Female	Male
.03431730	.03000999
.04596824	.04074297
.04730986	.04667436
.04815720	.04935760
.05006372	.05220440
.05069923	.05783101
.05945508	.06199710
.06333873	.06750482
.06873954	.06757543
.07174152	.07929698
.07414232	.07986767
.07851408	.08522836
.10446920	.08867495
.10683229	.09034987
.10906897	.10402494
.12098299	.10624500
.12433076	.10704683
.12483790	.10706202
.12927448	.11876417
.13184681	.11980151
Avg. = 0.08 ± 0.03	Avg. = 0.08 ± 0.03

Table 3 – 26. Relative anterior midbody area using radial-line method for *Macaca fascicularis* (cm^2)

Female	Male
.00255724	.00204684
.00318425	.00281529
.00354710	.00294263
.00374486	.00303630
.00378795	.00315084
.00397040	.00417368
.00412322	.00422005
.00431703	.00436226
.00435005	.00459684
.00470722	.00463726
.00489490	.00468407
.00504341	.00496624
.00557718	.00521694
.00571227	.00526537
.00609227	.00553516
.00700655	.00557969
.00709370	.00565387
.00714883	.00575892
.00720182	.00578216
.00732440	.00624367
Avg. = 0.005 ± 0.001	Avg. = 0.0045 ± 0.001

3.4.3.5 Rostral body

When the radial-line method is used to divide the corpus callosum it produces an area between the anterior midbody and the anterior portion of the genu. This area is referred to the rostral body (Fig. 3.7, section 5). For females the average area of this region is 0.14 ± 0.043cm^2 (range: 0.08cm^2 – 0.22cm^2), and for males the average area is 0.13 ± 0.042cm^2 (range: 0.08cm^2 – 0.22cm^2) (Table 3 – 27). Relative areas for this region yield

averages of 0.009 ± 0.002cm^2 for females and 0.008 ± 0.002cm^2 for males (Table 3 – 28). Both sets of areas suggest that there are no sex differences in this area for this group.

Table 3 – 27. Rostral body area using radial-line method for *Macaca fascicularis* (cm^2)

Female	Male
.08205635	.07598333
.08565779	.07697197
.09527235	.09434362
.09660335	.09745075
.09801568	.09832701
.10175835	.11065603
.10564226	.11086788
.11869653	.11263329
.12117788	.11326884
.12654474	.12240076
.13657228	.12534426
.14554057	.12714702
.16924450	.13585761
.17121083	.13869077
.17142319	.15119964
.17145418	.16974241
.17852124	.18100189
.19848771	.19788494
.20333119	.20701476
.21910443	.22052154
Avg. = 0.14 ± 0.043	Avg. = 0.13 ± 0.042

Table 3 – 28. Relative rostral body area using radial-line method for *Macaca fascicularis* (cm^2)

Female	Male
.00571474	.00491366
.00611464	.00530261
.00673767	.00548784
.00711631	.00620685
.00731790	.00639635
.00734881	.00664666
.00756528	.00691058
.00761172	.00715695
.00835452	.00719848
.00879872	.00760871
.00915324	.00787999
.00937967	.00814049
.00953763	.00826546
.00954544	.00835131
.00956334	.00871214
.00988929	.00873597
.01023146	.00962357
.01152029	.00980692
.01172855	.01113701
.01178839	.01159325
Avg. = 0.009 ± 0.002	Avg. = 0.008 ± 0.002

3.4.3.6 Genu

The anteriormost region of the corpus callosum as defined using the radial-line method is the genu (Fig. 3.7, section 6). For females the average area of the genu for this group is 0.22 ± 0.08cm^2 (range: 0.12cm^2 – 0.41cm^2). For males the average area of this region is 0.22 ± 0.08cm^2 (range: 0.11cm^2 – 0.36cm^2) (Table 3 – 29). The relative area of this region for females is 0.014 ± 0.004cm^2, and for males it is 0.013 ± 0.003cm^2 (Table 3 – 30). While the average absolute area for the genu indicates that there are no sex

differences in this area, the average relative area indicates that females may have slightly larger genu than males.

Table 3 – 29. Genu area using radial-line method for *Macaca fascicularis* (cm^2)

Female	Male
.11644658	.11489302
.12626227	.12654474
.13205282	.13558365
.13777276	.13593673
.14095050	.14073865
.14921263	.14900078
.15909893	.15479133
.15916955	.15535626
.18423840	.16594873
.20026834	.16757291
.22890285	.22501180
.23534550	.26218821
.23644873	.26527284
.26381953	.27316417
.27450493	.29306256
.28024575	.29655009
.28584947	.31077411
.29214500	.31831066
.34800000	.35830178
.41076740	.36294896
Avg. = 0.22 ± 0.08	Avg. = 0.22 ± 0.08

Table 3 – 30. Relative genu area using radial-line method for *Macaca fascicularis* (cm^2)

Female	Male
.00867732	.00797228
.00871817	.00882842
.00887620	.00898433
.01024780	.00923423
.01032963	.01005299
.01103823	.01010636
.01155216	.01016265
.01190618	.01081749
.01231557	.01091774
.01320734	.01117968
.01387271	.01260180
.01408424	.01402033
.01410986	.01425023
.01449182	.01427118
.01562084	.01549696
.01646568	.01580437
.01649047	.01619644
.01655959	.01673422
.01971689	.01751756
.02314845	.01775698
Avg. = 0.014 ± 0.004	Avg. = 0.013 ± 0.003

3.4.3.7 Statistical analysis of sex versus specific midsagittal regional areas of the corpus callosum defined via the radial-line method

The absolute and relative area values obtained for this group indicate that there are few or no sex differences in specific regions of the corpus callosum of *M. fascicularis*. Indeed statistical results from Student's t-test indicate that no statistically significant sex differences occur (Table 3 - 31). The degree of significance for each of the measures is well above the acceptable limit of $p < 0.05$. Student's t-test results on the absolute

regional areas are no more statistically significant than at the $p < 0.3$ level. In addition, Student's t-tests on the relative areas also do not show any statistical significance. While the degree of significance for relative areas indicates that sex differences are not present in the corpus callosum as a whole or its regions in *M. fascicularis*, the degree of significance for relative area of the rostral body ($p = .106$) indicates that there may be a trend towards females having a larger rostral body than males. It is, however, not possible to assign a level a certainty to which one can say that sex differences occur in this or any region of the corpus callosum of *M. fascicularis*, since no value approaches an acceptable level of significance.

Table 3 – 31. Statistical results of sex versus regional callosal areas defined by the radial-line for *Macaca fascicularis*

Measurement	t	Sig.
Splenium	-.702	.49
RELSplenium	.505	.62
Isthmus	-.18	.56
RELIsthmus	.67	.51
Posterior Midbody	-.30	.77
RELPosterior Midbody	.31	.76
Anterior Midbody	.44	.66
RELAnterior Midbody	1.26	.22
Rostral body	.48	.63
RELRostral body	1.66	.106
Genu	-.096	.92
RELGenu	.84	.41

n = 40 (20 females, 20 males);
relative area (REL) = area (cm^2) of callosal region/(brain weight)$^{2/3}$

3.4.4 Straight-line measures of regional areas of the corpus callosum in *Pan troglodytes*

As with *Macaca fascicularis* the corpus callosum of *Pan troglodytes* was also divided into regions (Fig. 3.8). Once the corpus callosum had been divided using the straight-line method each region was measured using SigmaScan Pro. These regions were then separately compared for the presence or lack of sex differences in *Pan troglodytes*. Below are the results of these measurements for each region. The statistical results for the raw and standardized measurements of each area are discussed in section 3.4.4.6.

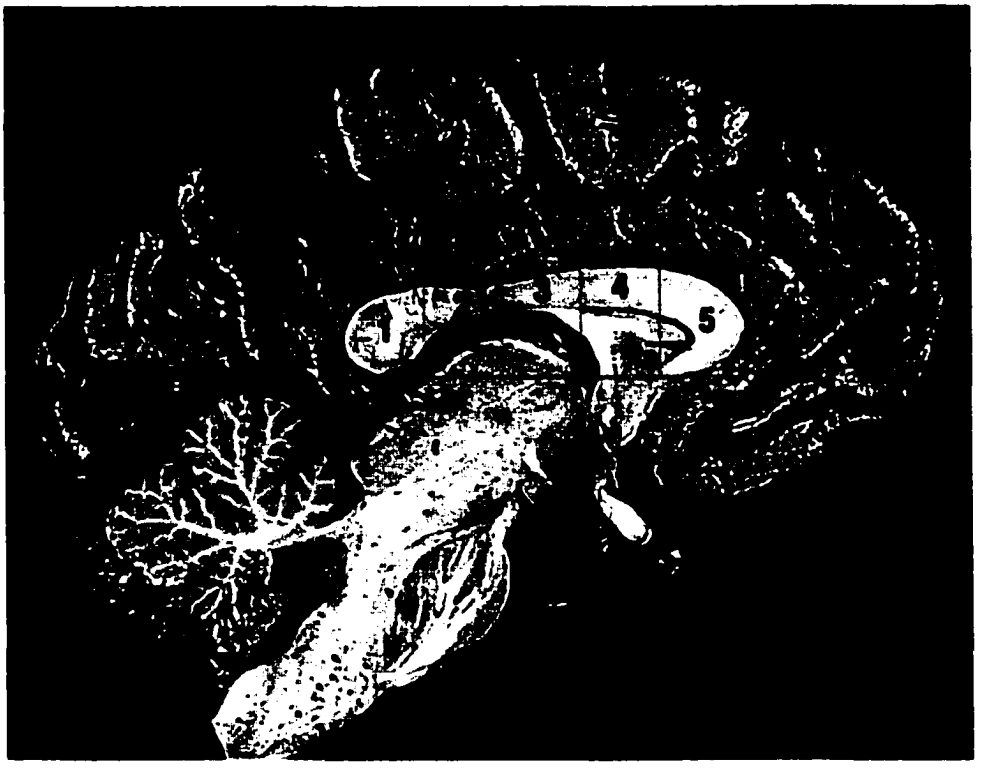

Fig. 3.8. Midsagittal view of the brain of *Pan troglodytes*, showing the straight-line method of callosal division. 1: splenium, 2: isthmus, 3: posterior midbody, 4: anterior midbody, 5: genu.

3.4.4.1 Splenium

Using the straight-line measurement of dividing the corpus callosum the splenium is identified as the posteriormost region (Fig. 3.8, section 1). Areas of the splenium measured using SigmaScan Pro yield a mean area for females of 0.64 ± 0.17cm^2 (range: 0.39cm^2 - 0.96cm^2), and 0.63 ± 0.14cm^2 for males (range 0.25cm^2 - 0.77cm^2) (Table 3 - 32). In this case females possess an absolutely larger splenium on average. However, there is a large degree of overlap between the two samples. When these measurements are standardized the difference between males and females becomes greater. Standardized measurements show that the average size of the splenium in females is 0.014 ± 0.003cm^2 and 0.013 ± 0.002cm^2 in males (Table 3 - 33). Statistical analyses of these results are presented in section 3.4.4.6.

Table 3 – 32. Splenial area using straight-line method for *Pan troglodytes* (cm^2)

Female	Male
.394824	.246949
.410301	.531522
.476285	.599040
.545911	.634380
.578081	.637731
.658740	.659180
.696159	.676612
.706104	.681926
.753466	.697209
.763404	.742568
.809514	.769917
.961934	
Avg. = 0.64 ± 0.17	Avg. = 0.63 ± 0.14

Table 3 – 33. Relative splenial area using straight-line method for *Pan troglodytes* (cm^2)

Female	Male
.010394	.007320
.010509	.012101
.010680	.012700
.011424	.012889
.012973	.013682
.014218	.013861
.014241	.014027
.014547	.014288
.015769	.015056
.016455	.015080
.016806	.016187
.020279	
Avg. = 0.014 ± 0.003	Avg. = 0.013 ± 0.002

3.4.4.2 Isthmus

The isthmus is the region just anterior to the splenium (Fig. 3.8, section 2). Area measurements on the isthmus as defined using the straight-line method yield an average area of 0.31 ± 0.09cm^2 (range 0.17cm^2 to 0.44cm^2) for females and 0.29 ± 0.09cm^2 (range: 0.15cm^2 and 0.47cm^2) for males (Table 3 - 34). As with the absolute area of the splenium, females exhibit a slightly larger isthmus on average than males. In addition, once the areas are standardized the average size of the isthmus is relatively larger in females. For females standardized measurements yield an average isthmus area of 0.007 ± 0.002cm^2, while for males standardized measurements yield an average isthmus area of 0.006 ± 0.001cm^2 (Table 3 – 35).

Table 3 – 34. Isthmus area using straight-line method for *Pan troglodytes* (cm^2)

Female	Male
.124917	.146980
.158652	.178684
.217074	.179925
.249999	.188099
.265432	.278807
.304532	.309961
.321480	.310561
.327875	.320452
.329324	.323983
.338820	.376932
.360880	.395363
.395748	
Avg. = 0.31 ± 0.09	Avg. = 0.29 ± 0.09

Table 3 – 35. Relative isthmus area using straight-line method for *Pan troglodytes* (cm^2)

Female	Male
.003289	.003531
.004064	.004110
.004619	.004333
.005207	.004357
.005430	.005233
.006262	.006545
.006330	.006592
.007237	.007080
.007250	.007383
.007789	.007607
.007896	.007883
.008343	
Avg. = 0.007 ± 0.002	Avg. = 0.006 ± 0.001

3.4.4.3 Posterior midbody

Just anterior to the isthmus is the posterior midbody (Fig. 3.8, section 3). Area measurements for this region in female *Pan troglodytes* yield an average area of 0.30 ± 0.065cm^2 (range: 0.18cm^2 – 0.4cm^2) (Table 3 – 36). For males the average area of the posterior midbody is 0.29 ± 0.096cm^2 (range: 0.14cm^2 – 0.44cm^2). The relative area of the posterior midbody in females is 0.0065 ± 0.001cm^2, while for males it is 0.0063 ± 0.002cm^2 (Table 3 - 37). There is little difference between males and females with regard to the relative area of the posterior midbody. While females possess a slightly larger posterior midbody on average, there is a large degree of overlap between the sexes.

Table 3 – 36. Posterior midbody area using straight-line method for *Pan troglodytes* (cm^2)

Female	Male
.176944	.138893
.196696	.153554
.275730	.246808
.281893	.261593
.288506	.262391
.304300	.279102
.309156	.334009
.318663	.371570
.320264	.371581
.322016	.373457
.390261	.447681
.400370	
Avg. = 0.30 ± 0.065	Avg. = 0.29 ± 0.096

Table 3 – 37. Relative posterior midbody area using straight-line method for *Pan troglodytes* (cm^2)

Female	Male
.004658	.003724
.005038	.004117
.005766	.005149
.005887	.005381
.006239	.006264
.006351	.006375
.006553	.006747
.006673	.007010
.007299	.007278
.007504	.008119
.007730	.008614
.008227	
Avg. = 0.0065 ± 0.001	Avg. = 0.0063 ± 0.002

3.4.4.4 Anterior midbody

The anterior midbody (Fig. 3.8, section 4) lies between the posterior midbody and the genu. Average absolute area measurements of the anterior midbody using the straight-line method are 0.43 ± 0.075cm^2 (range: 0.27cm^2 – 0.54cm^2) for females and 0.4 ± 0.1cm^2 (range: 0.22cm^2 – 0.53cm^2) for males (Table 3 – 38). Females have and average relative anterior midbody area of 0.0095 ± 0.0016cm^2 while the average relative area for males is 0.0086 ± 0.001cm^2 (Table 3 – 39). Although there is little difference between males and females with regard to absolute values, relative measures of this area indicate that females may have a relatively larger anterior midbody than males.

Table 3 – 38. Anterior midbody area using straight-line method for *Pan troglodytes* (cm^2)

Female	Male
.274277	.224040
.370987	.263935
.380969	.345092
.386985	.362031
.404413	.381688
.456104	.401322
.460367	.409725
.460367	.490488
.468793	.498811
.477218	.523320
.537567	.529779
.539781	
Avg. = 0.43 ± 0.075	Avg. = 0.4 ± 0.1

Table 3 – 39. Relative anterior midbody area using straight-line method for *Pan troglodytes* (cm^2)

Female	Male
.007150	.006400
.007220	.006641
.008007	.007851
.009138	.008269
.009296	.008277
.009330	.008759
.009611	.008769
.009883	.009598
.010135	.009607
.010378	.009823
.011791	.010397
.012579	
Avg. = 0.0095 ± 0.0016	Avg. = 0.0086 ± 0.001

3.4.4.5 Genu

As discussed above the genu, as defined using the straight-line method (Fig. 3.8, section 5), includes the genu and the rostrum. In females the genu occupies an area of $0.76 \pm 0.16cm^2$ (range: $0.45cm^2 - 0.96cm^2$) on average (Table 3 – 40). Males with an average genu area of $0.74 \pm 0.11cm^2$ (range: $0.57cm^2 - 0.88cm^2$) are slightly smaller than females in the average absolute area of this region. Relative values for the area of this region yield little difference between males and females. Females have an average relative genu area of $0.017 \pm 0.003cm^2$, while males have and average area of $0.016 \pm 0.002cm^2$ (Table 3 - 41).

Table 3 – 40. Genu area using straight-line method for *Pan troglodytes* (cm^2)

Female	Male
.447379	.570166
.494517	.616529
.721916	.620762
.726308	.675117
.751486	.675940
.774211	.771345
.782236	.775147
.796639	.834126
.831276	.849584
.916545	.853746
.962143	.880316
.965516	
Avg. = 0.76 ± 0.16	Avg. = 0.74 ± 0.11

Table 3 – 41. Relative genu area using straight-line method for *Pan troglodytes* (cm^2)

Female	Male
.011777	.013471
.012666	.013826
.013338	.015137
.016220	.015420
.016296	.015658
.016491	.015756
.017421	.016050
.017695	.016523
.018399	.016641
.018742	.017562
.019371	.020035
.021256	
Avg. = 0.017 ± 0.003	Avg. = 0.016 ± 0.002

3.4.4.6 Statistical analysis of sex versus specific midsagittal regional areas of the corpus callosum

As opposed to *Macaca fascicularis* the regional areas of the corpus callosum of *Pan troglodytes* exhibit a trend towards females having a larger splenium, isthmus, posterior and anterior midbody, and genu than males. The absolute and relative mean areas obtained for these regions appear to indicate that sex differences exist in the corpus callosum of *P. troglodytes*; however, the degree of overlap observed for absolute values as well as relative values demonstrates the converse. Indeed, statistical analysis of both absolute and relative areas demonstrate that no statistically significant sex differences exist in the different regions of the corpus callosum in *P. troglodytes*. Student's t-test results (Table 3 - 42) on the absolute regional areas are no more statistically significant

than the $p < 0.4$ level. In addition, Student's t-tests on the relative areas also do not show any statistical significance. While the degree of significance for relative areas indicates that sex differences are not present in the corpus callosum as a whole or its regions in *P. troglodytes*, the degree of significance for relative areas of the anterior and midbody ($p = .13$) indicate that there may be a trend towards females having a larger anterior midbody than males. However, since the degree of significance is well above the acceptable value of $p < .05$, to suggest sex differences may exist in these regions is merely conjecture.

Table 3 – 42. Statistical results of sex versus straight-line callosal regional area for *Pan troglodytes*

Measurement	t	Sig.
Splenium	.32	.75
RELSplenium	.57	.58
Isthmus	.54	.60
RELIsthmus	.88	.39
Posterior Midbody	.12	.90
RELPosterior Midbody	.44	.67
Anterior Midbody	.86	.40
RELAnterior Midbody	1.6	.13
Genu	.44	.66
RELGenu	.63	.53

n = 23 (12 females, 11 males)

relative area (REL) = area (cm^2) of callosal region/(brain weight)$^{2/3}$

3.4.5 Radial-line measures of regional areas of the corpus callosum in *Pan troglodytes*

The radial-line method divides the corpus callosum into six regions: splenium, isthmus, posterior midbody, anterior midbody, rostral body and genu (Fig. 3.9). The genu as designated here includes the genu and rostrum. In humans the rostrum recurves underneath the genu, which allows for it to be divided from the corpus callosum as a separate region. This is not the case, however, with *Pan*. Instead, as with the straight-line method, the genu and rostrum are combined due to the lack of recurve in the rostrum of *Pan troglodytes*. Other region designations are similar to those described in humans. Below are the results of absolute and relative area measurements of the regions created by the radial-line method. The statistical analyses of these results are discussed in section 3.4.5.7.

Fig. 3.9. Midsagittal view of the brain of *Pan troglodytes*, showing the radial-line method of callosal division. 1: splenium, 2: isthmus, 3: posterior midbody, 4: anterior midbody, 5: rostral body, 6: genu.

3.4.5.1 Splenium

Area measurements on the splenium, the most posterior region of the corpus callosum (Fig. 3.9, section 1), made using the radial-line method are similar to those obtained for the straight-line method. In females the area of the splenium (range: $0.4cm^2 - 0.85cm^2$) is $0.64 \pm 0.15cm^2$ on average. In males the average area of the splenium is $0.64 \pm 0.15cm^2$ (range: $0.38cm^2 - 0.86cm^2$) (Table 3 – 43). With regard to relative areas, females have an average relative area of $0.014 \pm 0.003cm^2$ and males have an average relative area of $0.014 \pm 0.0015cm^2$ (Table 3 – 44). Thus, for both absolute and relative areas there appears to be no difference between males and females.

Table 3 – 43. Splenial area using radial-line method for *Pan troglodytes* (cm^2)

Female	Male
.399179	.376825
.468557	.503240
.513721	.523767
.517770	.627824
.579561	.642617
.592833	.666446
.608205	.673168
.701989	.680143
.775664	.682442
.784780	.778426
.847800	.855529
.851166	
Avg. = 0.64 ± 0.15	Avg. = 0.64 ± 0.15

Table 3 – 44. Relative splenial area using radial-line method for *Pan troglodytes* (cm^2)

Female	Male
.010509	.011169
.010953	.012203
.011855	.012809
.012001	.012914
.012305	.013294
.012322	.013322
.013128	.013598
.015110	.014562
.016359	.014678
.016368	.015276
.017277	.016461
.017944	
Avg. = 0.014 ± 0.003	Avg. = 0.014 ± 0.0015

3.4.5.2 Isthmus

For females the average area of the isthmus (Fig. 3.9, section 2) using the radial-line method is 0.28 ± 0.08cm^2 (range: 0.12cm^2 – 0.4cm^2). For males the average area of this region is 0.27 ± 0.09cm^2 (range: 0.15cm^2 – 0.4cm^2) (Table 3 – 45). With regard to value of relative area of the isthmus, females have an average relative area of 0.006 ± 0.002cm^2, while males have an average relative area of 0.006 ± 0.002cm^2 (Table 3 – 46). These results indicate that, as with the results of the isthmus using the straight-line method, there are no differences between males and females in the absolute or relative area of this region.

Table 3 – 45. Isthmus area using radial-line method for *Pan troglodytes* (cm^2)

Female	Male
.124917	.146980
.158652	.178684
.217074	.179925
.249999	.188099
.265432	.278807
.304532	.309961
.321480	.310561
.327875	.320452
.329324	.323983
.338820	.376932
.360880	.395363
.395748	
Avg. = 0.28 ± 0.08	Avg. = 0.27 ± 0.09

Table 3 – 46. Relative isthmus area using radial-line method for *Pan troglodytes* (cm^2)

Female	Male
.003289	.003531
.004064	.004110
.004619	.004333
.005207	.004357
.005430	.005233
.006262	.006545
.006330	.006592
.007237	.007080
.007250	.007383
.007789	.007607
.007896	.007883
.008343	
Avg. = 0.006 ± 0.002	Avg. = 0.006 ± 0.002

3.4.5.3 Posterior midbody

The posterior midbody (Fig. 3.9, section 3) in females yields an average area of 0.19 ± 0.05cm^2 (range: 0.087cm^2 – 0.23cm^2). For males the average area is 0.19 ± 0.06cm^2 (range: 0.097cm^2 – 0.27cm^2) (Table 3 – 47). Relative area values for this region show a similar range of difference between males and females as well. For females the average relative area is 0.004 ± 0.001cm^2, and for males it is 0.004 ± 0.001cm^2 (Table 3 – 48). These averages suggest that there is no sex difference with regard to absolute or relative measurements of the posterior midbody.

Table 3 – 47. Posterior midbody area using radial-line method for *Pan troglodytes* (cm^2)

Female	Male
.086525	.095624
.116334	.097070
.149379	.142441
.186643	.179835
.189941	.186790
.199999	.194411
.208162	.209500
.209935	.210000
.213126	.225309
.222565	.261593
.223543	.269917
.233539	
Avg. = 0.19 ± 0.05	Avg. = 0.19 ± 0.06

Table 3 – 48. Relative posterior midbody area using radial-line method for *Pan troglodytes* (cm^2)

Female	Male
.002216	.002319
.003063	.002795
.003448	.002877
.003583	.003929
.003700	.003999
.004115	.004229
.004403	.004232
.004692	.004266
.004777	.005124
.004825	.005194
.004851	.005330
.004989	
Avg. = 0.004 ± 0.001	Avg. = 0.004 ± 0.001

3.4.5.4 Anterior midbody

In females the anterior midbody (Fig. 3.9, section 4) has an average area of 0.27 ± 0.08cm^2 (range: 0.08cm^2 – 0.37cm^2), and in males this region has an average absolute area of 0.275 ± 0.13cm^2 (range: 0.12cm^2 – 0.6cm^2) (Table 3 – 49). For relative area of the anterior midbody, females have and average area of 0.006 ± 0.002cm^2, and males have an average relative area of 0.006 ± 0.003cm^2 (Table 3 – 50). Like the area measurements mentioned above, there is no sex difference between the absolute and relative area measurements of the anterior midbody.

Table 3 – 49. Anterior midbody area using radial-line method for *Pan troglodytes* (cm^2)

Female	Male
.087714	.116355
.182358	.134853
.187779	.211058
.218539	.216524
.280976	.218182
.288869	.271406
.294925	.274052
.305456	.303210
.314507	.316187
.315262	.370987
.358368	.595698
.367970	
Avg. = 0.27 ± 0.08	Avg. = 0.275 ± 0.13

Table 3 – 50. Relative anterior midbody area using radial-line method for *Pan troglodytes* (cm^2)

Female	Male
.002247	.003270
.004219	.003449
.004504	.004341
.004801	.004374
.005337	.004767
.006141	.005378
.006186	.005834
.006788	.005935
.006873	.006199
.007259	.007266
.007527	.015120
.007555	
Avg. = 0.006 ± 0.002	Avg. = 0.006 ± 0.003

3.4.5.5 Rostral body

The rostral body as described above is designated as the region between the anterior midbody and the genu (Fig. 3.9, section 5). For females the average area of this region is 0.5 ± 0.14cm^2 (range: 0.24cm^2 – 0.697cm^2), and for males the average area is 0.45 ± 0.13cm^2 (range: 0.27cm^2 – 0.7cm^2). Relative areas for this region yield averages of 0.011 ± 0.002cm^2 for females and 0.01 ± 0.002cm^2 for males. Both sets of areas suggest that there are no sex differences in this area for this group.

Table 3 – 51. Rostral body area using radial-line method for

***Pan troglodytes* (cm^2)**

Female	Male
.244289	.270801
.268559	.295261
.375558	.307154
.476219	.405070
.501651	.448281
.533608	.465455
.563457	.482759
.575690	.511454
.579691	.535672
.584705	.564209
.649484	.701303
.696766	
Avg. = 0.5 ± 0.14	Avg. = 0.45 ± 0.13

Table 3 – 52. Relative rostral body area using radial-line method for

***Pan troglodytes* (cm^2)**

Female	Male
.006431	.006567
.006879	.007796
.009008	.008183
.010279	.008752
.011192	.009930
.011526	.010037
.011922	.010170
.011999	.010239
.012326	.010307
.012435	.011051
.012674	.013163
.013573	
Avg. = 0.011 ± 0.002	Avg. = 0.01 ± 0.002

3.4.5.6 Genu

The anteriormost region of the corpus callosum as defined using the radial-line method is the genu (Fig. 3.9, section 6). With the radial-line method, this region includes the genu and rostrum. For females the average area of the genu for *P. troglodytes* is 0.56 ± 0.15cm^2 (range: 0.35cm^2 – 0.81cm^2). For males the average area of this region is 0.59 ± 0.14cm^2 (range: 0.42cm^2 – 0.87cm^2). The relative area of this region for females is 0.012 ± 0.003cm^2, and for males it is 0.013 ± 0.002cm^2. As with the other measurements reported above, the absolute and relative areas of the genu demonstrate that there may not be any difference between males and females in the area of this region.

Table 3 – 53. Genu area using radial-line method for *Pan troglodytes* (cm^2)

Female	Male
.349531	.418224
.405821	.455714
.423525	.478413
.442534	.498320
.450786	.501029
.514269	.588099
.555563	.627229
.579904	.668847
.661516	.676576
.715980	.741616
.771900	.873386
.814208	
Avg. = 0.56 ± 0.15	Avg. = 0.59 ± 0.14

Table 3 – 54. Relative genu area using radial-line method for *Pan troglodytes* (cm^2)

Female	Male
.007498	.009404
.008664	.011382
.009202	.011567
.010713	.011601
.010822	.012285
.011100	.012396
.011335	.012850
.012225	.012869
.015719	.013917
.015762	.014981
.015867	.017140
.017988	
Avg. = 0.012 ± 0.003	Avg. = 0.013 ± 0.002

3.4.5.7 Statistical analysis of sex versus specific midsagittal regional areas of the corpus callosum defined via the radial-line method

There are no absolute or relative regional area sex differences in the corpus callosum of *Pan troglodytes* as defined using the radial-line method. Student's t-test results (Table 3 - 55) on the absolute area of different callosal regions are no more statistically significant than the $p < 0.4$ level. In addition, Student's t-tests on the relative areas also do not show any statistical significance. For these values the level of significance is no better than $p < 0.2$. Based on these results it is not possible to suggest that any sex difference exist in absolute or regional areas of the corpus callosum in *P. troglodytes*.

Table 3 – 55. Statistical results of sex versus radial-line callosal regional area for *Pan troglodytes*

Measurement	t	Sig.
Splenium	-.009	.99
RELSplenium	.20	.84
Isthmus	.27	.79
RELIsthmus	.39	.70
Posterior Midbody	-.08	.94
RELPosterior Midbody	.07	.94
Anterior Midbody	-.19	.85
RELAnterior Midbody	-.20	.85
Rostral body	.90	.38
REL Rostral body	1.4	.18
Genu	-.59	.56
RELGenu	.45	.66

n = 23 (12 females, 11 males)

relative area (REL) = area (cm^2) of callosal region/(brain weight)$^{2/3}$

CHAPTER 4

HISTOLOGICAL METHODS AND ANALYSIS OF THE SPLENIUM OF THE CORPUS CALLOSUM IN *MACACA FASCICULARIS* AND *PAN TROGLODYTES*

4.1 CHAPTER OVERVIEW

This chapter presents the results of a histological analysis of the splenium of the corpus callosum in *Macaca fascicularis* and *Pan troglodytes*. Morphological measurements of the midsagittal profile of the corpus callosum provide important information concerning the possible presence of sex differences in this structure. However, these morphological results provide only a partial understanding of sex differences in the corpus callosum.

Millions of axons of different diameters traverse the corpus callosum. Given the large number of axons occupying any given area in the corpus callosum, sex differences may be manifested in subtle aspects unavailable through purely morphological measurements. While it is prudent to assume that there are no sex differences in the fiber composition of the corpus callosum in the subjects examined here given the considerable lack of sex differences in the morphological dimensions of this structure (see chapter 3), it was determined that a histological study of a small number of select individuals would be performed in order to demonstrate this assumption in a timely manner. The region chosen for this portion of the study is the splenium, since it is this callosal region that has arguably undergone the most significant evolutionary change and is most sexually

dimorphic in modern humans (de Lacoste and Holloway, 1982; Holloway, 1990; see also chapter 1).

The histological methods used to examine the splenium, including the selection of samples, methods of sectioning and staining methods, are reported in section 4.2. Section 4.3 presents the microscopy techniques used to view histological slides of the splenium. Finally, section 4.4 presents the methods for obtaining the size and counts of axons in the splenium and the results of axonal measurements in each species sampled. (All procedures described below were performed by the author).

4.2 HISTOLOGICAL METHODS

Histology permits an examination of the splenium of the corpus callosum which reveals the composition of this structure. As discussed in section 1.3.3 the shape and size of the corpus callosum is dependent on the number and size of neuronal axons traversing the structure. A proper examination of these traversing axons and therefore the composition of the corpus callosum is dependent upon the methods used to examine it. Below are described the histological methods chosen and used in this project, including the selection of samples, sample preparation, histological staining and microscopy techniques.

4.2.1 Choice of splenium of the corpus callosum for histological study

As the results in section 3.4 demonstrate, there are no morphological sex differences in any region of the corpus callosum. Due to the degree of insignificance in regional areas

the possibility of sex differences being present at any level in the corpus callosum is remote. However, since there is little information concerning the composition of the corpus callosum in *Macaca* and no information regarding the composition of this structure in *Pan*, it was determined that a select region of the corpus callosum be sampled histologically.

Of the regions of the corpus callosum of modern humans the splenium exhibits the greatest degree of sexual dimorphism. De Lacoste and Holloway (1982) and Holloway et al. (1993) among others have described the splenium of modern humans as being more bulbous in females versus males. Although the differences were not statistically significant, Aboitiz et al. (1992a) report that females have more axons passing through the corpus callosum than males. More specifically, though, Aboitiz et al. (1992c) suggest that females have more axons passing through the anterior splenium than do males. These results are consistent with the findings of Highley et al. (1999), which show that females have more axons running through the splenium than do males. Moreover, the difference between males and females with regard to fiber composition is greater in this region than any other in the corpus callosum.

The importance of these morphological and histological findings for the splenium lies in the proposed projection areas for the fibers passing through this region. As discussed in section 1.3.3.2 the isthmus and splenium connect areas of the posterior cerebral cortex such as the posterior temporal, parietal, and occipital lobes. While projection sites of callosal fibers in humans are unknown, functional imaging and deficit studies indicate that the splenium serves an important role in language (Damasio and Damasio, 1983; Price, 2000). For example, Gazzaniga (2000) suggests based on deficit studies that an

intact splenium is necessary for visual language such as reading or describing a picture. These and other studies indicate that the splenium may have undergone a dramatic evolution throughout primate history, especially within humans. As a result it was decided that a cursory examination of the splenium be performed on *Macaca fascicularis* and *Pan troglodytes*. The histological results of this study are reported below.

4.2.2 Sample size and selection of samples

Unlike humans where sex differences in the corpus callosum likely exist, both *Macaca fascicularis* and *Pan troglodytes* do not exhibit sex differences in this structure or any of it regions. As a result it may be assumed that histological differences in these species do not exist. However, since little is known about the fiber composition of this structure in *Macaca* and nothing is known in *Pan* it was decided that a histological examination of arguably one of the more evolutionary important areas of the corpus callosum, the splenium, would be performed on a small sample.

For *Macaca fascicularis* two individuals of each sex (n = 2 males, n = 2 females) were chosen for histological examination of the splenium. The individuals used for histology were derived from Dr. Gannon's collection. The individuals in this collection have been perfused and refrigerated, thus providing samples with the greatest cellular integrity of any used in this study. The corpus callosum of each individual in the collection was examined for friable tissue along the dorsal and ventral borders of the splenium to detect any possible tissue damage or loss of cellular integrity. From the group examined two females (PGM40, PGM54) and two males (PGM43, PGM45) were chosen (Table 4 - 1).

Table 4 – 1. Specimens of *Macaca fascicularis* chosen for histological examination

Specimen	Sex	Brain weight (g)	Splenial area (R-L) (cm^2)
PGM 40	female	49.16	.129
PGM 54	female	53.65	.132
PGM 43	male	51.52	.165
PGM 45	male	49.53	.115

R-L: splenium defined using the radial-line method

While it is possible to readily obtain suitable tissue for histological examination in *Macaca fascicularis*, the availability of suitable tissue from *Pan troglodytes* is more difficult. As explained in the previous chapters *Pan troglodytes* is a protected species, and as such cannot be euthanized for research purposed. This means that the majority of tissue obtained from this species comes from individuals who have died of natural causes and were not euthanized. Under special circumstances when an individual is deemed terminally ill, it is possible to euthanize and perfuse a *Pan troglodytes* individual (National Research Council, 1996). However, these cases are extremely rare. As a result the allocation of suitable tissue from *Pan troglodytes* to a histological study is difficult.

Specimens of *Pan troglodytes* available for histological study were derived from Dr. Holloway's collection. Each brain and corpus callosum was examined for tissue integrity. Those brains that exhibited friable tissue were not considered possible candidates for histological study. Of the brains examined four individuals (n = 3 females,

n= 2 males) were chosen for further histological preparation[2] (Table 4 – 2). Only two males were determined to be possible candidates for histological examination. This was due to the poor quality of preservation exhibited by the other males in the collection. In addition, while the male brains (YN97-139, YN92-115) had not been perfusion fixed, the brains of these individuals were removed shortly after death and sent to Dr. Holloway. Thus, a short amount of time had lapsed between death, fixation, and tissue preparation. Although none of the female brains chosen (YN88-256, YN95-60, YN94-67) had been fixed by perfusion, each was chosen because it exhibited the highest degree of macrocellular tissue integrity among those in the collection.

Table 4 – 2. Specimens of *Pan troglodytes* chosen for histological preparation

Specimen	Sex	Brain weight (g)	Splenial area (R-L) (cm^2)
YN88-256	female	315.35	.608
YN95-60	female	370.00	.776
YN94-67	female	375.00	.848
YN97-139	male	374.67	.856
YN92-115	male	364.80	.680

R-L: splenium defined using the radial-line method

Once the specimens had been chosen the splenium was dissected out for histological examination. The radial-line line method was randomly chosen from the two methods for defining callosal regions as described in chapter 3 to define the splenium. Using

[2] The specimens chosen for histological preparation were further scrutinized after histological staining for evidence of cellular degradation. Only those individuals that displayed measurable and morphologically definable cellular content were included in the final histological analysis (see section 4.3.1.2).

overhead transparency acetate, a line was drawn tangential to the ventral border of the corpus callosum. From anterior-posterior length measurements the midline was calculated, and a vertical line, perpendicular to the horizontal, was drawn. With the midline determined, a series of lines were then drawn from the horizontal from 0° to 180° (Fig. 4.1, section 1). The splenium was defined as the posteriormost region of the corpus callosum. In each case the splenium was defined using transparency acetate and the radial-line method. The splenium as identified here was equivalent to the splenium as defined using the radial-line method via SigmaScan Pro.

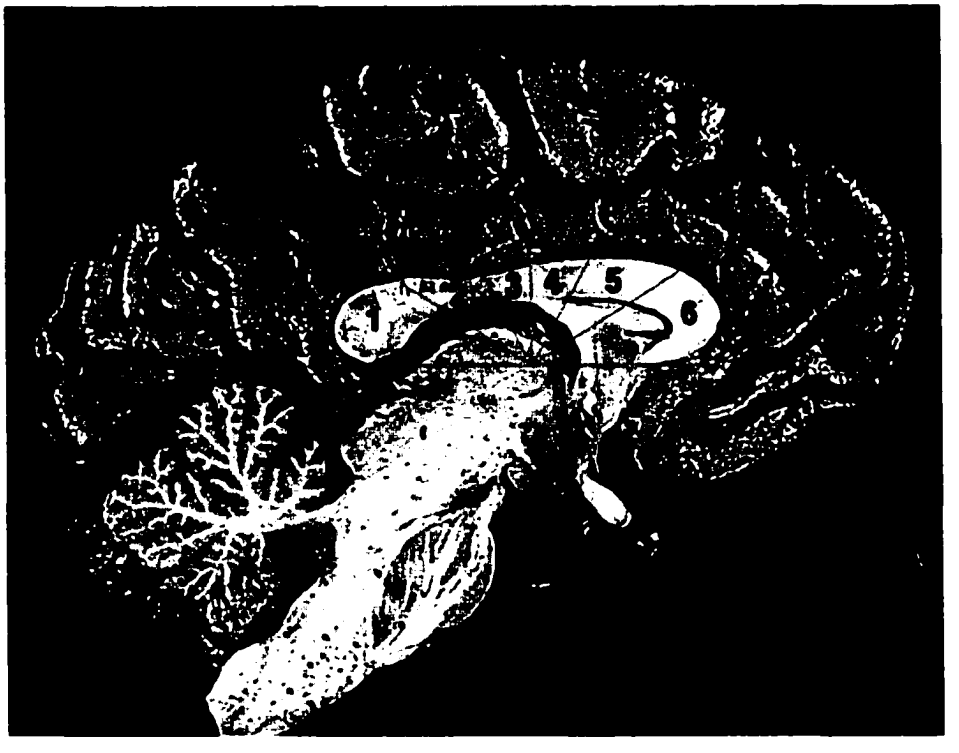

Fig. 4.1. Midsagittal view of the brain of *Pan troglodytes*, showing the radial-line method of callosal division. 1: splenium, 2: isthmus, 3: posterior midbody, 4: anterior midbody, 5: rostral body, 6:genu.

Once the splenium had been defined, it was removed for histological preparation. With the transparency acetate laying over the corpus callosum a cut to a depth of approximately 3mm was made through the acetate and into the corpus callosum at the 30° mark using a number 10 scalpel blade. After this initial cut was made along the anterior border of the splenium, the transparency acetate was removed and a second, parasagittal cut (approximately 2mm from the midline face) from the posterior edge of the corpus callosum to the anteriorly placed initial cut was made to remove the splenium. The parasagittal face of the splenium was distinguished by placing a lengthwise notch in it.

4.2.3 Tissue sample and Epon embedding procedures

With the splenium removed the excised tissue was prepared for histological examination. Following the model of LaMantia and Rakic (1990a) a sample area within the corpus callosum was chosen. The sampling region in *Macaca fascicularis* consisted of a 1-1.5mm^2 trapezoidal area positioned at the ventral boundary of the splenium (Fig. 4.2). In addition, a second sample consisting of a 1mm^2 block positioned at the mid-anterior edge of the splenium was removed. Although this second area has not been sampled before in other species, it was chosen here for its evolutionary interest as an area which may contain fibers connecting superior and inferior temporal lobes as well as posterior parietal cortex (Pandya and Seltzer, 1986).

Fig. 4.2. Midsagittal view of the brain of *Macaca fascicularis*, showing the radial-line method of callosal division. The shape and location of the blocks removed from the splenium for histological examination are indicated by the darkened areas in the splenium (posterior section) of the corpus callosum. (reference figure: fig. 3.7)

The same areas which were sampled in *Macaca fascicularis* were also sampled in *Pan troglodytes*. However, since the corpus callosum is larger in *Pan* than *Macaca*, larger sections were sampled in *Pan troglodytes* (Fig. 4.3). For example, the trapezoidal area sampled was 1.5-2.0mm^2, extending from the ventral border to the midpoint of the splenium. The block from the mid-anterior edge of the splenium was approximately 1.5mm^2 for *Pan*.

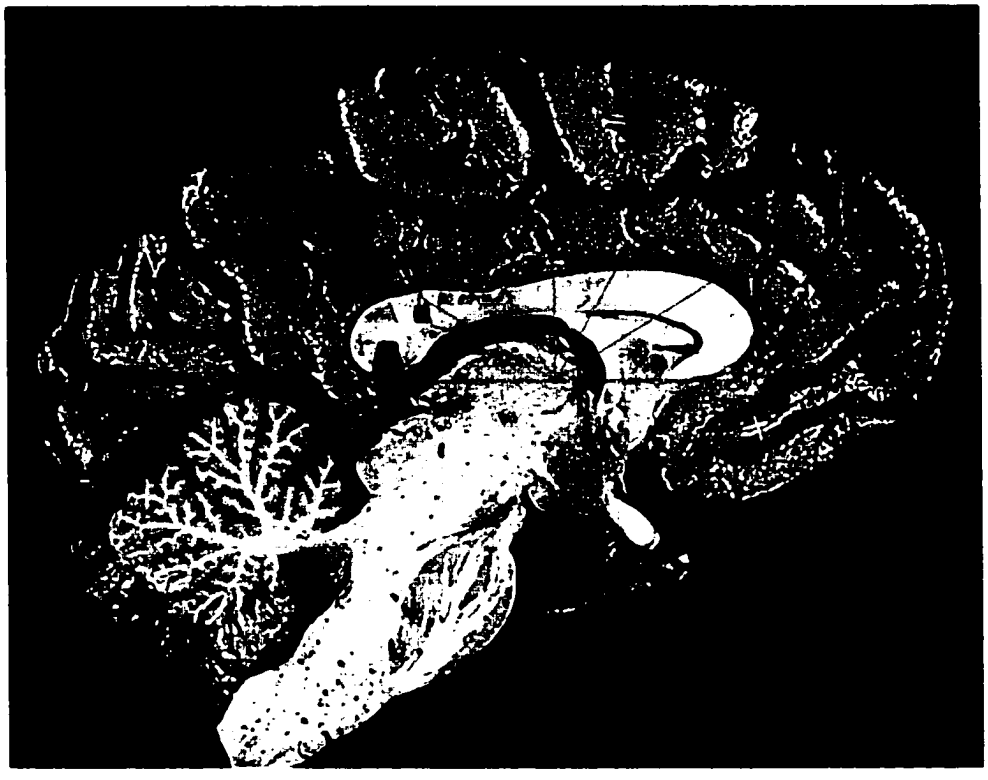

Fig. 4.3. Midsagittal view of the brain of *Pan troglodytes*, showing the radial-line method of callosal division. The shape and location of the blocks removed from the splenium for histological examination are indicated by the darkened areas in the splenium (posterior section) of the corpus callosum. (reference figure: fig. 3.9)

With the sections for histological examination chosen, the tissue could now be prepared for sectioning. The first set of tissue sections prepared were those of *Macaca fascicularis*. Since the cutting edge of the microtome blade is merely a portion of the entire cutting blade face, the splenial sample blocks were divided into smaller sections. For *Macaca fascicularis* the ventral splenial block was divided into four subsections (Fig. 4.4). The separate anterior block was not subdivided. After the *Macaca* tissue had been sectioned it was determined that the microtome blades could accommodate somewhat larger tissue blocks. Thus, for *Pan troglodytes* the ventral splenial block was only

divided into dorsal and ventral subsections (Fig. 4.5). Again, it was not necessary to divide the anterior splenial tissue block due to its small size. Each tissue block from both *Macaca fascicularis* and *Pan troglodytes* was marked on the parasagittal face as well as the superior surface, so that the orientation of the subsection could be determined relative to the other subsections. Additionally each subsection was stored and prepared for histology in a separate container, so that there was no intermingling of subsection blocks at any point.

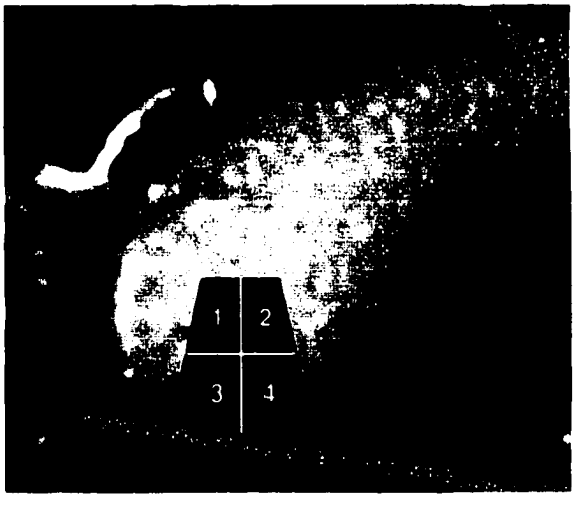

Fig. 4.4. Close-up midsagittal view of the splenium of *Macaca fascicularis*, showing the division of the ventral splenial block into four divisions. (reference figure: fig. 4.2)

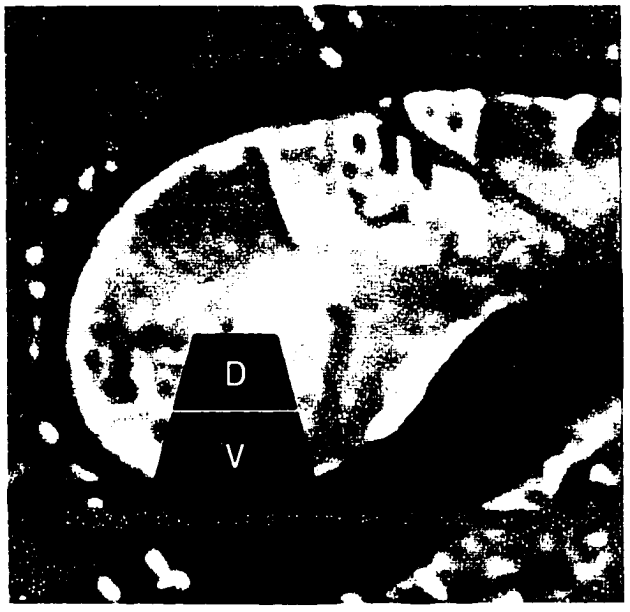

Fig. 4.5. Close-up midsagittal view of the splenium of *Pan troglodytes*, showing the division of the large splenial block into a dorsal (D) and ventral (V) portion. (reference figure: fig. 4.3)

Since small blocks of tissue were to be microtomed, the portions of the splenium to be sampled could not be sliced via frozen sectioning. Instead the section had to be Epon embedded to allow for thin sectioning without distortion. Epon embedding involves the plastination of tissue. This methodology allows for the tissue to be preserved as hard resin blocks which do not need to be stored under any special conditions, and which may be sliced into ultrathin sections. Traditionally, Epon embedding has been used to provide support for tissue that is to be sliced into ultrathin sections ($< 0.5\mu$m) (Bolam, 1992). Normally, these ultrathin sections are examined using electron microscopy techniques. Since the tissue examined here was not examined using electron microscopy a variation of the typical resin embedding procedure was performed (see below).

To examine tissue sections using light microscopy, as was done here, it is necessary to infiltrate the tissue with a water immiscible medium that penetrates all spaces in the tissue to even out the changes in refractive index of the tissue. To do so all of the water in the tissue must be removed prior to exposure to the mounting medium (Bolam, 1992). In this procedure the tissue is dehydrated in graded dilutions of ethanol (steps 1 – 7). Below are the processes for tissue dehydration and Epon embedding. Clean 20ml scintillation vials were used for each tissue block at the beginning of steps 1,2,3,8,10, 11 and 12. In addition, clean disposable pipettes were used for each fluid removal for each specimen tissue block.

1. Place tissue samples into cold phosphate buffered sucrose solution (20% sucrose in 0.1 Molar phosphate buffer) in order to remove any traces of fixative. Leave tissue samples in phosphate buffered solution refrigerated at 4°C for one week, vacuuming off the solution and replacing it with fresh solution each day. Tissue should be resting at the bottom of the vial indicating that the sucrose solution has completely infiltrated the tissue.
2. Wash tissue samples three separate times in deionized water with no significant time lapse between washes. This will clear the tissue of any other fluids remaining in it.
3. Place tissue samples in 50% ethanol for 10 minutes. Vacuum off the fluid and repeat the process twice more. This step is necessary to remove any remaining phosphate buffer so that it does not form a precipitate in later steps.
4. Place tissue samples in 70% ethanol for 10 minutes. Vacuum off the fluid and repeat the process twice more.

5. Place tissue samples in 80% ethanol for 10 minutes. Vacuum off the fluid and repeat the process twice more.
6. Place tissue samples in 95% ethanol for 10 minutes. Vacuum off the fluid and repeat the process twice more.
7. Place tissue samples in 100% ethanol for 10 minutes. Vacuum off the fluid and repeat the process twice more.

Once the above steps have been completed the tissue should be sufficiently dehydrated. Now the tissue may be infiltrated with resin. The first step in the process involves the use of an appropriate link reagent, a substance that is miscible in both ethanol and the resin. This link reagent permits the gradual replacement of the dehydrating solution (ethanol) with the resin (Epon). In addition, this process allows for the tissue to be gradually infiltrated and polymerized by the resin. The ideal resin is one that is soluble in the dehydrating solution, polymerizes evenly, does not change volume when polymerized, can be cut easily, and has good optical quality (Glauert, 1975; Bolam, 1992). The epoxy resin commonly used for neural tissue, and chosen for this study is Epon.

The following steps, 8 – 13, are used to embed the tissue in resin. This process is most often used to provide support for the tissue during the preparation of ultrathin sections for electron microscopy. One of the advantages of this procedure is that it clears the tissue and results in a preparation suitable for high resolution light microscopy.

8. Under a fume hood, place tissue samples in a 100% ethanol: propylene oxide mixture (1:1 ratio) for 20 minutes. Vacuum off the mixture and repeat the process.

9. Under a fume hood, place tissue samples in 100% propylene oxide for 20 minutes. Vacuum off the mixture and repeat the process.
10. Under a fume hood, place tissue samples in a 100% propylene oxide: Epon mixture (1:1 ratio). Leave the sample vials in a rotator for 10-12 hours so that the tissue is completely submerged and evenly coated with the mixture.
11. Under a fume hood, blot the tissue samples and place them in 100% Epon. Leave the sample vials in a rotator for 10-12 hours so that the tissue is completely submerged and evenly coated with the Epon mixture.
12. Under a fume hood, vacuum off the Epon used in step 11, blot the samples, and place tissue samples in clean vials with a 100% Epon. Leave the sample vials in a rotator for 8-10 hours so that the tissue is completely submerged and evenly coated with the Epon mixture.
13. Under a fume hood, remove the tissue samples from their vials, blot each to remove Epon from step 12, and position them in individual molds so that the cutting face of the tissue block is positioned next to the edge of the narrow end of the mold. Fill the boats with Epon and place the mold trays in a vacuum for several minutes to remove any air pockets.
14. Place the mold trays into a 60°C oven for 24 – 48 hours. Once the molds have hardened remove the resin blocks from the tray for sectioning.

4.2.4 Microtome sectioning

Light microscopy can be used to view sections up to 40μm thick. A disadvantage of using such thick sections, however, is that since there is no finite plane of view portions of the tissue slice above and below the desired plane are within the field of view. While this is non-obtrusive to certain forms of data, it is obtrusive to others. The purpose of this study is to understand the composition of the splenium of the corpus callosum in *Macaca fascicularis* and *Pan troglodytes*. In order to assess the size of axonal fibers passing through this structure and to obtain accurate axonal counts it is necessary to view a single refractive plane of the splenium. To do this ultrathin sections (0.5μm) of the splenium were required. 0.5μm sections were made using glass knives on an ultramicrotome (LKB Ultramicrotome III).

Before ultrathin sections can be made the Epon resin blocks must first be prepared for the ultramicrotome. First the blocks were removed from their trays and coded according to specimen number and the orientation of the splenial tissue. Next the blocks were placed into the ultramicrotome chuck, and positioned for direct viewing of the block surface. Surplus resin was trimmed away with a razor blade under stereo-microscope guidance. Under the microscope the block is trimmed to produce a face that is suitable for ultrathin sectioning. Once the block has been trimmed, the chuck with the trimmed block is placed in the ultramicrotome. The chuck is adjusted to make the block face parallel to the cutting edge of the knife. The knife is advanced to the block face by coarse and fine manual feed. Fine adjustments to line up the block face and the knife edge are also made. These adjustments can be made in three dimensions, so that the

cutting edge and the block face are in complete alignment with each other. Once the knife edge and the block face are properly aligned, the block may then be sectioned.

With the controls on the ultramicrotome set to advance the specimen 0.5μm after each pass, the cutting face of the tissue block was carried across the cutting face of the glass knife, using the manual advance controls on the machine. After the first few slices had been observed to have been properly removed from the block face, the ultramicrotome was set to automatically create a series of ten tissue slices. In order to prevent curling of the tissue slices and to prevent them from sticking to the glass knife, a water-filled boat was used behind the cutting face of the knife to catch tissue slices as they were removed from the block. As the ultrathin tissue sections were floated on water as they came away from the cutting edge of the glass knife, they were picked up with a drop of water with a fine-wire loop. This allows for the tissue slice to be moved to a coated glass slide while still floating on water. Tissue sections were placed on glass slides in sequential order, beginning at the upper, left hand corner closest to the frosted edge of the slide. Once a slide had been filled with two to three rows of tissue slices it was dried with gentle heat on a hotplate. This allowed for the water droplets to evaporate, adhering the tissue slices evenly to the glass slides. All specimens were sectioned according to this procedure. A new glass knife was used after every forty sections or sooner if it was observed that the edge of the glass knife had become dull.

4.2.5 Histological staining

Once the tissue sections were properly adhered to the slides, the slide was removed from the hotplate and allowed to cool. Once the slides had cooled for a few minutes, the

sections could then be stained. Section 4.2.5.1 reports the type of histological staining used. Section 4.2.5.2 reports the staining procedure.

4.2.5.1 Choice of toluidine blue stain

The morphology of myelinated fibers in the corpus callosum as well as other white matter structures in the nervous system is usually judged by cross-sectional profiles. Such profiles reveal size and morphological features of the axon and the myelin sheath. However, myelinated fibers are delicate structures, making conservation of the ultrastructure of the axon a priority when performing morphological studies as the type undertaken here (Feirabend et al., 1994). To accomplish this conservation, adequate tissue preservation procedures are imperative. In cases when tissue perfusion is not a viable option, rapid immersion in fixatives such as aldehyde fixatives is sufficient (Bolam, 1992; Feirabend et al., 1998). In addition to tissue fixation, the proper staining technique is required to maintain tissue conservation as well as to view those morphological characteristics that are of interest.

Toluidine blue is a metachromatic dye that demonstrates Nissl substance. When used on tissue which has been fixed only in an aldehyde-based fixative toluidine blue dyes the myelin dark blue while the axon appears clear or unstained (Feirabend et al., 1994, 1998). While the tissue samples obtained from *Pan troglodytes* for this study were initially immersion fixed in an aldehyde-based solution, later fixation was in non-aldehyde based solutions. In addition, none of the *Macaca fascicularis* specimens were fixed with aldehydes. However, these fixation techniques do not pose any histological technical

difficulties. Instead they provide tissue which may be stained to view a variety of axonal structures.

When used on non-aldehyde fixed tissue that has not been treated with osmium tetroxide, toluidine blue stains both the axons and myelin sheaths blue, where the myelin sheaths are slightly darker (Fig. 4.6). Staining of this nature permits the morphological aspects of the axon to be measured reliably, since the myelin surrounding the axon can be easily distinguished, and other neuronal cells such as nonmyelinated axons and glial cells can be identified. In addition, toluidine blue is an excellent stain for light microscopy analysis, especially in semithin (1μm) and ultrathin (< 0.05μm) Epon sections (Feirabend et al., 1998). Toluidine blue also has an advantage over other types of stains in that it is easy to use; it is reversible; it can be produced and applied at low cost; and tissue is not discarded from the study because the stain did not take hold, as occurs with immunocytochemical stains. Thus, immunohistochemical procedures do not represent a viable option for examining rare tissue with limited availability, as is the case for *Pan troglodytes* tissue.

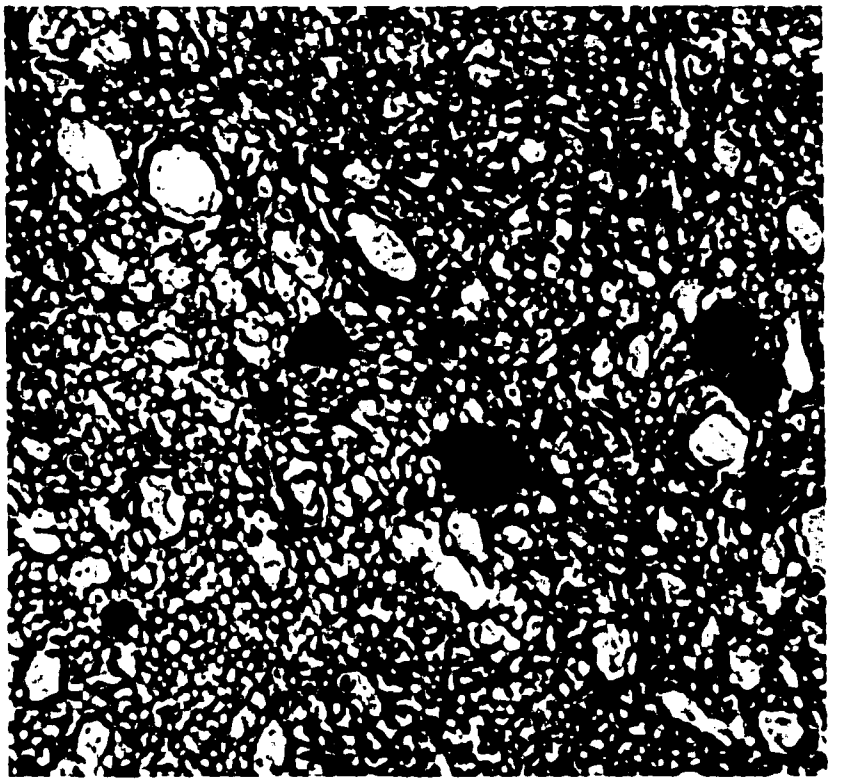

Fig. 4.6. Greyscale image of a histological section of the splenium of *Macaca fascicularis*. Myelin is stained dark. (1000x).

4.2.5.2 Staining procedures for toluidine blue stain

Once the ultrathin (0.05μm) sections had been made of the splenium from the Epon blocks and placed onto glass slides, they could be stained with toluidine blue. Below is the stepwise procedure used for staining with toluidine blue.

1. Produce a solution 50ml of 0.5% toluidine blue and 0.25% boric acid. Mix thoroughly, then place into a vacuum for several minutes to remove any introduced air from the solution. Store in an opaque container away from light.

2. Using a disposable pipette, place the toluidine blue solution on the specimens which have been mounted on glass slides, making sure to entirely cover each section with stain.
3. Warm the slide at 70°C for 1-2 minutes, or until the stain has begun to evaporate.
4. Rinse the slides briefly in two subsequent changes of distilled water at room temperature.
5. Allow the slides to air dry.
6. Coverslip the slides using Permount. Blot any excess mounting medium, and allow the slides to dry in a darkened location. (Permount is a slide-mounting medium that is non-permanent and also helps to preserve the dye.)
7. Once the slides have sufficiently dried, they are ready for microscopic viewing.

4.3 ANALYSIS OF STAINED SECTIONS

The study of sex differences with regard to fiber composition of the corpus callosum is dependent on three factors: the choice of tissue and area samples, the histological staining technique used, and the method of microscopy. This section discusses the choice of microscopy used to study fiber composition in the splenial samples chosen from *Macaca fascicularis* and *Pan troglodytes* (section 4.3.1.1), and the methods used to assess the axonal composition of the splenium.

4.3.1 Microscopy technique

Microscopy techniques vary according to the requirements of the data desired. For example, electron microscopy is useful for examining intracellular and nuclear structures, but is often unnecessary when examining certain extracellular structures. The choice of a microscopy method is important to all histological data collection schemes. This section discusses the microscopy method chosen as well as the data acquisition methods used.

4.3.1.1 Choice of light microscopy

Three basic methods of microscopy are available for the study of nervous tissue: light, electron, and confocal microscopy. For this study light microscopy was deemed the preferred method of data acquisition. This choice was made by assessing the advantages and limitations of each of the above microscopy methods.

Confocal microscopy is a new technology that presents several advantages over conventional methods of microscopy, depending on the constraints of the study. For example, in light microscopy an entire sample is illuminated. This causes areas above

and below the focal plane in 5 – 40μm sections to be included within the viewed image. The result is a blurred image, making it difficult to discern various cellular structures. The illumination of the sample via laser light in a confocal microscope results in sequential illumination of the sample, not simultaneous illumination as in light microscopy. In addition, utilizing a pinhole aperture, out of focus light is eliminated. The result is an image with a narrow depth of field (~0.5μm), sampled from a single plane. The image that is created with confocal is digitized and thus can be manipulated in a variety of ways (Majlof and Forsgren, 1993; Wright et al., 1993).

Confocal, however, does have limitations. Its use in a morphological study such as this depends on immunohistochemicals designed to label specific intra- or extracellular structures. For example, if myelin is to be distinguished from the rest of the axon, then the myelin must be treated with an antibody to a myelin protein. Tagged to the antibody is a chemical that will fluoresce when exposed to the laser light of the confocal microscope. The specific immunohistochemicals, procedures, and fluorescent qualities of the tagged antibodies present separate issues when they are considered for use in a study on neuronal tissue.

Three specific problems must be assessed when considering the use of confocal microscopy. The first deals with the structures being studied. Confocal is useful for studying single structures or a limited number of cells within an area. Part of this is due to the specificity of the antibodies used, but another part is related to the fluorescent tags applied (Majlof and Forsgren, 1993). While confocal microscopy is useful for examining cells that are spaced throughout a sample, it is of limited usefulness in studies where the target cells have high packing densities. Since the cells fluoresce, the divisions between

specific cells or cellular components can become obscured when the cells are packed closely together such as in the corpus callosum. This is especially problematic when there is a desire to view every cell within a sample, such as in the case of this study. When this happens an obscured view or "wash-out" effect can create artificially large cells when it is not possible to delineate between two adjacent cells. In addition, smaller cells can become obscured or absorbed by larger cells, and thus would not be counted (Wright et al., 1993). The effects produced by the fluorescent tags prohibited their use in studies on the composition of the corpus callosum.

The second problem that arises with regard to the use of confocal microscopy is the availability of antibodies that will stain the tissue to be studied. Most antibodies are produced to react with tissue from a specific species or genera (Majlof and Forsgren, 1993; Wright et al., 1993). In addition, tissue samples should be from perfusion fixed specimens only, since the receptor proteins that react with the staining antibodies begin to fragment soon after death. At the time the histology for this study was performed there were no antibodies available that worked with neuronal tissue in *Pan*, albeit antibodies for *Macaca* were available. In addition, none of the available tissue samples from *Pan troglodytes* were perfusion fixed. While certain antibodies could have been tested on the *Pan troglodytes* tissue, the low supply of available tissue prohibited the use of unproven antibodies. For reasons of consistency it was decided that both the *Macaca fascicularis* and *Pan troglodytes* samples would be examined using the same histological techniques.

Another method of microscopy to consider is electron microscopy. Unlike confocal microscopy electron microscopy does not suffer from the introduction of fluorescent artifacts. In addition, it offers superior magnification. Electron microscopy, though, does

have certain limitations. Depending on the desired results of a histological study electron microscopy can be either insufficient or overkill. In the case of this study these limitations were considered to be not mutually exclusive. Electron microscopy is ideal for viewing three dimensional objects microscopically (e.g., insects); opaque objects such as bone and teeth; and cellular and subcellular components. However, it does not represent the best microscopic method for viewing immersion fixed tissue at the extracellular level. Instead confocal and light microscopy are more viable options when viewing tissue at 100 – 1,000x. In addition and more importantly, tissue prepared for electron microscopy is subject to fixation artifacts, especially in the case of immersion fixed specimens. Since the *Pan troglodytes* tissue was fixed by immersion, electron microscopy was not a viable option for histological examination.

Light microscopy provides a path to analyze sometimes large areas of tissue (Feirabend et al., 1998). In addition, the use of immersion fixed tissue is less problematic when staining methods for light microscopy are applied. Besides these particular issues, which solve some of the problems that arise in confocal and electron microscopy, light microscopy provides other benefits. Principally among these are issues concerning the resolution of the microscope. Traditionally, light microscopy has suffered from the possible inclusion of out-of-focus information and its low resolution (0.2μm) (Wright et al., 1993). However, advancements in optics and digital imaging have reduced many of these resolution errors.

A light microscope has a maximum magnification of 1200x. This provides ample magnification for viewing the composition of the splenium of the corpus callosum. In addition, certain techniques can be used to enhance the optical resolution of the

microscopic image. As mentioned above, tissue viewed with light microscopy has usually suffered from the introduction of image artifact from out-of-focus information (Wright et al., 1993). A solution to this optical resolution problem is to incorporate some electron microscopy techniques in the preparation of histological sections. As discussed above in section 4.2.4, this study used ultrathin (0.5μm) histological sections. By using sections of this thickness, the out-of-focus artifact effect was essentially eliminated. Thus, as with confocal microscopy the tissue could be viewed along a single plane.

Unlike tissue prepared and stained for electron microscopy, the tissue as prepared for ultrathin sectioning and light microscopy avoided the potentially artifact introducing step of osmication of immersion fixed tissue. In addition, the tissue was stained with an often-used electron microscopy stain, toluidine blue. These preparation and staining procedures produced histological sections of superior quality that could be viewed with light microscopy without the concern for artifact, which may have occurred had they been prepared for electron microscopy.

A final feature available for light microscopy today essentially eliminates the possibility of the application of confocal or electron microscopy. This feature is digital image capture. Both electron and confocal microscopy were designed to use digital image capture. Light microscopy, though, had always suffered from observer error, since images could only be studied optically (Wright et al., 1998). However, digital image capture techniques have lately been employed in light microscopy. These digital image capture techniques not only provide images that are of similar quality to confocal, but they also permit a second degree of magnification (see section 4.3.1.2, below). This has allowed for the viewing of smaller fibers often unseen in optically viewed images. Thus,

through the use of ultrathin sectioning and enhanced digital image capture techniques light microscopy represents the most sound method of viewing and examining the histological samples of the splenium of the corpus callosum in *Pan troglodytes* and *Macaca fascicularis*.

4.3.1.2 Light microscopy image capture

Microscopy of toluidine stained, ultrathin histological sections was performed using a Zeiss Axioskop light microscope. Using bright field emission and a 40x objective lens, histological samples were examined for myelin and cellular integrity. Samples which did not meet specific criteria for myelin integrity were rejected. Each of the *Macaca fascicularis* individuals chosen for study was deemed appropriate for study, since none of these individuals exhibited any myelin or cellular degradation. The stability of these tissue samples most likely was the result of the original tissue preparation. Since all of the specimens chosen for histological examination were perfusion fixed, and maintained in formalin at 4°C, there was little reason to assume that the integrity of the tissue had been significantly compromised. The *Pan troglodytes* specimens, however, required additional scrutiny, since these specimens had been immersion fixed, and the quality of tissue preservation could not be specifically confirmed.

As noted above, myelin is extremely fragile and begins to lose its constitution soon after the death of the organism unless it is quickly preserved. Using a 40x objective, the histological sections from *Pan troglodytes* were examined for myelin and cellular integrity. Specimens YN88-256, YN92-115, and YN95-60 were all rejected for data collection on cellular composition of the splenium, since all exhibited significant and

severe myelin and cellular degradation. Thus, a single female (YN94-67) and single male (YN97-139) were selected for an examination of the fiber composition of the splenium of the corpus callosum in *Pan troglodytes*.

After the final specimens for inclusion into the histological study were selected, histological samples could then be examined for the number and kinds of myelinated fibers occupying a given area. Each histological slide was examined for these attributes using a 100x oil immersion objective. Thus, each specimen was viewed at approximately 1200x magnification.

Under the limits of optical observation it is often only possible to examine a portion of any given histological section, since the size of the area examined is dependent upon the constraints of hand-counting cellular features. However, using digital image capture and analysis, as was done here, it is possible to examine larger areas. Once the histological sample had been brought into view, the optical aperture was closed and a separate aperture leading to a digital camera mounted above the objective lens was opened. Using controls leading to the objectives on the digital camera and the computer to which the camera is connected, the histological area to be sampled was brought into focus. Once the cellular borders could be visually defined, the image was captured by the computer and coded according to specimen, histological slide number, and sample area location. These images were then saved for later analysis. Adjacent areas within the samples were defined according to grid coordinates and morphologically identifiable structures such as large glial cells. These numerous and large, morphologically obvious features allowed for adjacent areas within a section to be sampled without overlap. Once a section had

been thoroughly sampled and the images captured and saved, it could be analyzed for axonal density and composition.

4.4 MEASURE OF AXONAL DENSITY AND SIZE IN THE SPLENIUM OF THE CORPUS CALLOSUM OF *MACACA FASCICULARIS* AND *PAN TROGLODYTES*

After light microscopic images of the histological sections had been digitally captured, they could be analyzed for axonal composition and density. The computer-aided means of image capture and analysis was through the same software program (see section 4.4.1). Within this program not only could the number of axons in the splenium of the corpus callosum be sampled, but the types of fibers comprising a sample could also be derived. While the sample size of this histological study provides only limited information on the fiber composition of the corpus callosum in *Macaca fascicularis* and *Pan troglodytes*, it, for the first time, gives insight into the brain's interhemispheric highway of these two nonhuman primate species. Moreover, this is the first study to examine sex differences in the fiber composition of the corpus callosum in any nonhuman primate. Below are the results for the histological study of the composition of the splenium of the corpus callosum in *Macaca fascicularis* and *Pan troglodytes*.

4.4.1 Selection and application of analysis software

Several software programs exist for the analysis of histological sections. The software used for this portion of the study was IPLab 3.1 (Scanalytics, Inc.). In general, this software is designed to capture images through a digital camera and analyze the

components of the image in a variety of ways. Specifically designed for microscopic use on Apple computers, IPLab is a program applicable to a variety of fields from engineering to biology. Due to its unique design application, IPLab is capable of making a variety of measurements on shapes of varying dimensions. This feature in particular makes this software program useful for measuring the irregular shapes of closely packed axonal fibers. Since axons in the corpus callosum do not possess regular areas it is not possible to classify them by hand according to diameter. Instead the area of the axon must be computed, and from the resultant area only then can the diameter of the axon be determined. Such analysis can only be performed with a computer, especially when fiber counts approach the tens of millions.

Before axons are counted and measured the image can, if necessary, be enhanced so that the edges of the cells are more distinctive. While these artificial enhancements affect the intensity of the background and axonal edges, they do not alter the overall morphological features of the axons. In addition, it is possible to magnify the image if necessary.

Like other software programs, such as SigmaScan Pro, which are designed to measure and identify objects within an image, IPLab is capable of discriminating objects within an image. This is accomplished through pixel differentiation. Objects within an image are defined according to the varying light intensities they emit (Fig. 4.7). For example, a line drawing of a circle is viewable because the pixel intensity of the edge of the circle is different from the background. In IPLab, as with other image analysis software, the edge of an object is selected. With the pixel intensity of a cell selected the software can then automatically identify and measure all the axons occupying a single section.

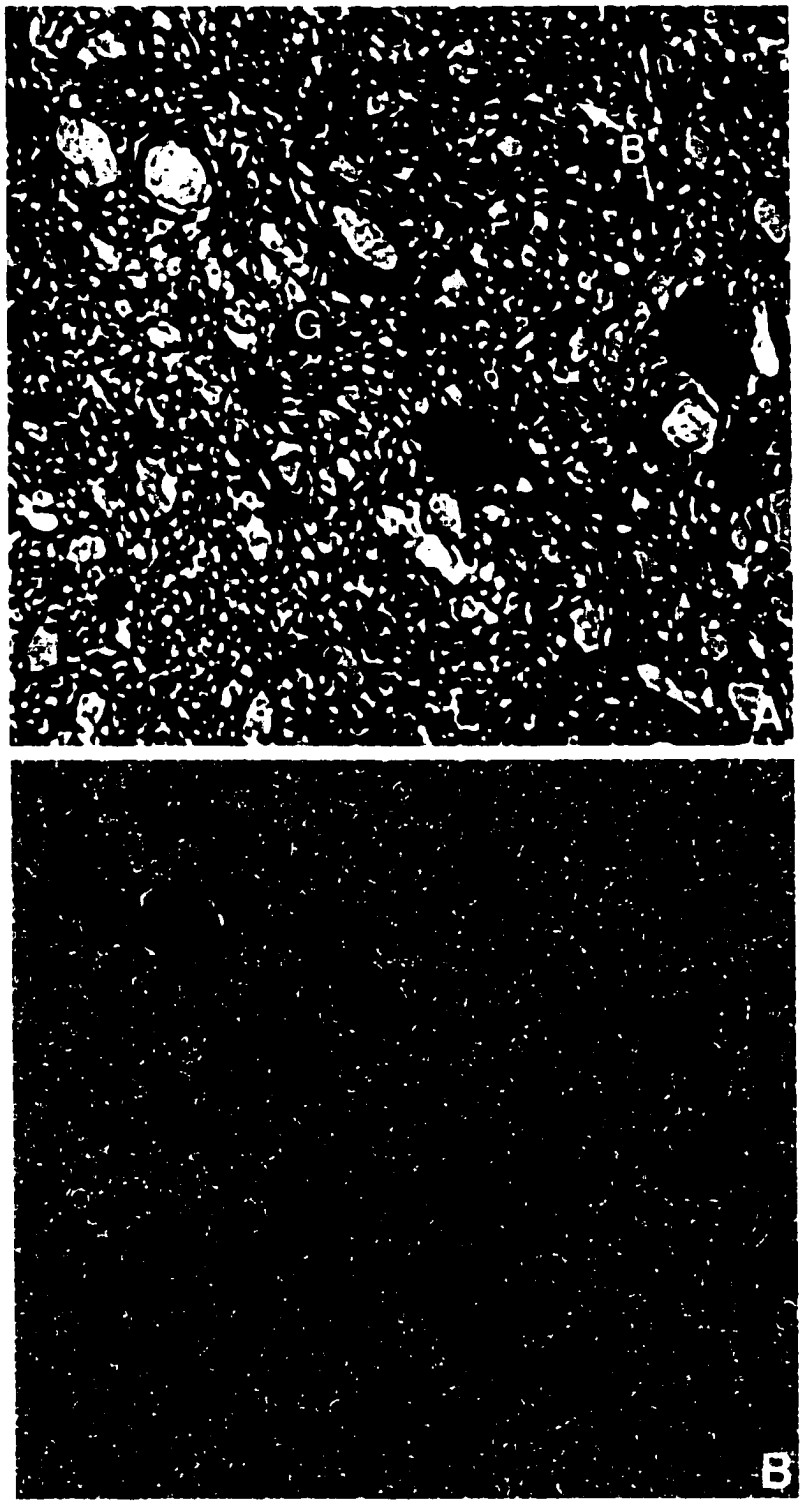

Fig. 4.7. Histological section (1000x) from the splenium of *Macaca fascicularis*, showing the counting regime of the IPLab software. Figure A is of an unsampled section. Figure B is the same section indicating the cells counted by IPLab. G: glial cell, B: air bubble. The latter features are manually removed before the end count is made.

IPLab is a suitable microscopy image capture and analysis program for Apple computers. However, as with other image analysis software it cannot logically discriminate among complete single axons, axons whose membranes contact each other, or partial cells along the edge of the image. Fortunately, the error is small due to the presence of interstitial space created by glial cells. However, before the final analysis of an image can be accepted the image must be carefully examined for counted cells that should be divided, because they are comprised of two or more cells, and partial cells along the edge of the image. In this process the image was first analyzed using IPLab. After the software had processed the image, outlining each cell, the image was magnified in IPLab and examined for incorrectly identified axons. Any partial cells along the edge of the image were rejected from further analysis, since the actual size of these axons could not be reliably determined. In addition, axons with contacting edges that were identified by the software as a single axon instead of two or more axons were divided and the edge of each cell was defined under high magnification. With the cells in an image suitable identified, IPLab was used to measure the number of axons with in an image as well as the diameter and area of each axon. The axonal data for each image was combined with the data from other adjacent images within the same sample and specimen, and compared to other individuals in the sample.

4.4.2 Number of axons in the splenium of the corpus callosum in *Macaca fascicularis* and *Pan troglodytes*

For *Macaca fascicularis* the mean axon density of the splenium of the corpus callosum is 65.68 axons per 100μm^2, placing the total number of axons in the corpus callosum of the *Macaca fascicularis* individuals sampled at approximately 34.3 million on average. Based on the size of the corpus callosum these results are consistent with those obtained by LaMantia and Rakic (1990a; average axon density = 76.6axons/100μm^2; range 38 – 98 axons/100μm^2). Specifically, females exhibit an axon density per 100μm^2 of 64.96. From this average it can be assumed that 10.69 x 10^6 axons can be found in the splenium and 34.24 x 10^6 axons may be found in the corpus callosum as a whole. More specifically, female PGM40 showed 62.73 axons/100μm^2 and female PGM54 showed 70.15 axons/100μm^2 (Table 4 - 3). For males PGM43 displays an axon density of 65.44 axons/100μm^2, while PGM45 displays an axon density of 67.68 axons/100μm^2. These results are discussed in chapter 5.

Table 4 – 3. Splenial axon number for *Macaca fascicularis*

Specimen	Sex	Area sampled (μm^2)	Total # axons in sample area	Axon density (/100μm^2)
PGM 40	female	119638	75047	62.73
PGM 54	female	51533	36154	70.15
PGM 43	male	76589	50118	65.44
PGM 45	male	70520	47731	67.68

Pan troglodytes possesses a larger corpus callosum than *Macaca fascicularis*; however, an increase in the size of this structure does not necessarily indicate an increase the number of axons traversing it. Indeed, *Pan troglodytes* exhibits lower axon densities than *Macaca*. The female specimen (YN94-67) displays an axon density of 45.36 axons/100μm^2, while the male specimen (YN97-139) displays an axon density of 52.07 axons/100μm^2 (Table 4 –4). These values suggest that the corpus callosum of *Pan troglodytes* contains 145 million axons on average (average for YN97-139 = 152 million; average for YN94-67 = 137 million axons), while the splenium contains 41.5 million axons on average (YN97-139, 38.8 million; YN94-67, 44.1 million axons).

Table 4 – 4. Splenial axon number for *Pan troglodytes*

Specimen	Sex	Area sampled (μm^2)	Total # axons in sample area	Axon density (/100μm^2)
YN94-67	female	2396.51	1087	45.36
YN97-139	male	2963.48	1543	52.07

4.4.3 Axonal size types in the splenium

The number of axons traversing the corpus callosum provides some information concerning the composition and possible function of this structure. However, total fiber numbers do not address the role of the corpus callosum in certain cognitive functions, since the integration of those functions between left and right cerebral hemispheres may depend more on the types of fibers carrying information as opposed to the numbers of axonal fibers involved. To understand some of the possible differences between males

and females of the species studied here, the total fiber composition of the splenium has been deconstructed and categorized into fiber groups based on size.

4.4.3.1 Types of axonal size

It is possible to create an infinite number of categories based on the diameter of axons. Axons with diameters smaller than 0.4μm generally fall into the class of unmyelinated axons, while those larger than 0.4μm are always myelinated axons (LaMantia and Rakic, 1990a). Thus, it is possible to create at least two separate and distinct classes. To merely use two categories of classification, though, ignores the variation in transmission time exhibited by myelinated axons of different diameters. For example, larger myelinated axons carry an impulse more quickly along a given length than smaller myelinated axons. Such a difference in transmission time is directly related to an axon's diameter (Kandel et al., 2000). While possessing very large axons may appear favorable, limitations in the size of the corpus callosum or other brain structures limits the number and size of the axons occupying a given region. In humans, Aboitiz et al. (1990a) found many very large axons with diameters exceeding 5.0μm. However, LaMantia and Rakic (1990a) report that while axons of this diameter can be found in the corpus callosum of *Macaca mulatta*, their frequency is so low that there is no need to place them in a separate category ala Aboitiz et al. (1992a).

Using categories defined by Aboitiz et al. (1992a) for humans and LaMantia and Rakic (1990a) for *Macaca mulatta*, five different axonal categories were defined according to axonal diameter. For *Pan troglodytes* the following axonal diameter categories were defined: 1) small axons (myelinated and unmyelinated) whose diameters

are less than 0.4μm; 2) medium axons (myelinated) whose diameters are greater than or equal to 0.4μm and less than 1.0μm; 3) large axons whose diameters are greater than or equal to 1.0μm and less than 2.5μm; 4) very large axons whose diameters are greater than or equal to 2.5μm and less than 5.0 μm; and 5) giant axons who diameters are greater than or equal to 5.0μm. In *Macaca fascicularis* four axonal diameter categories were defined with categories 1 through 3 being the same as those described above for *Pan*, and category 4 defined as the following: 4) very large axons whose diameters are greater than or equal to 2.5μm.

4.4.3.2 Number of splenial fibers within each type of axonal size

With regard to axon diameter categories for *Macaca fascicularis*, it was found that as a percent of the total there is virtually no difference between males (n=2) and females (n=2) in medium (32% of the total number of axons in females to 34% of the total number of axons in males), large (28% in females to 31% in males), or very large axon diameters (12% in females to 13% in males) (Table 4 – 5). The number of small axons as a percent of the total number of axons, however, differs between females (28%) and males (21%), albeit the difference is not statistically significant ($p > 0.8$).

Table 4 – 5. Splenial axon number based on axonal size for *Macaca fascicularis*[1]

Specimen	Sex	Very large axons (≥ 2.5μm)	Large axons (1 - 2.5μm)	Medium axons (0.4 – 0.99μm)	Small axons (< 0.4μm)
PGM 40	F	7073 (.09)	20768 (.28)	27250 (.36)	19956 (.26)
PGM 54	F	4875 (.13)	10831 (.30)	13001 (.36)	7447 (.21)
PGM 43	M	6017 (.12)	14050 (.28)	16136 (.32)	13915 (.28)
PGM 45	M	6220 (.13)	14999 (.31)	16606 (.35)	9906 (.21)

[1]Total number of axons for each axon category. Percentage to the total number of axons in the sampled area is listed in parentheses. Percentages are rounded up. M = male, F = female.

In *Pan troglodytes* there was little difference between the female (YN94-67; n=1) and male (YN97-139; n=1) sampled (Table 4 – 6). As a percentage of the total number of fibers counted, the female had slightly more small (18.4% vs. 16.7%) and large (28.4% vs. 25.5%) diameter axons in the splenium than the male. The male, though, possessed slightly more medium (24% vs. 22%) and giant (5.7% vs. 3.2%) axons than the female. For very large diameter fibers there is no difference between the male and female, since these types of fibers represent 18% of the total in both individuals.

Table 4 – 6. Splenial axon number based on axonal size for *Pan troglodytes*[1]

Specimen	Sex	Giant axons (≥5μm)	Very large axons (2.5 - 5μm)	Large axons (1 - 2.5μm)	Medium axons (0.4 – 0.99μm)	Small axons (< 0.4μm)
YN94-67	F	62 (.06)	197 (.18)	277 (.25)	370 (.34)	181 (.24)
YN97-139	M	50 (.03)	276 (.18)	438 (.28)	495 (.32)	284 (.22)

[1]Total number of axons for each axon category. Percentage to the total number of axons in the sampled area is listed in parentheses. Percentages are rounded up.

While the size of the sample here for both species is too small to compare reliably the sexes statistically, the results can be discussed descriptively. The two individuals of *Pan troglodytes* that were suitable for histological sampling described here demonstrate that the corpus callosum of this species is uniquely different from that of Old World monkeys. *Pan* unlike *Macaca* possesses a larger percentage of the total number of axons greater than 2.5μm in diameter. Although the splenium of the corpus callosum of *Pan troglodytes* is distinct from that of its primate cousin, it is not distinct in displaying any possible development of sex differences in this structure. While the differences in the fiber composition of the splenium of *Pan troglodytes* and *Macaca fascicularis* indicate that one sex may possess more of a particular type of fiber than the other, it is unlikely that any of the differences would be statistically significant. These results will be discussed further in chapter 5.

CHAPTER 5

DISCUSSION AND CONCLUSIONS ON SEX DIFFERENCES IN THE CORPUS CALLOSUM OF *MACACA FASCICULARIS* AND *PAN TROGLODYTES*

5.1 CHAPTER OVERVIEW

In this chapter the results reported in chapters three and four are discussed. Section 5.2 discusses the methods and sampling described in chapters 2 – 4 applied to this study and how they represent improvements compared to previous studies, and/or acceptable means of data collection. Section 5.3 discusses the specific morphological and histological results of this study, while section 5.4 discusses what conclusions may be drawn from the combined morphological and histological results. Finally, section 5.5 briefly discusses the results in terms of primate brain evolution.

5.2 ADVANCES IN METHODS AND SAMPLING OF THE PRIMATE CORPUS CALLOSUM

Neuroscience, morphological, and anthropological studies have the ability to employ a variety of data acquisition techniques. With the aid of computers it is not only possible to assess more data points, but it is possible to peer deeper into structures than ever before. For example, since the advent of functional MRI (fMRI) some fifteen years ago, researchers have sought to disclose the structural-functional relationships of the brain. In addition, improvements in laser, mechanical, and magnetic field computational

methodologies have improved the likelihood of morphometrics as a dominant force in taxonomy and systematics (e.g., Bookstein, 1997; Delson et al., 2001). The methods employed in this study have sought to employ the most advanced technologies applicable.

The corpus callosum in its midsagittal profile is a complex shape not easily measured by conventional means. The first attempts to measure this structure using computer software were by Denenberg et al. (1991a). The software designed for their measurements (KSS Stereology) used digitized line drawings of the profile of corpus callosum. These digitized images were then divided into 99 equal sections based on anterior – posterior callosal length, which was determined as the distance from the terminal end of the rostrum to the terminal end of the splenium. Each region of the corpus callosum was defined as the area occupied by ten sections. As Denenberg et al. (1991a) explain, this methodology allows for factor analysis of morphological measurements depicting features of circularity, genu asymmetry based on curvature, and apex asymmetry. However, as the results of Denenberg et al. (1991b) attest, these features have little meaning with regard to intraspecies differences. The many prior and subsequent studies mentioned here have instead relied on proportional divisions of the corpus callosum when examining intraspecies variation.

For this study computer software (SigmaScan Pro) was used to assess morphological differences between males and females in *Macaca fascicularis* and *Pan troglodytes*. This software represents a definite advantage over other morphological methodologies employed. Namely, the ability to measure photographic images with dense pixelation permits more precise measurements of the corpus callosum and its regions than any other

current method. Such data acquisition methods are necessary when minor variations in size may yield major differences in morphology, composition and function. In addition, morphological measurements of the corpus callosum of the species sampled here are necessary, since they represent the only reasonable means of comparison to the numerous studies reporting sex differences in the human corpus callosum.

Unlike most bony elements that yield functional information from morphological measurements, morphological measurements on the nervous system, especially the brain, often provide only cursory information concerning function. To interpret differences between individuals with regard to nervous functions it is often necessary to decipher the composition of the structure of concern. This study presents an examination of the composition of the corpus callosum in order to determine whether differences in cognition between males and females are the result of the number and/or types of fibers comprising the corpus callosum. The importance of this approach is that no other study has attempted to examine sex differences in the corpus callosum of a nonhuman primate using both morphological and histological approaches.

The histological methods employed here represent traditional and not novel techniques in histology. However, the methods used for counting and comparing the axons comprising the corpus callosum do represent methodological advancements. Using digital image capture and analysis, it was possible to examine areas twice the size of those sampled by LaMantia and Rakic (1990a). This meant that it was possible to sample a larger number of fibers, and thus gain a better estimation of the true composition of the splenium of the corpus callosum. In addition, the use of image analysis software, specifically designed for the analysis of histological sections, removes much of the error

created by hand counts of cells. For example, the axons in a single image used in this study were counted by hand as well as the IPLab software. The hand count observed more than a hundred fewer cells than the software. Most of these axons were small and visually difficult to identify due to the density of cells in the section. Since the specific importance of the various axonal size classes is unknown, it is important that any study examining the fiber composition of a structure such as the corpus callosum utilize methods that are capable of accounting for every axon as this study does.

This study attempts to make use of the most advanced and applicable methods available for assessing sex differences in the corpus callosum. The morphological analysis, while using established definitions for defining callosal regions, employed advanced measurement techniques capable of providing accurate results. In addition, the histological methods used here attempt to employ reliable methodologies combined with advanced analysis in order to produce reliable, repeatable results.

5.3 DISCUSSION OF THE RESULTS OF THIS STUDY

This section discusses the results for morphological areas of the corpus callosum in each species sampled. Section 5.3.1 discusses differences or lack thereof in total callosal area in *Macaca fascicularis*. Section 5.3.2 offers independent discussion of the area differences between males and females for each callosal region. Sections 5.3.3 and 5.3.4 provide similar discussions for these area measurements in *Pan troglodytes*. Finally, section 5.3.5 discusses the meaning of the histological results for the individuals and species sampled.

5.3.1 Sex differences in total midsagittal callosal area of *Macaca fascicularis*

Measurements of total callosal area (section 3.3.1.1) indicate that males on average possess a slightly larger corpus callosum than females. This difference, however, is only relevant if absolute measurements of the corpus callosum are deemed the sole satisfactory indicators of sexual differences as argued by Fitch and Denenberg (1998) and Bishop and Wahlsten (1997, 1999). While the statistical approach of these two reports are arguable, the evidence for the presence of allometric differences between males and females of sexually dimorphic species is incontrovertible (Holloway, 1979, 1980; Holloway and Post, 1982; Martin, 1982; Peters et al., 1998). *Macaca fascicularis*, like most other primate species, exhibits sexual dimorphism. In general, males are absolutely larger with regard to body size than females. The result of such a size difference is that males possess absolutely larger brains than females. From this observation it can be assumed that these allometric effects are exhibited in brain constituents (Finlay and Darlington, 1995; Semendeferi and Damasio, 2000; Clark et al., 2001). Moreover, the presence of resultant allometric differences for many nonhuman primates with regard to the corpus callosum is well documented (Holloway and Heilbroner, 1992; Franklin et al., 2000). Acceptance of these allometric differences between males and females of a given species requires the standardization of morphological measurements when comparing males and females.

For *Macaca fascicularis*, males on average possess an absolutely larger corpus callosum then females. However, when these results are standardized according to brain weight the differences between males and females for total callosal area disappears and are statistically insignificant. Such a result indicates that any cognitive differences

between males and females of this species are probably caused by the overall structure of the corpus callosum. In addition, they would also suggest that sex differences in this structure as seen in modern humans (see de Lacoste and Holloway, 1982) likely do not have their origins within the cercopithecoid clade. However, this conclusion may be somewhat presumptuous at this stage when discussing the results of this research, because it assumes that evolution of the corpus callosum results in an overall change in this structure rather than a mosaic alteration. To give consideration to the idea of mosaic evolution within the corpus callosum it is necessary to discuss the results for regional callosal areas first. These are discussed below.

5.3.2 Sex differences in regional callosal area of *Macaca fascicularis*

Male and female *Macaca fascicularis* do not differ from each other with regard to the relative size of the corpus callosum. However, the corpus callosum is an enormous and complex structure within the brain. As such it is necessary to investigate the possibility of sex differences within different callosal regions. The results of the regional measurements for this species are discussed here.

5.3.2.1 Genu and rostral body

The genu as defined using the straight-line method is roughly equivalent to the genu and rostral body as defined through the radial-line method. As such these areas occupying the anterior one-fifth of the corpus callosum will be referred to as the genu here. The genu represents one region of the corpus callosum that exhibits significant evolution based on shape. In *Macaca fascicularis* the genu ends in an inferiorly

positioned spit of tissue referred to in humans as the rostrum. However, while the rostrum in humans is easily demarcated, since it recurves under the genu, the supposed rostrum of the macaque lack any recurved portion that may permit its designation as a distinct callosal region.

Macaca fascicularis does not exhibit any statistically significant difference between males and females either in the absolute or relative size of the genu. The lack of sex differences in this area is unsurprising, since the genu is thought to connect portions of the motor cortex as well as areas within the prefrontal cortex (Pandya et al., 1971; de Lacoste, 1981; Seltzer and Pandya, 1983; Barbas and Pandya, 1984). Differences in this region should not be suspected, simply because there is little information to suggest that male and female members of *Macaca fascicularis* differ from each other with regard to motor skills. In addition, while fibers traversing this region of the corpus callosum connect portions of the orbitofrontal cortex, which is important for both memory and behavior (Parker et al., 1997), there is little information in *Macaca* suggesting that males and females differ significantly in these tasks (Lacreuse et al., 1999).

5.3.2.2 Midbody

The anterior and posterior midbodies represent the two divisions of the midbody defined using the straight and radial line methods. The anterior portion of this region contains interhemispheric fibers connecting the primary, secondary, and supplementary motor cortices, while the posterior portion connects primary and secondary sensory areas (Pandya et al., 1969; de Lacoste, 1981; Pandya and Seltzer, 1986). In addition, the posterior portion of the midbody possesses fibers connecting the postcentral and posterior

parietal lobe as well as portions of the superior and inferior temporal lobes (de Lacoste, 1981; Seltzer and Pandya, 1983; LaMantia and Rakic, 1990a). Despite the complexity of the connections passing through this region, the midbody areas for males and females of *Macaca fascicularis* do not differ significantly from each other (see sections 3.4.2 and 3.4.3). Albeit these results are not unusual when compared to the human data (Oppenheim et al., 1987; Allen et al., 1991; Witelson, 1989; Matano and Nakano, 1998), they are somewhat unexpected given the role of callosal axons passing through the midbody in sexually dimorphic tasks. Further discussion on why the midbody should exhibit variation between males and females is discussed in section 5.4.

5.3.2.3 Isthmus

This study found that there are no sexual differences in the area of the isthmus of the corpus callosum in *Macaca fascicularis* using either the straight-line or radial-line method (see sections 3.4.2 and 3.4.3). The fibers passing through this region are presumed to connect portions of the superior and inferior temporal lobe along with a small portion of the posterior parietal lobe (Pandya et al., 1971; Pandya and Seltzer, 1986; LaMantia and Rakic, 1990a; see also de Lacoste, 1981). Based on these topographic maps and deficit studies (see Gazzaniga, 2000), it is presumed that these callosal fibers in humans are important for language and somatosensory integration and cognitive functioning. It is not likely, though, that these regions perform the same cognitive functions in macaques as they do in humans. For example, both Witelson (1989) and Steinmetz et al. (1992) report sex differences in the overall area of the isthmus, and Davatzikos and Resnick (1998) report sex differences in the posterior

portion of the isthmus in humans. If the isthmus serves to connect certain language-related areas in the human brain, then these results are unsurprising. While macaques may possess features and connections in the brain homologous to language areas in humans, there is no evidence suggesting that macaques possess any language-related skills approaching those of modern humans (Deacon, 1997; Parr et al., 2000). It is possible that the sex differences seen in humans with regard to isthmus area are dependent upon the advent of sex differences in language or visuospatial areas of the brain, which may not have arisen until later in hominin evolution (Broadfield et al., 2001).

5.3.2.3 Splenium

The splenium represents the region of the corpus callosum that has often been found to exhibit sexual dimorphism in humans (Holloway, 1990; Holloway et al., 1993; Davatzikos and Resnick, 1998). Moreover, area differences between males and females do not appear to be the result of isometric expansion of the splenium in one sex versus the other. Instead the relatively larger splenium of human females is also more bulbous than that of males (de Lacoste and Holloway, 1982; Holloway et al., 1993; Davatzikos and Resnick, 1998). Since the splenium is responsible for connecting occipital, temporal, and posterior parietal areas of the brain (Pandya and Seltzer, 1986; Gazzaniga, 2000), it is possible that these area and form differences may be related to sex differences in visuospatial, language, and somatosensory cognitive functions.

The splenium of the *Macaca fascicularis* sample used in this study did not exhibit any sex differences (see sections 3.4.2 and 3.4.3). While males possessed absolutely larger

splenia using the straight and radial-line methods, this difference was eliminated when brain size was taken into account. In fact, the samples overlap entirely. Since the composition and form of the corpus callosum appears to be the result of cortical size and function (see section 1.3), it is unlikely that the hypothesis that there are sex differences in the corpus callosum of cercopithecines would be true.

Cercopithecines do not appear to show the same level of cortical development that has been exhibited in the hominin lineage over the past three million years (Holloway, 1968; Jerison, 1973; Radinsky, 1975; Heilbroner and Holloway, 1989; Allman, 1982). While the basic callosal connections through the splenium and other callosal regions appear to be similar between humans and macaques, and the cortical regions they connect appear to perform the same basic functions (e.g., hearing, vision, tactile sensation); there is no evidence suggesting that macaques and humans share the ability for higher level cognitive functions. It is probable that humans demonstrate certain sex differences in the splenium due to the presence of fibers involved in higher level cognitive processes, such as the integration of visuospatial-related information. However, the manifestation of these sex dimorphic cognitive functions in the splenium is argumentative with some researchers expressing that sex differences exist in humans in this region (e.g., Holloway et al., 1993, see also section 1.5.1), and others contending their absence (e.g., Witelson, 1989). Since the presence or of sex differences in the splenium for modern human populations is arguable, it is less likely that sex differences in this region of the corpus callosum should be expected in macaques or other cercopithecine groups. Indeed, the evidence from this study suggests that sex differences with regard to the area of the

splenium are not present in the *Macaca fascicularis*, suggesting that sex differences in this region would be lacking in other cercopithecine groups.

5.3.3 Sex differences in the total midsagittal area of the corpus callosum in *Pan troglodytes*

Sex differences or the lack thereof in the corpus callosum of *Macaca fascicularis* provide information regarding the evolution of sex differences in this structure as well as implications for the evolution of sex differences in cognitive function. However, the lack of sex differences in this species merely implies the development of sex differences in the corpus callosum early in cercopithecine evolution. Since hominoid and cercopithecoid lineages diverged from each other approximately 27 – 30 million years ago (Fleagle, 1999), the question of when sex differences in the corpus callosum came about is left unanswered due to the gapping space in time between the this divergence and the advent of hominins. To close this gap it is necessary to examine a more recent relative of modern humans. Because of the lack of complex cerebral architecture and cognitive behaviors in macaques, the species chosen to answer the above question should possess cortical and cognitive features that are most similar to humans. For this reason *Pan troglodytes* was examined in this study.

Female *Pan troglodytes* possess an absolutely and relatively larger corpus callosum. However, these differences are statistically insignificant (see section 3.3.2). Despite the lack of a significant difference between males and females, it is worth noting that unlike *Macaca fascicularis* the corpus callosum in *Pan troglodytes* trends toward being larger in females. There are several reasons why this result was observed here. First, the female

brains (*Pan troglodytes*, n=12) obtained for this study tended to be fresher than the male brains (*Pan troglodytes*, n=11), that is access to the brain was available shortly after death as opposed to some of the males which where incorporated into the study many months or years after initial fixation. For example, due to the general lack of male brains available for purchase, several male brains from the Smithsonian collection were incorporated into the morphological assessment phase of this study. These brains were collected during the course of several hunting expeditions in the early 1900s. While the brains have remained in good condition, one must be aware that the weight and dimensions of the brains have decreased over the eighty years they have been immersed in fixative. A comparison of the skeletal material of these individuals demonstrates the typical dimorphism exhibited in this species with males being larger than females. The fixed tissue, however, does not demonstrate the same degree of dimorphism. This is principally due to the differential shrinkage of tissue. Based on initial brain weights collects on these specimens it is apparent that the brains have shrunk to approximately seventy-five percent of their initial size. While this may appear to render the results gained from these particular individuals invalid, it is important to note that based on cursory data examined here as well as information obtained on humans, the corpus callosum shrinks proportionally to the brain (Peters et al., 2000). Thus, the comparison of relative values between males and females should provide a reasonable measure of sexual dimorphism.

A second possibility for the differences, albeit insignificant, observed here between males and females may be the result of age. Age is a variable that is of concern in human studies, since it has been shown that the corpus callosum functions and atrophies

differently as males and females age (Hayakawa et al., 1989; Jeeves and Moes, 1996; Aboitiz et al., 1996; Salat et al, 1997). It is, however, uncertain how aging affects the chimpanzee brain. The sample used for this study is comprised of individuals equally distributed across adult age groups. Thus, the sample does not possess an overrepresentation of young adults or aged individuals. With regard to aged chimpanzees it is worth noting that none of the individuals used in this study from Yerkes Regional Primate Center appeared to have suffered from cognitive decline related to illness or age (Yerkes colony and necropsy files, 1997; Dahl, pers. comm.). Based on this information it is unlikely that age would be a factor. Moreover, comparisons of similarly aged individuals indicate that females at every age group possess a slightly larger corpus callosum than males.

A third possibility for the results observed here is that females do possess a corpus callosum that is slightly larger than that of males. While the results are statistically insignificant, the raw values of absolute and relative size of the corpus callosum indicate a trend towards females possessing larger callosa. This would also imply that males and females had begun to develop cognitive and/or structural differences in the brain around the time of the ape-human split some seven million years ago. However, due to a paucity of data it is difficult to discern how different male and female chimpanzees are cognitively. While male and female *Pan troglodytes* display different behaviors (Wrangham, 1980; Goodall, 1990), it is nevertheless difficult to determine how these behavioral differences are manifested in the brain. Moreover, it is hard to say how sex differences in the brain of this species affect the composition and structure of the corpus

callosum. For this reason, regional areas were measured to determine whether certain regions express sexual dimorphism. These regional results are discussed below.

5.3.4 Sex differences in regional areas of the corpus callosum in *Pan troglodytes*

Measurements of total callosal area provide some information regarding the presence of sexual differences of the structure, but they do not provide specific information that may be useful for the assessment of possible lobular or cognitive differences in the brain. To gain insight into such differences when examining the midline profile of the corpus callosum it is necessary to examine callosal regions. Below the results for each region are discussed.

5.3.4.1 Genu and rostral body

The genu as defined using the straight-line method is roughly equivalent to the genu and rostral body as defined through the radial-line method. As such these areas occupying the anterior one-fifth of the corpus callosum will be referred to as the genu here. For both the straight and radial-line methods females possess and absolutely larger genu than males. However, the relative values of this structure do not indicate any difference between males and females. In addition, there is no statistical difference between males and females in the genu. The lack of a significant difference between males and females in the genu means that sex differences in this region as displayed in humans (Witelson, 1989) must have evolved after the ape-human split.

5.3.4.2 Midbody

Both the absolute and relative values for midbody area differences between males and females are statistically insignificant for *Pan troglodytes*. The averages for the anterior and posterior midbody using the straight-line and radial-line methods display significant overlap, such that there is no apparent trend towards one sex possessing a slightly larger midbody than the other. For example, the greatest difference between males and females occurs when the averages of the relative size of the anterior midbody as defined using the straight-line method are compared. The average relative size of the anterior midbody is $0.0095cm^2$ for females and $0.0086cm^2$ for males. However, the standard deviation of the sample is large, and thus there is a significant degree of overlap. The lack of sexual differences in this area, though, is expected, since the areas of the brain connected by fibers passing through this region have not become highly specialized over the course of primate evolution. Moreover, humans do not display any sexual differences in this area (Witelson, 1989), and as such it is not expected that *Pan* would.

5.3.4.3 Isthmus

The isthmus of the human corpus callosum displays sexual dimorphism with females possessing a relatively larger isthmus than males (Witelson, 1989; Steinmetz et al., 1992; see also Davatzikos and Resnick, 1998). Due to this relationship it is hypothesized that female *Pan troglodytes* may also possess a relatively larger isthmus. Indeed females possess an absolutely and relatively larger isthmus on average as defined using the straight-line method. However, there is a large degree of overlap between the two samples, and thus there is not a statistically significant relationship between sex and

isthmus size. This means that any statistically significant sex differences in this region are unique to humans, and must have evolved after the ape-human split. Alternately, chimps may have retained the earliest trends toward such a dimorphism.

5.3.4.2 Splenium

The splenium of the corpus callosum has been an area of intense interest in human studies (de Lacoste and Holloway, 1982; Oppenheim et al., 1986; Holloway, 1990, 1993; see also section 1.5.1). Bean (1906) had first described the splenium of females as being different from males. Later studies found similar differences and described the female splenium as more bulbous. This general description has become useful in identifying the corpus callosum of human females, although the functional significance of this morphology has not been deciphered. Some researchers, though, have suggested that despite this general morphological dissimilarity between males and females sex differences in the human splenium do not exist (e.g., Witelson, 1989; see also section 1.5). Due to the disparity of splenial data from humans it is not possible to predict the presence of sex differences in this region in *Pan troglodytes*. Indeed, there is not a significant difference between male and female *Pan troglodytes* for area measurements of the splenium using the straight or radial line method.

5.3.5 Measures of histological sections of the splenium

Regional and total area measurements of the corpus callosum provide useful information of the overall structure of this interhemispheric highway. Not only is it possible to assess sexual differences based on area, but it is also possible to assess disease from callosal morphology. For example, schizophrenia (Narr, 2000), dyslexia (Rumsey et al., 1996), Alzheimer's (Vermersch et al., 1996; Thompson et al., 1998), autism (Hardan et al., 2000) and attention-deficit hyperactivity disorder (Lyoo et al., 1996) produce changes in the midsagittal profile of the corpus callosum that distinguishes a person with one of these diseases from a normal individual. However, individuals with a disease like one of these do not differ from the normal population based merely upon the general size and shape of the corpus callosum. Instead these diseases are defined according to the cognitive or neural deficits they produce in an otherwise normal individual. In turn the function of a cortical structure or its relationship to cognitive functioning cannot always be discerned from its dimensions. For example, to determine the reason for the difference between a normal and schizophrenic corpus callosum it is necessary to determine the compositional differences in the structure. The same can be said for determining the differences between the male and female corpus callosum.

In their study of the composition of the corpus callosum, LaMantia and Rakic (1990a) found no appreciable difference between a single male and female *Macaca mulatta* for the total number of fibers comprising this structure. Concurring with LaMantia and Rakic (1990a), this current study found no difference in the number of fibers comprising the splenium as well as the total number of fibers inferred to compose the entire corpus callosum in both *Macaca fascicularis* and *Pan troglodytes*. This is consistent with results

on morphological measurements of total and regional callosal area (LaMantia and Rakic, 1990a; Aboitiz et al., 1992a; see also Highley, 1999). However, contra to LaMantia and Rakic, (1990a) who suggest that males have slightly more axons in the corpus callosum than females, this study found that females possess slightly more axons than males. From this it can be assumed that there is a large degree of variability expressed in *Macaca* with regard to the total number of axons in the corpus callosum. In addition, this study concurs with the conclusion of Aboitiz et al. (1992a), which states that if the overall area of a callosal region does not demonstrate sexual dimorphism then one would not expect to find a difference in the number of fibers comprising that area.

In humans, Aboitiz et al. (1992a) found no appreciable difference between males and females in the number of fibers comprising the corpus callosum. This would indicate that even apparent sexual differences in the size of the corpus callosum do not impart any correlation to its composition. Aboitiz et al. (1992a) predict that the area of the corpus callosum is a good indicator of the number of fibers contained in it. However, they go on to acknowledge that this predictive hypothesis may not be accurate for estimating the number of gigantic fibers (> 3μm in diameter). For this reason it is not possible to propose the presence or lack of sex difference in the corpus callosum by merely estimating the total numbers of fibers it contains. Instead it is necessary to additionally account for the types of fibers comprising the corpus callosum. Thus, counting the total number of fibers in the corpus callosum is only one step to the conclusion of assessing sexual dimorphism in this structure.

5.3.5.1 Sex differences in the types of axons passing through the splenium of the corpus callosum in Macaca fascicularis and Pan troglodytes

Axons of varying diameters traverse the midline of the corpus callosum. To determine the presence or lack of sex differences in the splenium of the corpus callosum in *Macaca fascicularis* and *Pan troglodytes* the types of fibers comprising this structure were counted. Fibers types were determined according to the diameter of the axon. Definitions for each class can be found in section 4.4.3.

There is no significant difference between *Macaca fascicularis* males and females with regard to fiber type (see section 4.4.3.2). While these results combined with those from other aspects of this study conclusively show that there are no sex differences in the corpus callosum of this species, they can be discussed descriptively to provide information that may be useful for drawing a hypothesis on the evolution of sex differences in the brain. Males of this species tend to possess more medium, large, and very large axons than females. Females conversely tend to possess more small axons than males. While the differences between males and females are not statistically significant, this descriptive information does offer some insight into relative differences between males and females.

The differences between the male and female *Pan troglodytes* sampled do not appear to be significant. Proportionally, the female possesses a greater number of fibers than the male, but based on data from macaques (LaMantia and Rakic, 1990a) and humans (Aboitiz et al., 1992a; Highley et al., 1999) this type of variation between individuals for total callosal axon number is not unusual. While the difference in the total number of axons in the corpus callosum between the male and female sampled demonstrate that sex

differences in this structure most likely do not exist, it is, nevertheless, possible to discuss the general differences in the types of fibers found in the splenium of these individuals .

The male *Pan troglodytes* sampled possesses more small and large diameter axons than the female, while the female possesses more medium, very large, and giant axons than the male (see section 4.4.3.2). While this cannot be tested statistically due to the small sample size, it can be assumed by examining the number of axons in each category and their percentage to the total number of axons that there is not sexual dimorphism with regard to types of fibers in the splenium of the corpus callosum. This data can provide basic descriptive information regarding possible sex differences in this species and the evolution of sex differences in general. For example, the female *Pan troglodytes* possessed more medium and very large fibers than the male, while the female *Macaca fascicularis* were found to possess only more small axons than the males. This difference as expressed in *Pan* is similar to the result obtained by Aboitiz et al. (1992a) for humans, speculatively implying that the structure of the corpus callosum in *Pan* is more similar to humans than to cercopithecoids.

5.4 CONCLUSIONS REGARDING SEX DIFFERENCES IN CALLOSAL AREAS AND SPLENIAL AXON COMPOSITION IN *MACACA FASCICULARIS* AND *PAN TROGLODYTES*

The conclusion of this study is that based on measurements of the total midsagittal area of the corpus callosum, midsagittal regional areas of the corpus callosum, and the number and type of axons in the splenium of the corpus callosum, there are no sex differences in this structure in *Macaca fascicularis* or *Pan troglodytes*. Indeed, neither species exhibits a statistical trend, indicating that one sex may possess a larger callosum, more axons, or more of a particular type of axon. From these results it is also possible to conclude that modern humans are the only extant primate group that exhibits any sexual dimorphism in the corpus callosum or its regions. In some ways these results are consistent with the literature suggesting specialized lateralization of the human brain, and sex differences exhibited in lateralized cortical processes (e.g., Witelson, 1977; Kimura, 1980, 1983; Hugdahl et al., 1993; Eviatar et al., 1997; Crucian and Berenbaum, 1998; Halpern et al., 1998; Hausmann and Gunturkun, 1999; Vallortigara et al., 1999; Amunts et al., 2000; also see review by McGlone 1980). This is because many lateralized processes often are related to functions of speech and language, which have never been isolated in nonhuman primates.

Despite the apparent lack of lateralization in the nonhuman primate brain with regard to language, there have been other studies that indicate the brain of nonhuman primates may be lateralized (e.g., Gannon et al., 1998). However, many of these studies depend on correlations between handedness and a given task (Note: the author disagrees with the usage of the term handedness as it has been applied in many of the following

psychological studies, and prefers the term hand preference). For example, Bard et al. (1990) found that *Pan troglodytes* displays a general right hand preference during feeding behaviors. At the same time Hopkins (1990) found that *Pan* and *Pongo* display a general right hand preference in an experimental model requiring subjects to manipulate a joystick (see also review by Hopkins and Morris, 1993). Later, Hopkins and his colleagues have correlated hand preference to birth order (Hopkins and Dahl, 2000), gestural communication (Hopkins and Leavens, 1998), and other manipulation tasks (Hopkins and Pearson, 2000). Although these particular studies do not provide definitive data on the lateralization of the nonhuman primate brain, they do provide a means to understand the origins of laterality.

Recent anatomical asymmetries have been noted in the brains of great apes but not Old World or New World monkeys (Hopkins and Rilling, 2000; Hopkins and Marino, 2000). In their study on petalial patterns in primates using left and right anterior frontal, posterior frontal, parietal, and occipital cerebral width measurements on axial magnetic resonance images[3], Hopkins and Marino (2000) found that the great apes (*Pan*, *Gorilla*, *Pongo*) display a right-frontal, left-occipital directional asymmetry or petalia pattern. While there was an individual from each taxon that displayed the converse asymmetry, the results for these genera were more consistent than for other groups. That is, Old and New World genera did not display directional asymmetry, albeit certain individuals within the *Macaca mulatta* sample did. Working from the same dataset Hopkins and

[3] Measurements were taken on an image derived from the first axial slice superior to the third ventricle. Left and right cerebral hemispheres were defined by drawing a line along the interhemispheric cleft from the frontal interhemispheric cleft point to the occipital interhemispheric cleft point. Measurement points were defined as follows: anterior-frontal (10% from frontal pole), posterior-frontal (30% from the frontal pole), parietal lobe (30% from occipital pole) and the occipital lobe (10% from occipital pole).

Rilling (2000) report that measured asymmetries in neocortical surface area and brain volume indicate that the brains of the great apes are more asymmetrical than those of Old and New World monkeys. Moreover, this particular study suggests that individuals that possess a more leftward asymmetric brain had a smaller corpus callosum than those individuals that displayed rightward or no asymmetry. Handedness (hand preference) data collected by Hopkins (1995) and Westergaard et al. (1998) suggest that there is a general shift in primates from population-level left-hand preference to population right-handedness for quadrupedal and bipedal reaching such that *Pan* more often displays a preference for right handed reaching and manipulation than Old and New World primate groups. Moreover, individuals that display right-handedness or right hand preference possess a smaller corpus callosum as a function of neocortical surface area and brain volume (Hopkins and Rilling, 2000). While this finding cannot confirm the presence of lateralized brain function in any of these species studied, especially *Pan*, it does suggest an early evolution for the development of lateralization.

Lateralization of the primate brain is important for understanding the evolution of sex differences in the brain as well as sex differences in the corpus callosum. It is unfortunate that Hopkins and Rilling (2000) do not report the measurements of each male and female in their species specific data, since this data would provide information concerning sex differences in lateralization and the size of the corpus callosum. However, since Hopkins and Marino (2000) utilize the same data set of MRIs it is possible to glean certain information regarding sex differences in lateralization. Based on the reported asymmetry quotients (Table 5 – 1) it seems that *Pan troglodytes* males and females do not differ from each other with regard to asymmetry of the neocortex based

on petalial pattern. This is somewhat unexpected, since some published reports suggest a sex difference for certain behavioral and motor tasks.

Table 5 – 1. Individual asymmetry quotient (AQ) data for *Pan troglodytes* from Hopkins and Marino (2000)

Sex	AF	PF	PAR	OCCP
M	0.104	0.086	0.036	-0.043
M	0.030	0.010	-0.058	-0.019
M	0.074	0.080	-0.037	-0.018
M	0.008	0.024	0.039	-0.011
M	-0.074	-0.103	-0.094	0.180
F	0.041	0.076	-0.048	-0.080
F	0.035	0.030	-0.007	-0.034
F	0.040	0.040	-0.054	-0.081
F	0.022	0.052	0.034	-0.137

AF • anterior frontal, PF • posterior frontal, PAR • parietal lobe, OCCP • occipital lobe. For each subject and region, an asymmetry quotient (AQ) was derived following the formula [AQ=((R-L/R+L)*0.5)]. Positive AQ values reflected a rightward bias and negative values reflected a leftward bias. The absolute value of the AQ score reflected the magnitude of the bias. (after Hopkins and Marino, 2000)

Experiments designed to test cognitive skills in nonhuman primates, such as handedness, provide important data that can be used to formulate hypotheses concerning the origins of brain lateralization as well as the development of sex differences in the brain. In addition to handedness or hand preference studies, other behavioral experiments have been report that may enhance these evolutionary and cognitive hypotheses. Data collected from memory and cognitive performance studies on nonhuman primates indicate that certain male-female differences occur. In particular, several studies have

found that male and female *Macaca mulatta* differ from each other with regard to facial discrimination tasks (Buccafusco et al., 2000; Lacreuse et al., 1999; Parr et al., 2000). For example, Buccafusco et al. (2000) reports that male *Macaca mulatta* performed better on memory-related tasks compared to females, although these tasks required simple memory recall, and not recall of complex subjects.

Complex subject recall requires the individual to not only recall specific subject matter, but also associated features of the item in question. In humans such complex tasks are usually associated with language tasks (Hugdahl et al., 1993; Hadar et al., 1998; Hausmann and Gunturkun, 1999). For example, when an individual is required to recognize familiar faces prefrontal and lateral temporal regions are bilaterally activated. However, when an individual is exposed to newly learned or unfamiliar faces hippocampal, parahippocampal, parietal and anterior temporal activation is observed (Clark et al., 1998; Leveroni et al., 2000). Observations such as these are significant, since these tasks, except for the hippocampal responses, require the participation of callosal axons. Moreover, males and females are dissimilar from each other for these and many cognitive tasks involving language areas (Shaywitz et al., 1995; Levin et al., 1996; Gur et al., 1999; see also Kimura, 1983, 1987).

Although macaques do not possess cognitive abilities approaching those of humans, studies on these nonhuman primates indicate that they possess some ability to perform tasks such as facial recognition and recognition of facial cues (Vermeire et al., 1998; Parr et al., 2000). While it is not currently possible to test nonhuman primates with PET or fMRI to determine the specific functional areas of their brain, it is possible to use topographic studies to draw some correlations between cortical anatomy and possible

cognitive functions. Work by de Lacoste (1981), Pandya and his colleagues (Pandya et al., 1969; Seltzer and Pandya, 1983; Barbas and Pandya, 1984), and LaMantia and Rakic (1990a,b) indicate that humans and macaques share many functional areas within the cerebral cortex. From such correlations it is possible to hypothesize that if sex differences exist with regard to certain cognitive functions that males and females may demonstrate differences in the callosal fibers associated with those tasks. For facial recognition tasks these fibers likely, in part, pass through the midbody of the corpus callosum. Thus, it is probable that the midbody would be different between males and females. The data presented here, though, concur with measurements on humans indicating there is no difference between males and females in the area of the midbody of the corpus callosum (Oppenheim et al., 1987; Allen et al., 1991; Witelson, 1989; Matano and Nakano, 1998).

The above behavioral studies are restricted to *Macaca*, but other data also provide important information suggesting the presence of sex differences in the brains, and possibly the corpus callosum, of nonhuman primates. Two recent studies involving *Pan troglodytes* suggest that this species possesses memory and recall abilities that exceed those displayed by *Macaca mulatta*. In the first study, Menzel (1999) reports the ability of a single female *Pan troglodytes* that retained the ability to recall the locations of randomly hidden objects for up to sixteen hours. In the second study, Parr et al. (2000) report that *Pan troglodytes* displays a greater recall of conspecifics facial features than *Macaca mulatta*. In this last report chimpanzee individuals were required to match similar pictures of conspecifics. While the both *Macaca mulatta* individuals and chimpanzees displayed an equal ability to discriminate conspecifics, the *Macaca mulatta*

individuals required significantly more trials to be able to perform the task successfully. Although these reports could be described as rudimentary behavioral studies, they do still suggest the possible presence of specialization (and possibly lateralization) in the nonhuman primate brain.

Three final studies that are more relevant to the current study than many of those discussed above include spatial experiments performed on *Macaca mulatta*. This is because both of the following studies not only discuss the likely presence of lateralized function in parts of the nonhuman primate brain, but also the presence of sex differences on spatial tasks. In an experiment on twenty-six split-brain *Macaca mulatta*, Vermeire et al. (1998) found that faces were better remembered by the right hemisphere than the left. In addition, they also found that females monkeys were more lateralized for learning to discriminate faces than were males. A later study by Kavcic et al. (2000) agrees with the above findings that left hemisphere dominance for certain visual-memory tasks occurs in *Macaca*. Finally, work by Lacreuse et al. (1999) shows that *Macaca mulatta* displays sex differences with regard to spatial ability. However, it should be noted that Lacreuse et al. (2000) found a decline in spatial ability among males as they age, such that old males perform no better than old females. Yet for any given age class, except this late one, males outperform females in spatial cognitive tasks.

The studies discussed above report provocative results that suggest the presence of lateralization for certain tasks in nonhuman primates. While chimpanzees seem to possess greater asymmetry and cognitive abilities than macaques, macaques do appear to exhibit some lateralization in cognitive function. Moreover, males and females differ in some of these functional tasks. This later point, though, is contradicted by the results of

this study and those of reports such as Hopkins and Rilling (2000). Hopkins and Rilling (2000) suggest that the brains of macaques are not as lateralized as those of chimpanzees. This would imply that spatial, memory, or other cognitive tasks are not lateralized in Old World monkeys. In addition, the information provided by this dissertation suggests that males and females should not perform differently for these tasks. However, these hypotheses assume that the corpus callosum must be integral to all cognitive tasks. This, though, is not the case.

First, the various reports that suggest lateralization of the nonhuman primate brain rely upon what has been described as handedness (more properly hand preference) and visual capabilities. While tasks related to these features may be useful in understanding cognitive tasks and callosal function, there is no known study that adequately demonstrates the existence of higher cognitive processes in nonhuman primates. Because of this disparity between human and nonhuman primate studies, many of the results that suggest laterality in function may be explained as proving not the existence of complex pathways traversing the corpus callosum or specific lateralization of the neocortex, but as lateralization in basic mammalian cognitive tasks involving more primitive pathways such as the superior colliculus, anterior commissure, and hippocampal commissure, all of which are capable of carrying the type of information investigated in the afore mentioned reports.

Secondly, the studies that report sex differences in cognitive performance utilize visual information. While the splenium of the corpus callosum is important for relaying visual information, the type of visual discrimination described by Kavcic et al. (2000) and Vermeire et al. (1998) can occur via the superior colliculus (Wright and Craggs, 1976;

Sommer and Wurtz, 1998). In addition, results showing sex differences in throwing among capuchin monkeys (Watson, 2001) may occur via sex differences in the anterior commissure (see Noonan et al., 1998). Although this does not eliminate the likelihood of lateralization of visual and motor components of the cerebral cortex in nonhuman primates, the possibility that these sex differences occur as the result of other hemispheric pathways explains why it is possible to suggest lateralization of and sex differences in the brain of nonhuman primates, yet to not find sex differences in the corpus callosum.

In general, there is a wealth of information that implies the presence of lateralized function within the brains of macaques and chimpanzees (see above discussion). These studies, though, lack the sophistication to ally simple visual and motor functions of the nonhuman primate brain with higher cognitive processes involving the integration of data as seen in humans. It is probable that some lateralization exists within the nonhuman primate brain, albeit not at the level present in modern humans. Indeed, the results of Hopkins and Rilling (2000) study would say that the degree of lateralization is different between macaques, chimpanzees and humans with humans displaying the most asymmetric brains in this group and macaques the least. However, the question still remains, is the level of asymmetry seen in great ape brains sufficient to produce human-like cognitive functions?

Based on behavioral data the answer remains unresolved. A lack of cerebral laterality in nonhuman primates, though, does not preclude one from suggesting that the corpus callosum would not be expected to display sexual dimorphism in either midsagittal area or axonal composition until the brain is sufficiently lateralized in function. This can be assumed, because none of the above studies examines cognitive functioning at a level

sufficient to assume the corpus callosum has been co-opted for the task of interhemispheric integration of cognitive information. Such information could only be approached through invasive retrograde histology, or PET and fMRI studies. To conclude, the above studies are useful in understanding the evolution of the brain and sex differences within it (discussed in section 5.5), but they do not contradict the results of this study, which concludes that sex differences do not exist in the midsagittal area or axonal composition of the corpus callosum of nonhuman primates.

5.5 EVOLUTIONARY IMPLICATIONS

The uniqueness of the human brain has been discussed for thousands of years since the times of the Egyptians, Aristotle, and Descartes with little resolution (see Finger, 1994). Moreover, its has been a contentious topic in anthropology since the days of eminent neuroscientists/anatomists/anthropologists such as Broca, Smith, Dart and Anthony (see Holloway, 1997). There is, however, still disagreement concerning the advent of human-like features in the brain, which eventually led to human cognitive abilities. Recently, Ambrose (2001) has revived an idea first proposed by Holloway (1970) and later revisited by Calvin (1983, 1993) and Wilson (1998) hypothesizing that the need for accurate throwing and tool making skills created selective pressures for advancement of the hominin brain, and in turn the development of sex differences in the cerebral cortex. These selective pressures also aided the development of sex differences in the modern human brain. While there are other hypotheses for the evolution of the human brain (e.g., Tobias, 1971; Jerison, 1973; Gould, 1977; Gould and Lewontin; 1979; Falk, 1990), few

have been visited as frequently as Holloway's "throwing theory". This, though, has not quelled the debate of general human brain evolution or the development of sex differences in the brain, since the data that may be used for such studies is merely corroborative. The paucity of endocasts in the fossil record and the limitations of endocasts restrict their ability to provide conclusive answers of primate brain evolution. In addition, behavioral data on human and nonhuman primate subjects can provide information on cortical and cognitive functions of extant brains. However, an examination of both types of data, fossil and living, can be used to develop robust theories of brain evolution. In the case of this study it is possible to propose a hypothesis about the advent of sex differences in the corpus callosum of the primate brain.

5.5.1 Advent of sex differences in the corpus callosum of the primate brain

The results of this study indicate that sex differences in the corpus callosum did not develop until after the ape-human split some 7 – 5 million years ago. Indeed, sex differences in this interhemispheric pathway may not have developed until the advent of our own species some 250,000 - 100,000 years ago. Neither *Macaca fascicularis* nor *Pan troglodytes* display sex differences in total callosal area or the area of individual callosal regions. Moreover, neither species shows a difference between males and females for the number and types of fibers comprising the splenium of the corpus callosum. One would be inclined to conclude that these statements are possible, since the results do not exhibit statistical significance or a statistical trend.

From the results obtained here it seems apparent that sex differences in certain cognitive features represent an evolutionarily recent phenomenon. However, the finality

of these results should be questioned, since it is difficult to assume that sex differences in the corpus callosum and cognition must be statistically significant. While the results reported here are not significant, lending confidence to the conclusions discussed above, the general patterning of sex differences in *Macaca fascicularis* versus *Pan troglodytes* may provide important clues as to when sex differences resulting in differences in cognitive performance came about. The results for *Macaca fascicularis* show that there is complete overlap in the relative size of the corpus callosum and its regions between males and females. From this it is possible to conclude that the corpus callosum is not wholly responsible for the differences between males and females in the performance of certain tasks (see section 5.3.5). The results for *Pan troglodytes*, though, do show a tendency for females to possess a slightly larger corpus callosum, genu and isthmus than males, albeit these distinctions are not statistically significant. In addition, distribution of the types of axons passing through the splenium in *Pan troglodytes* is similar to the distribution seen in modern humans in that the female possesses more medium, very large, and giant axons than males (Aboitiz et al., 1992a). While this does not suggest that the corpus callosum of humans and chimpanzees are similar in their composition and fiber distribution, it does pose an interesting question. What level of uniqueness in the human corpus callosum is required to separate its features of form, function, and sexual dimorphism from that of chimpanzees?

As discussed in section 5.3.5, the corpora callosa of great apes and humans are smaller relative to neocortical surface area and brain volume. From this it is assumed that the brains of great apes and humans are more lateralized than either Old or New World monkeys (Rilling and Insel, 1999; Hopkins and Rilling, 2000; see also Gannon et al.,

1998). In addition, the findings of Hopkins and Marino (2000) suggest that the great apes possess a torque pattern similar to modern humans. Despite these general comparisons, though, these results do not imply that the brains of great apes and humans are alike. More importantly they indicate that the evolution of the human brain has been largely the result of a long, continuous evolution throughout primate history, and not rapid punctuated change, albeit this is conjecture. These studies as well as those testing for lateralization of the brain for certain cognitive and motor functions do suggest that *Pan* possesses a more lateralized brain than its cercopithecoid relatives. However, data on *Pan* behavioral, motor, and visual tasks do not suggest that *Pan* possesses a degree of lateralization in the cerebral cortex that would permit cognitive functioning beyond the level of a modern human two year old child (Deacon, 1997; Savage-Rumbaugh et al., 1998). The fact that *Pan* may possess a degree of lateralization approaching but not mimicking the human condition helps to explain why *Pan* would display a callosal morphology and composition similar to humans yet not possess similar cognitive characteristics. This observation that the brain and corpus callosum of *Pan* are similar but not the same as those of modern humans also explains why one does not find sex differences in the corpus callosum. That is, the brain of *Pan* has not become sufficiently specialized at the species level to permit the development of measurable sex differences in neocortical components and the corpus callosum.

5.5.2 Advent of sex differences in cognitive processes of the primate brain with a special emphasis on language

There are several cognitive differences between males and females. These include differences with regard to visuospatial, motor, and language skills (see section 1.4). While it is likely that visuospatial and motor skills contributed to the expansion and reorganization of the hominin brain (Holloway, 1970), one can argue that the most significant consequence of human evolution in general and human brain evolution specifically has been the development of complex language abilities. The below discussion uses language as one example of the development of sex differences in the brain.

The similarities between the brain and corpus callosum of *Pan* and humans can be used to express the uniqueness of each species. As discussed above, *Pan* appears to approach the neocortical condition of humans but does not mimic it. This explains why sex differences in the brain and corpus callosum of *Pan* do not approach statistical significance. It also explains why certain brain structures such as the planum temporale and petalial pattern may display asymmetry in *Pan*, but do not confer human-like cognitive functioning (Gannon et al., 1998; Hopkins et al., 1998; Rilling and Insel, 1999; Hopkins and Marino, 2000; Hopkins and Rilling, 2000). This difference between human nonhuman primates is best understood by examining the issue of language.

Several studies have attempted to assign some level of language to *Pan* (Savage-Rumbaugh et al., 1998). However, regardless of the displayed "intelligence" of study subjects, none have ever been able to express communicative abilities beyond those capable in a normal two and a half year old child. This is not to imply that *Pan* does not

express some level of intelligence, but instead indicates the mere differences between the brain of *Pan* and the brains of modern humans. For example, Gannon et al. (1998) found that human-like asymmetry can be found in the planum temporale of *Pan troglodytes*. While this level of asymmetry in humans is thought to result in or represent a product of the laterality of language, the authors do not express any intent to align the language skills of *Pan* and humans. This is because it is difficult to assign advanced cognitive functions such as language to asymmetry in one single structure. In this case asymmetry in the planum temporale may confer laterality in certain cognitive processes in both *Pan* and humans, but it does not presume language in both species.

The role and relationship of the corpus callosum in speech and language has been well established (O'Kusky et al., 1988; Zaidel et al., 1995; Rumsey et al., 1996; Moffat et al., 1998; Gazzaniga, 2000; Habib, 2000; Preis et al., 2000; Shevtsova and Reggia, 2000). The size of the corpus callosum has been shown to be related to the lateralization of language function (Witelson, 1995; Zaidel et al., 1995). In addition, women, who are thought to be less lateralized than men for language, possess a larger corpus callosum and more bulbous splenium (de Lacoste, 1981; de Lacoste and Holloway, 1982; Kimura and Harshman, 1984; Witelson, 1991, 1995; Holloway et al., 1993; Moffat et al., 1998; see also section 1.5). The presence of continued argument as to the existence of sex differences in the corpus callosum of humans attest to the degree of difference between males and females, which in some cases is small. However, it is still uncertain how much of a difference must occur between the brains of two individuals or the sexes to obtain significant differences in cognitive features. For example, it is generally accepted that males and females differ from each other in certain cognitive skills (Kimura, 1987;

Hugdahl et al., 1993; Halpern et al., 1998; de Courten-Myers, 1999; Hausmann and Gunturkun, 1999; Amunts et al., 2000). Yet, each sexually dimorphic skill does not correlate to an equally sexually dimorphic neuroanatomical area, albeit certain areas such as the motor cortex do exhibit direct correlations (de Courten-Myers, 1999; Amunts et al., 2000). Nevertheless, these gaps in human research leave the question of how sexually dimorphic the splenium must be to permit one sex to possess greater integrative capabilities with regard to language and visuospatial skills remains unresolved. Without the resolution of these particular issues the specific role of sex differences in the corpus callosum will remain uncertain.

The when, where, why and how of the evolution of language are questions that are not easily answered. This is because data relevant to these questions must be derived from at least three mutually exclusive categories: living nonhuman primates, living humans, and endocasts of fossils. As mentioned above communicative information in nonhuman primates like that being produced by Sue Savage-Rumbaugh and others attest to the level of skill in species such as *Pan troglodytes* and *Pan paniscus*. However, these studies do not specifically prove the existence of language and language areas in nonhuman primates or *Pan* in particular. They do, though, shed some light on the development of language. Based on these behavioral studies and the anatomical studies mentioned above, it is possible that *Pan* possesses certain brain structures and a degree of cerebral lateralization that permit *Pan* to communicate at a level beyond other nonhuman primates. Though this level of cerebral and cognitive development is not the same as displayed by humans, it does provide provocative evidence for the existence of a cerebral

archetype early in human evolution rather than the arise of areas such as Broca's and Wernicke's *de novo* in *Homo sapiens.*

The fossil record appears to support this claim. Although endocasts of australopiths do not appear to be significantly different from *Pan*, later species such as *Homo habilis* and *Homo erectus* do begin to display human-like proportions and features (Tobias, 1975; Holloway, 1981a,b; Broadfield et al., 2001). The presence, though, of human-like features does not necessarily confer the capacity for modern human speech and language on any species other than modern humans. However, they do indicate that the development of neuroanatomical features related to speech, language, and visuospatial skills may have existed long before the arrival of *Homo sapiens.* As to the role of these features for the development of sex differences in the corpus callosum, in particular the splenium, the development of certain higher cognitive features of the brain should precede the development of sex differences in those functions (speech, language, and visuospatial skills) as well as sex differences in the neuroanatomical structures related to those functions. Sex differences in the corpus callosum would thus not be expected in taxa such as *Pan* and *Macaca*, since neither species possesses the neuroanatomical substrate for modern human speech, language, and visuospatial skills or the degree of lateralization of the cerebral cortex required to produce the specialized features of language. Due to the role of the splenium in connecting modern human language areas, it is suspected that if a particular species is to possess communicative features comparable to humans then this area may display sex differences as it does in humans. However, since *Pan*, as mentioned above, does not possess human communicative abilities, visuospatial skills, or the neuroanatomical substrate that would lead one to propose the

ability for human-like communication or visuospatial skills, one would not expect to find sex differences in this particular callosal region. Humans, therefore, are unique among living primate taxa in possessing a highly lateralized, sexually dimorphic brain and corpus callosum.

5.6 PATHS FOR FUTURE RESEARCH

In sum, the corpus callosum of *Macaca fascicularis* and *Pan troglodytes* does not display any sex differences. This provides important information with regard to human brain evolution, since various studies give the impression that chimpanzees may be more similar to humans in a behavioral sense. However, the brains of *Macaca fascicularis* and *Pan troglodytes* do not possess the level of cerebral specialization found in modern humans, although *Pan* shows a directional shift toward the human cerebral condition. Moreover, the disparity between behavioral, morphological, and histological data for these groups can only be taken to assume that certain features of hominin evolution such as the specialization of the brain have their origins in early or late hominin evolution. In addition, the lack of sex differences in prominent cerebral structures such as the corpus callosum indicates that sex differences in the brain that are responsible for certain significant cognitive differences between males and females are relatively new features of hominin brain evolution, which must have occurred after the development of certain neuroanatomical and cognitive features such as speech and language.

The final analysis of the role, importance, and sexual dimorphism of the corpus callosum is far from complete. This is because many other forms of data are required to

resolve the questions asked here. First, further histological data for *Pan*, *Pongo*, *Gorilla*, and *Homo* are necessary to fully assess the composition of the hominoid corpus callosum, the brain's primary interhemispheric pathway. This is a difficult task to achieve, since it requires the acquisition of perfusion fixed specimens. Second, functional data is required to determine the specialization of the brain of nonhuman primates, particularly *Pan*. This is best achieved through fMRI and PET. However, it will be some time before adequate data can be obtained using these methods, since both require the animal to be calm and alert, and capable of spending several minutes in the machine. Finally, more data is necessary from human studies to pinpoint the areas of the brain that are responsible for specific functions. While these future research pathways will permit a further understanding of the corpus callosum, the data provided by this study represent a significant contribution to understanding of the origin of sex differences in the corpus callosum and the brain, and general human and nonhuman primate brain evolution.

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