SEX DIFFERENCES AND THE EFFECTS OF SEX HORMONES ON THE STRUCTURE OF THE CORPUS CALLOSUM

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ABSTRACT

OBJECTIVE

This thesis examines sex differences in the structural properties of the corpus callosum (CC) during adolescence and investigates the possible role of sex hormones in the development of these sex differences, as well as hormonal effects on the inter-individual variations in the structure of the CC in young women.

DESIGN

This thesis is conducted on three separate study samples: 1) the Saguenay Youth Study (SYS) sample (n=737); 2) the IMAGEN Study sample (n=1,979); and 3) the Cycle Study sample (n=26). The SYS and IMAGEN studies are large-scale studies carried out with magnetic resonance imaging (MRI) in typically developing adolescents from Canada (12 to 18 years) and Europe (13 to 15 years), respectively. In these studies, the "sex hormone-CC" relationship is explored by examining the association between the CC and: testosterone level, duration of sex hormone exposure (since menarche), puberty stage and contraceptive use. The Cycle study examines the CC of 13 freely cycling and 13 oral contraceptives (OCP) using young women (18 to 30 years) scanned using MRI across four separate phases of their menstrual cycle. Freesurfer-based computational anatomy is used to estimate the volume of the total corpus callosum and its segments in all three studies. Magnetization transfer imaging (MTI) is used to assess microstructural properties of the corpus callosum in the Cycle study.

RESULTS

The relative volume of the corpus callosum is seen to be sexually dimorphic in both the SYS and IMAGEN adolescents with a female versus male advantage that is particularly

significant for the anterior, central and posterior segment of the corpus callosum; the mid-anterior segment is larger in males versus females.

Pubertal stage of adolescent boys (SYS) demonstrates a negative correlation with the relative volume of the anterior CC and a positive correlation with the relative volume of the mid-anterior CC. These associations are consistent with the sex differences observed (anterior: F>M; mid-anterior: M>F), thus suggesting that male sex hormones that are responsible for inducing pubertal development of boys may play a role in generating the sexually dimorphic volume of the corpus callosum in a region-specific manner.

Contraceptive use in adolescent girls (SYS and IMAGEN) is negatively associated with the relative volume of the corpus callosum. In addition, the Cycle study demonstrates a trend for lower MTR values in women using contraceptives versus freely cycling women, thus suggesting that the natural sex steroids suppressed by use of oral contraceptives may exert a positive effect on the volume of the CC, possibly by increasing the degree of myelination.

The Cycle study demonstrates an increase in the relative volume of the total corpus callosum of freely cycling women from the ovulatory to the luteal phase with corresponding decreases in the MTR value of the total CC. This finding suggests that increased production of progesterone during the luteal phase may cause an increase in the relative volume of the CC, possibly by increasing the axonal calibre.

CONCLUSION

Sex steroids influence the structure (relative volume and MTR) of the corpus callosum.

ACKNOWLEDGEMENTS

I dedicate this thesis to mama and papa, my parents, who have always encouraged my inquisitive nature, instilled enthusiasm in me for all my educational endeavours and taught me innumerable lessons. I am eternally grateful for the dedication with which they have raised me and for their selfless love and affection.

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1 GENERAL INTRODUCTION

1.1 INTRODUCTION TO THE THESIS

This thesis explores sex differences in the volume and structural properties of the corpus callosum assessed with magnetic resonance imaging (MRI) during adolescence. It also investigates the possible role of sex hormones in the development of these sex differences during adolescence and on inter-individual variations in the structure of the corpus callosum in young women.

This thesis has two parts. The first part re-examines sex differences in the structure of the corpus callosum (CC). The results of previous studies investigating sexual dimorphism in the CC fail to yield a consensual conclusion. The importance of this topic and inconsistencies in existing literature provide the impetus for re-examining these sex differences. There are multiple areas of improvement in this thesis, compared with previous studies, relating to sample sizes and methodological approaches. These include:

- Sex differences are examined in two large-scale studies (n=737 and n=1,979) that afford high statistical power necessary for detecting subtle differences.
- Sex differences are examined in 3D volume instead of 2D surface area of the corpus callosum, used conventionally. This enables more appropriate normalization of CC volume with the total brain volume.

- Improved statistical methods are employed that control for inter-segmental dependence.

The second part of this thesis poses a novel question regarding the origin of these sex differences in the corpus callosum. The possible role of sex hormones as one of the potential factors responsible for the development of these sex differences is investigated. This is done using two main methodological approaches:

- Observational Studies: These are cross-sectional studies that examine the differences in callosal volume associated with differences in the level of endogenous sex hormones (bioavialable testosterone) in adolescent boys and their exposure period (exposure to gonadal estrogen and progesterone since menarche in years) in adolescent girls. Additionally, CC volume changes associated with sex hormonal variation across the menstrual cycle of young women is also investigated using a longitudinal study.
- Pseudo-experimental Studies: These are cross-sectional studies that examine the variations in callosal volume associated with exogenous suppression of female sex hormone production caused by the use of oral contraceptive pills in adolescent girls and young women.

Understanding the role of sex hormones in relation to CC development during adolescence can have beneficial long-term consequences with respect to the cognitive and mental health. This is because the corpus callosum is the largest

inter-hemispheric white-matter tract in the brain (Aboitiz et al. 2003) that enables transfer, integration and processing of information necessary for a variety of brain functions (Hines et al. 1992, Sauerwein et al. 1994, Kennedy et al. 2000, Luders et al. 2007, Paul et al. 2007, Hutchinson et al. 2009, Voineskos et al. 2010, Sullivan et al. 2006, Johansen-Berg et al. 2007, Meutzel et al. 2008, Moes et al. 2008, Mueller et al. 2009). Additionally, white-matter diseases, such as multiple sclerosis (Whitacre et al. 1999 and 2001) and other neurological disorders, such as schizophrenia (Hafner et al. 2003), autism and attention deficit hyperactivity disorder (Hutchinson et al. 2008, Gershon et al. 2002) that involve the CC show sexual dimorphism. Studying the role of sex hormones in the development of sexual dimorphism, using the corpus callosum as a model structure, may help to identify the biological basis for these sexually dimorphic white-matter diseases.

1.2 CHAPTER INTRODUCTION

This introductory chapter begins by describing the basic structure of the corpus callosum, its size, shape, location in the brain and its sub-sections (Section 1.3). The topographic distribution of cortical regions connected by callosal fibres of different callosal segments (Section 1.3.3) and some of the functions of the corpus callosum (Section 1.4), are discussed subsequently. Knowledge of the different sections of the corpus callosum and their functional topography is relevant to this thesis because it examines sex differences in the structure of the callosal segments (chapter 4) and their functional correlates (Chapter 5). Next,

the *ex-vivo* and *in-vivo* multi-modal MRI techniques used to examine the structure of the corpus callosum in this thesis and previous studies are described (Section 1.5). Subsequently, existing literature on the relationship of sex with the volume of the corpus callosum is reviewed (Section 1.6). This literature is examined in an age-dependent fashion, separately for the following age groups: prenatal to infants, childhood to adolescence, adulthood and elderly. Reviewing the literature in this fashion aids the elucidation of the specific period during which sex differences begin to manifest. Lastly, the current knowledge about the relationship of sex hormones, oral contraceptive use, and menstrual cycle phase with global and regional brain volume is described (Section 1.7). In brief, this introductory chapter provides the necessary background knowledge on the CC, including its structure, function and sexual dimorphism. This information helps set the stage for the aims of this thesis, why they are generated and what they are meant to achieve. These aims are described in detail at the end of this chapter (Section 1.8).

1.3 CORPUS CALLOSUM: ANATOMY AND MICROSTRUCTURE

This section gives an introduction of what the corpus callosum is and describes in detail its shape, structure, cortical connectivity and microstructural properties.

1.3.1 Definition

Corpus Callosum (CC) is the largest cerebral commissure connecting the two cerebral hemispheres to allow interhemispheric communication necessary for

the processing of information. There are four main cerebral commissures: anterior and posterior commissures, hippocampal commissure and the corpus callosum.

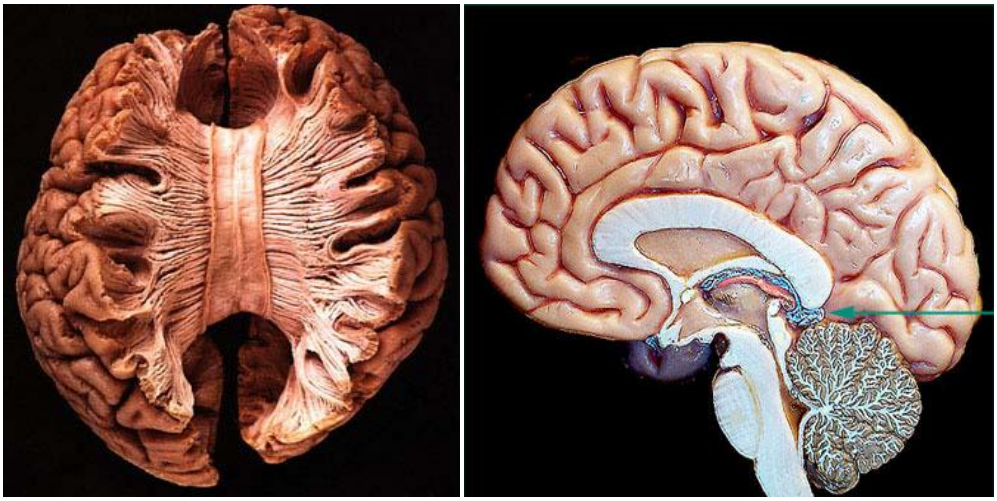

Figure1.1: Commissural fibres of the corpus callosum passing from one hemisphere to the next (left figure). Mid-sagittal view of the corpus callosum (right figure). Pictures from the following websites: http://diamondhead.net/mnc1.htm *(left) and* http://www.mlahanas.de/Greeks/Med.htm *(right).*

1.3.2 Anatomy of the Corpus Callosum

The corpus callosum is composed of bundles of white-matter fibres running from one hemisphere to the other. Most of the callosal fibres are homotopic, that is, they connect functionally and structurally similar regions of the two hemispheres; heterotopic fibres connecting different cortical areas represent a small fraction of all callosal fibres (Aboitiz et al. 2003). These homotopic and heterotopic fibres collectively form the corpus callosum, which appears at midline as a wide arched structure beneath the cortex, around 10 cm long and 2.5 cm wide in the adult human brain (Kiernan et al. 2009).

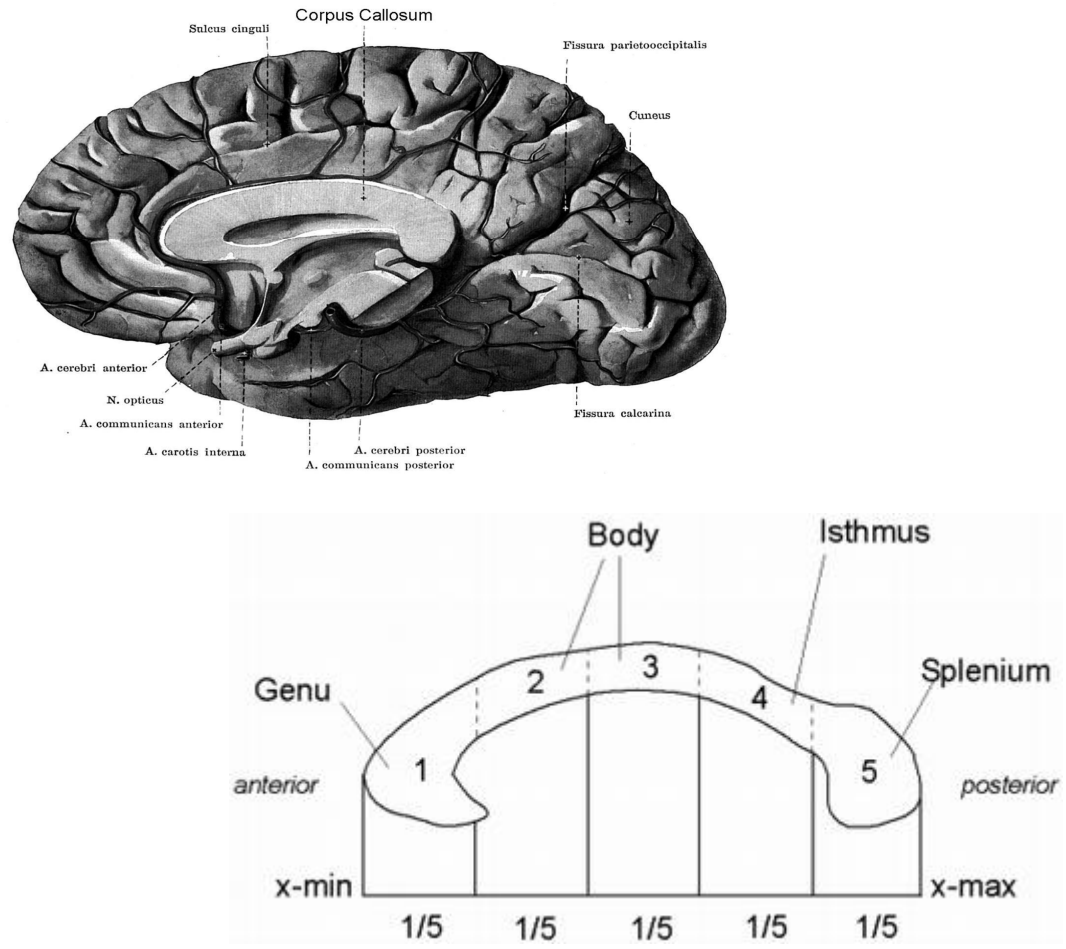

Figure 1.2: Mid-sagittal view of the CC (top figure). Arbitrary segmentation of the CC into equal fifths along the anterior-posterior axis (anterior fifth: genu; mid-anterior fifth: genu and body; central fifth: body; mid-posterior fifth: body and isthmus; posterior fifth: splenium; bottom figure). Schematic from Fine et al. 2007.

The corpus callosum is surrounded superiorly by the supracallosal and cingulate gyri and inferiorly by the septum pellucidum, body of the fornix and lateral ventricles (Kiernan et al. 2009). The CC appears as a continuous, homogenous structure both in post-mortem brains and MR images. There are no gross anatomical landmarks that delimit the corpus callosum into distinct sections along the anterior-posterior axis. For the ease of its study, however, the CC has been divided into different subsections. The anterior most region of the CC is called the genu. The narrow middle region is called the body. The narrowest callosal section which appears at the junction of the body with the caudal-most

region is called the isthmus. Lastly, the broad caudal most region of the CC is referred to as the splenium.

CC Topography based on Anatomic Studies: This section explains the rough topographic representation of cortical areas along the anterior-posterior axis or length of the corpus callosum, determined by tracer-studies in non-human primates (rhesus monkeys). This is relevant vis-à-vis possible functional consequences of the sex differences identified in callosal segments (Chapter 4) and also the possible brain functions that sex hormones may influence by virtue of their association with callosal segments (Chapter 6 and 7).

There exists a large body of work describing a rough topographic representation of axons connecting different cortical areas along the corpus callosum. This includes neuroanatomical tracing studies carried out in non-human primates (rhesus monkeys and macaque monkeys), post-mortem studies in humans, diffusion tensor imaging (DTI)-based tractography studies and most recently functional magnetic resonance imaging (fMRI) studies. Some of the earliest and perhaps the most comprehensive and reliable work in this context was done by Pandya et al. (1971, 1983, 1984). They used two main neuroanatomical techniques to elucidate the intracallosal topography in the rhesus monkey: 1) Tracer studies where radiolabelled amino acids were injected into discrete cortical areas and axonal transport of the tracer then observed under light microscopy; and 2) Transection induced degeneration studies where terminal fibre degeneration was examined in cortical areas that is affected by transection of different callosal regions. Based on these studies it was deduced that the genu

carried fibres between the prefrontal and premotor areas (in the frontal lobe) of the left and right hemispheres. The rostral half of the body of the CC carried fibres between the left and right primary motor areas (M1 in the precentral gyrus) and supplementary motor areas (M2 in the superior frontal gyrus). The somatotopic representation was such that fibres from the primary motor areas representing the face lay rostral to those representing the arms and the legs. The caudal half of the body of the CC carried fibres between the left and right primary somatosensory areas (S1 in the postcentral gyrus) and secondary somatosensory areas (pericentral and parietal opercula). The isthmus of the CC carried fibres between the left and right primary and secondary auditory areas (A1 and A2 in the supratemporal gyrus). Lastly, the splenium carried fibres between the left and right temporoparietal association areas (in the superior and inferior temporal gyri and parahippocampal gyri) rostrally and visual association areas (Broadman area 19 and 18 in the occipital cortex) caudally. De LaCoste et al. (1985) conducted a similar post-mortem study in 13 human brains. They delineated callosal topography by correlating callosal areas of Wallerian degeneration with focal cortical lesions (infarctions and contusions). They observed that topography of the human corpus callosum was very similar to that of the rhesus monkey stated above. Furthermore, most of the callosal fibres between the left and right primary sensori-motor areas and visual areas connect cortical areas representing the midline of the body (face and trunk) and visual field, respectively.

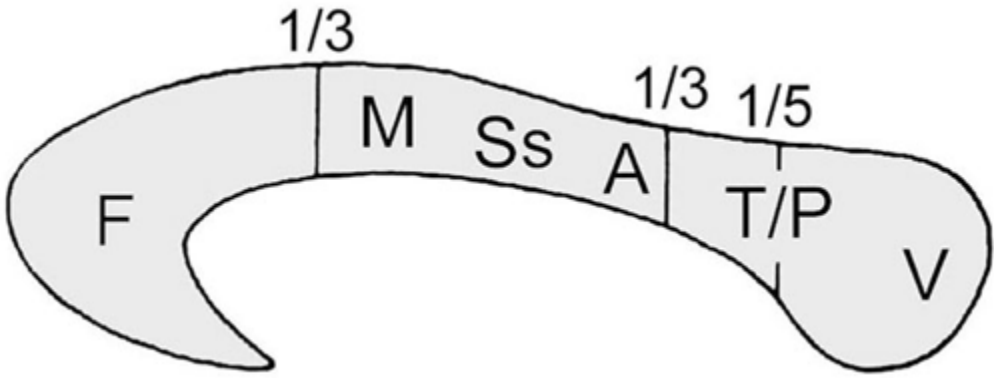

Figure 1.3: Topographic representation of cortical areas connected by the different callosal segments (F: Frontal cortex; M: Motor cortex; Ss: Somatosensory cortex; A: Auditory cortex; T/P: Temporo-parietal cortex; V: Visual cortex; 1/3: one third subdivision; 1/5: one fifth subdivision). Schematic from Aboitiz et al. 2003a.

CC Topography based on MR Techniques: Animal tracer and degeneration studies described in the above section are considered to be the gold-standard for studying callosal topography. Modern alternatives (described below) are now available to study callosal topography *in-vivo* in human subjects. This section discusses callosal topography highlighted by these modern techniques, and differences in comparison to the results of the gold-standard animal studies.

Callosal Topography via DTI studies: Recent MRI studies used tractography techniques based on diffusion tensor imaging (DTI) to create maps of white-matter tracts connecting the two hemispheres via the CC. This technique measures the diffusion of water molecules in different directions. Then based on the principle that the diffusion of water molecules is maximal along the direction of the axons it determines the prevailing orientation of fibres and consequently generates "connectivity" maps. Note, however, such DTI-based connectivity maps are but a mathematical model of water diffusion and do not necessarily represent true point-to-point (axon-based) connectivity (for discussion on the

interpretation of DTI-based tractography, see for example Nucifora et al. 2007, Alexander et al. 2007 and Mori et al. 2006). Hofer and Frahm (2006) used DTI based tractography to identify the trajectories that cross individual pixels within the entire CC and penetrate different cortical regions in eight adult participants. They observed that the genu contains fibres projecting to the prefrontal cortex. The anterior body contains fibres projecting to the premotor and supplementary motor cortical areas. Connections of the primary motor cortical areas (M1) were observed to be located in the posterior body. Connections to the primary somatosensory cortical areas (S1) were located in the isthmus. Lastly temporoparietal connections and occipital connections were located in the anterior and posterior part of the splenium respectively. Similarly Zarei et al. (2006) produced a two dimensional sagittal map of the cortical tracts within the CC of 11 healthy adult participants using probabilistic DTI tractography. The callosal topography identified by this in-vivo study was consistent with that of Hofer and Frahm (2006). Wahl et al. (2007) combined fMRI and DTI fibre tracking techniques to examine the topography of callosal motor fibres in 12 healthy adult participants. In this study the callosal motor fibres connecting the primary motor areas (M1) of the two hemispheres were mapped onto the posterior body and isthmus. Moreover, in addition to topographic representation a somatotopic representation of the callosal fibres was also identified. Hand callosal motor fibres were located significantly more anterior and ventral than the foot callosal motor fibres. Westerhausen et al. (2009) conducted a DTI-based tractography study on 17 males aged 19 to 46 years. They observed that the callosal fibres originating from the posterior superior temporal gyrus and Heschl's gyrus

(containing the auditory areas) were located in the splenium. Saenz et al. (2010) conducted a DTI study to identify the topography of the V1 vertical midline projections through the splenium of six adult participants (aged 21 to 41 years). Fibres from the ventral V1 (representing the upper visual field) were observed to be projecting to the inferior anterior corner of the splenium. Fibres from the dorsal V1 (representing the lower visual field) were observed to be projecting to the superior posterior end of the splenium.

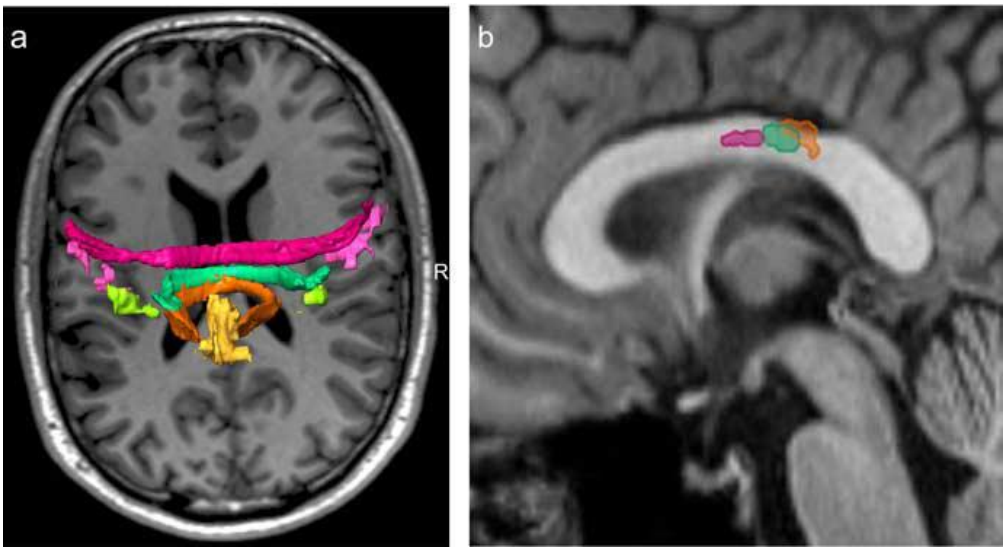

Figure 1.4: fMRI defined M1 representations (lip: light red; hand :light green; foot: yellow) and DTI tracked callosal motor fibres (lip: dark red; hand: dark green; foot: orange) passing through the posterior body and isthmus of the corpus callosum on axial (a) and sagittal (b) MR views. Schematic from Wahl et al. 2007.

Overall, if the callosal topography highlighted by these DTI studies is compared with that of human post-mortem and rhesus monkey tracer studies, all the cortical tracts were seen to have a relatively more posterior location. Moreover, considerable overlap was seen between adjacent cortical tracts.

CC Topography via fMRI Studies: Fabri et al. (2011) were among the first few researchers who described callosal topography using functional MRI (fMRI)

technique. They did so by detecting the blood oxygenation-level dependent (BOLD) signal within the corpus callosum during the presentation of different sensory stimuli and a performance of motor tasks. Taste stimuli evoked a BOLD response in the anterior CC. Unimanual and bimanual hand movements evoked response in the CC midbody. Tactile stimuli to the peripheral body (hands and legs) evoked response in the posterior CC body whereas that to the midline body (trunk, shoulder and thigh) evoked response within the isthmus. Lastly, visual stimuli evoked a BOLD response in the CC splenium.

Overall, the main difference in the topographic map highlighted by this fMRI study and previous human post-mortem and rhesus monkey tracer studies was that callosal fibres to motor and somatosensory cortices were located in a relatively more caudal position. Additionally, greater inter-individual differences in callosal topography were observed in the fMRI maps as compared with the DTI and post-mortem maps.

1.3.3 Microstructure of the Corpus Callosum

In this section the microstructure of the corpus callosum is discussed. Understanding the fibre composition of the CC helps to conjecture the possible cellular underpinnings of the volumetric differences observed the CC. Furthermore, this thesis also examined the association of sex hormones with the magnetization transfer ratio (MTR) value of the total corpus callosum and its segments (Chapter 7). MTR is an MR technique that generates contrast based on the microstructural properties of the white matter. Knowledge of the

microstructural makeup of the corpus callosum is, therefore, important to understand the microstructural properties represented by the MTR values.

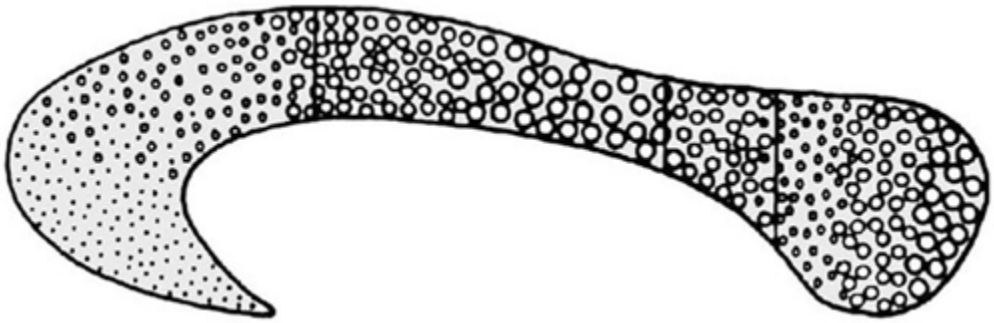

Figure 1.5: Variation in fibre composition (axon diameter) along the length of the corpus callosum. Schematic from Aboitiz et al. 2003a.

The CC is composed of around 200 to 300 million axons. There is considerable variability in fibre composition along the length of the CC (rhesus monkey: La Mantia and Rakic 1990a and 1990b; human: Aboitiz et al. 1992). The variability is in the size (diameter) of the axons, their density and degree of myelination. Aboitiz et al. (1992) conducted a post-mortem study to investigate fibre composition of corpus callosum in 20 human brains. They analyzed the density of different sized fibres (fibre=axon + myelin sheath) along the length of a 5 µm thick stained section of the CC under light microscopy. Most of the fibres within the CC were seen to be small diameter (0.4 to 1 µm). Fibres above 1 µm, 3 µm and 5 µm formed only 20%, 0.1% and 0.02% of the total callosal fibre population respectively. Moreover, no sex differences were observed in the fibre composition; given the small sample size, this finding should be interpreted with caution. The density of small diameter fibres (0.4 to 1 µm) was highest in the genu; it decreased gradually from the genu to the posterior midbody after which it increased once again (see Fig. 1.5). Density of the medium sized fibres (1 to 3

µm) was more or less constant throughout the length of the CC. Density of the large (3 to 5 µm) and very large (above 5 µm) diameter fibres followed a trend opposite that of the small diameter fibres: it was lowest in the genu and kept increasing till the posterior midbody, after which it declined. Overall, density of small diameter fibres was highest in the genu, anterior midbody and anterior part of the splenium, whereas density of large diameter fibres was highest in the posterior midbody, isthmus and caudal end of the splenium. Most of the CC fibres were myelinated. The fraction of unmyelinated fibres in each CC section was below 5% except for the genu where 15% of the fibres were unmyelinated.

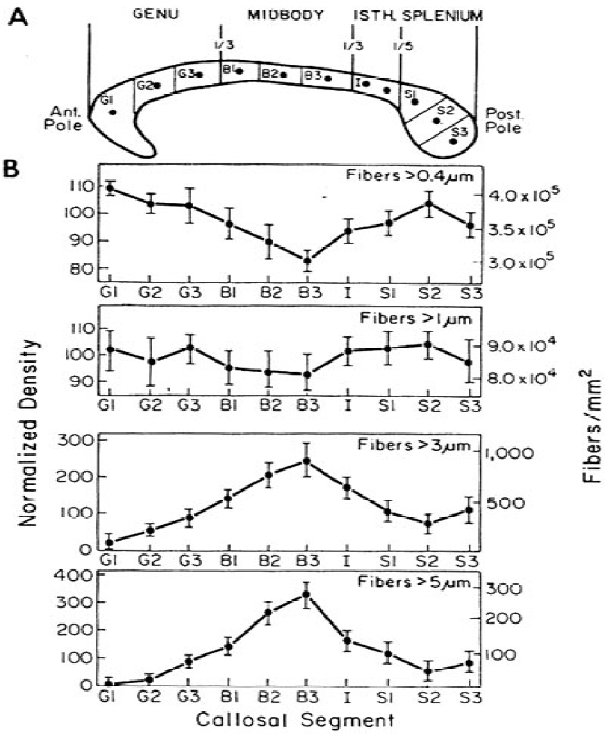

Figure 1.6: Density of callosal fibres >0.4 µm, >1.0 µm, >3.0 µm and >5.0 µm (top to bottom, respectively) across the length of the corpus callosum. Schematic from Aboitiz et al. 2002.

The variability in fibre composition along the length of the CC is thought to be related to the topographic representation of cortical areas along its (anterior-

posterior) length. Fibre composition determines the speed at which information can be transferred between the two hemispheres. In general, large-diameter myelinated fibres conduct action potentials at faster rates than small- diameter unmyelinated fibres. The highest density of large-diameter fibres was in the posterior midbody followed by the caudal end of the splenium, allowing conduction velocities of up to 40 to 70 mm/ms (La Mantia and Rakic 1990a and 1990b, Aboitiz et al. 1992). These callosal sections are responsible for transmitting information between the somatosensory cortices, auditory cortices and visual association cortices, respectively. The highest concentration of small-diameter unmyelinated fibres was in the genu and anterior splenium (La Mantia and Rakic 1990a and 1990b, Aboitiz et al. 1992); they afford slower conduction velocities of up to 3 to 15 mm/ms (La Mantia and Rakic 1990a and 1990b, Aboitiz et al. 1992). These callosal sections are responsible for transmitting information between the high order association areas of the prefrontal and temporoparietal cortices, respectively. Thus, as fibre composition changes along the length of the corpus callosum so does the presumed rate of interhemispheric transfer of information. In general, it is important to note that there were a significantly greater proportion of small diameter fibres in the CC as compared with large diameter fibres (La Mantia and Rakic 1990a and 1990b, Aboitiz et al. 1992). This is probably associated with the higher proportion of association areas within the cortex as compared with primary sensori-motor areas.

1.4 CORPUS CALLOSUM: FUNCTION

This section describes some of the functions that appear to depend on the integrity and/or structural properties of the corpus callosum. This knowledge helps to support the hypothesis regarding functional correlates of the corpus callosum. Additionally, in a larger sense it also helps to conjecture the possible functions that sex hormones may influence by virtue of their association with callosal segments (Chapter 6 and 7).

The corpus callosum (CC) connects most of the cortical areas of the two hemispheres, enabling transfer, processing and integration of information. Since the corpus callosum transfers information between multiple cortical areas, it is likely to be involved in multiple cortical functions. This possibility has been explored in detail by many researchers using a wide variety of approaches.

Classically, researchers studied callosal function by investigating the functional deficits present in individuals lacking corpus callosum, usually due to agenesis or surgical transection of the CC. In addition they explore the functional specialization of callosal segments by observing and identifying deficits in individuals with partial agenesis and partial callosotomies. A review of acallosal case reports (both agenesis and transection) by Chiarello et al. (1980) and Sauerwein et al. (1993) showed that most acallosal individuals function at the lower end of the normal range of general intelligence (IQ). A small proportion, however, fell in the high average IQ range. Moreover majority of the cases (69%) did not have any significant difference in their verbal and performance IQ scores;

this suggested that the CC is equally important for both aspects of IQ. In 1994, Sauerwein et al. conducted a study to compare the IQ and motor skills of nine agenesis individuals (10 to 24 years old) with two age- and sex-matched, control groups; one group with healthy individuals and one with individuals having low IQ. They observed that, compared with healthy individuals, the agenesis individuals performed significantly poorly in all Wechsler's subscales, Purdue pegboard and simple finger tapping tasks. In comparison with individuals having low IQ, however, agenesis individuals performed significantly poorly only in the similarities subset of Wechsler Intelligence Scales (WAIS-R, Wechsler D. 1981 and WISC-R, Wechsler D. 1974) and Purdue pegboard. Thus, based on this study, verbal abstract reasoning skills (similarities test) and fine motor performance (Purdue pegboard) were the only skills impaired in acallosal individuals compared with individuals having intact CC. Paul et al. (2000) have done extensive work to explore cognitive deficits in agenesis individuals. They observed that agenesis individuals as compared with healthy individuals having intact CC are significantly impaired in reasoning, problem solving, concept formation and interpretation of literal and non-literal language comprehension skills (Review by Paul et al. 2007). Moes et al. (2008) collected information on 720 children (aged 5 to 15 years) with agenesis of the corpus callosum and 219 healthy siblings via caregiver-reported questionnaires. They reported that children with CC agenesis, as compared with their healthy siblings, performed significantly poorly in tasks requiring bimanual coordination such as biking, brushing, bathing and dressing. This finding is significant considering the size of the sample. But note that performance was estimated by caregiver reports and not corroborated by direct

testing. This method of measuring performance is subject to possible bias and omission of facts. Mueller et al. (2009) tested bimanual motor coordination of 13 agenesis individuals on the computerized version of etch-a-sketch task. They observed that agenesis individuals as compared with healthy individuals having intact CC are less accurate and slower. Additionally, Callie et al. (2005) tried to identify specific callosal segments associated with bimanual coordination by studying motor deficits in individuals with partial callosotomies. They observed that section of the anterior portion of the genu caused a deficit in motor coordination and middle portion caused a deficit in motor planning.

At present, MR-based imaging techniques are used to explore the function of the CC in healthy individuals. These techniques include structural (typically T1-weighted) MRI, diffusion tensor imaging and most recently functional MRI. In MRI studies, images are used to obtain different measures of callosal size such as area, volume, thickness, length. Relevant structure-function relationships are then evaluated by correlating the anatomic measures with performance on various tasks. Using this approach, Hutchinson et al. (2009) correlated IQ of 71 healthy participants (aged 19 ±3 yrs), measured using Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler D. 1999), with their callosal area. They observed a negative correlation between IQ and area of the posterior half of the CC. Detailed analysis revealed that this relation was mainly between midbody area and performance IQ, not verbal IQ. Similarly, Luders et al. (2007) correlated full scale IQ, performance IQ and verbal IQ of 62 healthy participants (aged 28 ±7 yrs), measured using Wechsler Adult Intelligence Scale (WAIS-R; Wechsler D.

1981), with their callosal thickness. They observed a positive correlation between full scale IQ and thickness in the anterior third and body of the CC. Hines et al. (1992) correlated the verbal fluency of 28 healthy female participants (29 ±1 yr) with their CC area. They observed that posterior callosal area positively predicted verbal fluency.

In DTI studies, microstructural integrity of white matter is indexed by values of fractional anisotropy (FA) and mean diffusivity (MD). Callosal function is subsequently investigated by correlating its FA and MD value with task performance. Using this study design, Voineskos et al. (2010) correlated the cognitive performance of 64 healthy elderly volunteers (aged 49 ±17 yrs) in memory, executive functioning, motor speed, set shifting/flexibility and visuospatial construction with participants' callosal FA and MD values. They observed that the FA value in the posterior fifth of the CC broadly predicted working memory and executive function only. Similarly, Kennedy et al. (2000) correlated the cognitive performance of 55 healthy elderly volunteers (aged 54 ±19 yrs) in processing speed, working memory and executive function with the FA and MD values of participants' CC. They observed a positive correlation between FA of the genu and both working memory and executive function. They also observed a negative correlation between MD of the genu and processing speed. Muetzel et al. (2008) correlated performance of 92 healthy volunteers (aged 9-24 yrs) in an altered finger-tapping task with callosal FA and MD value. They observed a significant correlation between FA of the splenium and bimanual performance on the altered finger tapping tasks. Sullivan et al. (2006)

conducted a similar study in 49 healthy volunteers (aged 44 ±15 yrs) and likewise observed a significant correlation between FA of the splenium and performance on altered finger tapping task. Johansen-Berg et al. (2007) observed a positive correlation between the bimanual performances of 10 healthy volunteers (aged 21-45 yrs) on an altered finger tapping task and the FA of the callosal midbody.

Recent fMRI studies show that performing bimanual coordination tasks is associated with BOLD responses within the body of the corpus callosum (Fabri et al. 2011). Additionally the strength of the BOLD response in the mesial frontal cortex during bimanual movements was correlated with the area of the genu and posterior midbody of the CC (Stancak et al. 2003).

In summary, studies investigating the functional consequences of callosal connectivity were largely acallosal (agenesis and transection) studies. These studies demonstrated that absence of the corpus callosum was associated with reduced performance in various aspects of cognitive and motor functioning, including: verbal fluency, language comprehension, working memory, non-verbal reasoning and problem solving, processing speed, emotional and social processing, finger dexterity and bimanual coordination (Chiarello et al. 1980, Sauerwein et al. 1993 & 1994, Moes et al. 2008, Mueller et al. 2009). Reliability of the results of these acallosal studies was limited, however, since they were conducted with small samples of individuals with additional neurological conditions and developmental disorders. MR studies investigating the functional correlates of corpus callosum are few and their results inconsistent and contradictory. Some of these studies demonstrate a positive (Luders et al. 2007)

and some a negative (Hutchinson et al. 2009) correlation between intelligence (FSIQ) and the corpus callosum. Bimanual coordination has not been associated with callosal volume but positive correlations with callosal microstructure have been demonstrated (Callie et al. 2005, Sullivan et al. 2006, Meutzel et al. 2008, Johansen-Berg et al. 2007).

1.5 ASSESSMENT OF THE CORPUS CALLOSUM MORPHOLOGY

In this section the different techniques used to study the structure and function of the corpus callosum are discussed. Firstly, the different *ex-vivo* techniques that have been used classically by various researchers to study the following are discussed: 1) the volume of the corpus callosum in autopsied human brains; 2) connectivity of callosal fibres in animals (rhesus monkeys); and 3) microstructural properties of fibre composition along the length of the CC. Although these techniques have not been employed by this thesis, they are the means that have been used to gain knowledge about callosal structure. Secondly, the modern magnetic resonance imaging (MRI) techniques employed by this thesis to evaluate the volume of the corpus callosum and its microstructure are described.

1.5.1 *Ex-Vivo* Techniques

Prior to the advent of modern in vivo imaging techniques, the standard means of studying structural properties of specific regions within the brain was by postmortem examination of autopsied brains. Here the postmortem means used to study the size of the corpus callosum, its anatomic connectivity and its

microstructural properties such as myelination, axon density and axon size in both animal and human studies are discussed. These techniques form the basis of many well-established facts on callosal macrostructure and microstructure and are considered to be the gold-standard for examining callosal anatomy.

Size of the Corpus Callosum: Most preliminary studies demonstrating sex differences in the size of the corpus callosum were conducted on photographs or drawings of fixed mid-sagittal sections of the corpus callosum, dissected from autopsied brains. The basic preparatory steps involved in creating these mid-sagittal sections began with removal of the brain from the skull of the deceased individual within 24 hours following death (Witelson et al. 1991). The extracted brains were then immersed in a fixative material such as formalin (10%) and kept in cold storage (for up to three weeks). In rodent studies, subjects were anesthetized and directly perfused with fixative material such as formalin; their brains were then removed the day following perfusion and kept in formalin (Berrebi et al. 1988). Subsequently the formalin fixed brains were bisected in the sagittal plane to expose the corpus callosum (Witelson et al. 1991, Berrebi et al. 1988). In humans, the exposed medial surface of each hemisphere was photographed and magnified prints of the photographs were produced. The outline of the corpus callosum was traced on these magnified prints (Witelson et al. 1991). These tracings were then viewed on a computer screen and their mid-sagittal area calculated through specially designed software (Witelson et al. 1991). Rodent studies used a slightly different approach to measure the mid-sagittal area of the corpus callosum. They sectioned the formalin fixed brains to

produce thin sagittal slices (45 to 60 µm). These slices were mounted on slides and then a microscope with camera-lucida attachment was used to create their drawings (Berrebi et al. 1988). Camera-lucida is an optical device that enables the microscopic view of the corpus callosum to be superimposed on a drawing surface.

Anatomic Connectivity of the Corpus Callosum: Today DTI-based tractography enables us to "trace" the paths of different white-matter tracts and explore their connectivity in a non-invasive fashion. The invasive, neuroanatomical methods originally used, however, continue to be the gold standard for studying the projection patterns of various white-matter tracts. They allow for more accurate identification of the tract pathways. Of these neuroanatomical methods, two of the most common, comprehensive and reliable techniques used to elucidate the intracallosal topography are the tracer and transection studies done in rhesus monkeys. The tracer technique investigated callosal topography by injecting tracers directly into specific cortical regions of anesthetized rhesus monkeys and subsequently studying the distribution of terminal label within the corpus callosum via dark field microscopy. Callosal fibres pathways were mapped by correlating the terminal label location in callosal sub-regions with the cortical sites of label injection (Pandya et al. 1969a, 1969b). Similarly, transection studies examined fibre degeneration in cortical areas that results from transection of different callosal sub-regions of rhesus monkeys mapped; CC pathways were then mapped by documenting the terminal fibre degeneration observed in cortical areas with the callosal sub-regions sectioned (Pandya et al. 1971).

Microstructural Properties of the Corpus Callosum: Fibre composition of the corpus callosum has been examined in detail by electron and light microscopic analysis techniques. Fibres below 0.4 µm diameter cannot be seen by light microscopy due to limitations in the resolution of the light microscope. Electron microscopy has a higher resolution and allows (unmyelinated) fibres below 0.4 µm to be visualized. Specific stains are used along with microscopy to allow clear visualization of the individual fibres of the corpus callosum and their myelin coats. In particular, Holmes dye was used for identification of the neurofibrils and Loyez dye for identification of the myelin sheath (Aboitiz et al. 1992). Photomicrographs of these myelinated and unmyelinated fibres were then compared with a calibrated grid for estimation of their size and density.

1.5.2 *In-Vivo* Magnetic Resonance Imaging

This section describes the MR-based techniques employed by this thesis to evaluate the volume of the corpus callosum. Understanding the biological properties of the callosal tissue that MRI uses to generate contrast is important. This explains how the white matter of the corpus callosum is highlighted and subsequently targeted for volumetric analysis, by this thesis. Sound knowledge of this technique, its advantages and limitations enables better comprehension of the results of this thesis and their potential consequences.

Magnetic resonance imaging (MRI) is an *in-vivo* imaging technique used to generate greyscale images of the anatomy of the human body, particularly soft tissues, by utilizing the magnetic property of the body protons. The human body

is about 60 percent water. Each water molecule contains two hydrogen nuclei or protons, which behave like small independent magnets with their own magnetic moments and spin or angular momentum (Boesch et al. 1999, NessAvier et al. 1997). In order to generate an MR image, the body is first exposed to a strong static magnetic field (B_o) generated in a large electromagnet. This forces the magnetic moment of the body's protons to align along the direction of the magnetic field (B_o). Next a radiofrequency transmitter is switched on to release energy known as 'resonance frequency' (RF). This resonance frequency knocks the protons out of their aligned state and changes their spin (Boesch et al. 1999, Brown et al. 1999). The degree to which the aligned spin of the protons is affected depends on the intensity and duration of application of the RF (Boesch et al. 1999, Brown et al. 1999). The RF is then switched off and this causes the protons to return to their original equilibrium state by releasing energy (difference in the two energy states). It is this released energy which forms the final MR signal detected to generate the images. The MR signals acquired are initially stored in a temporary image space called 'k space', before being reconstructed (Boesch et al. 1999). A mathematical model known as the Fourier transformation is applied to the raw k space data to produce the final image. It is important to note that during the scanning process additional magnetic gradient fields (G_x, G_y and G_z) are also applied. These time varying gradient fields are needed for the spatial localization of the MR signal (Boesch et al. 1999).

The images are produced as greyscale images coding different intensities of the recorded MR signal. The greater the difference in the MR signal intensity of the

different tissue types, the better the contrast in the image. Image contrast is dependent on three main factors: tissue properties, sequence type and sequence parameters (Boesch et al. 1999, Brown et al. 1999). The differences in the physical and biological properties of different tissue types cause their protons to return to their equilibrium state at different rates. This difference is responsible for variations in the MR signal intensity of different tissue types that generate contrast. Sequence type basically refers to the time constant used to describe the decay and recovery of the MR signal. There are two main relaxation time constants: T1 and T2 (Boesch et al. 1999). T1 or spin-lattice relaxation time refers to the rate at which the longitudinal component of the magnetization vectors recovers exponentially to its original equilibrium state. T2 or spin-spin relaxation time refers to the rate at which the transverse component of the magnetization vectors decays exponentially towards zero. The T1 and T2 values of the tissues affects the intensity of their MR signals. Tissues with longer T1 appear hypointense and those with longer T2 appear hyperintense. The two main sequence parameters that influence contrast are TE and TR (Boesch et al. 1999). Echo time or TE refers to the time between the application of the RF pulse and signal acquisition. Repetition time or TR refers to the time between applications of repetitive RF pulses. By adjusting the TR and TE parameters of a certain scanning protocol one can alter the contrast of the resultant images. Short TR and TEs yield T1 weighted images. These T1 weighted images form the basic anatomic scans where water containing tissue, such as, CSF appears dark and fat containing tissue, such as, white matter appears bright. Long TR and TEs yield T2 weighted images. These T2 weighted images are often regarded as “pathology

scans" where water containing tissue, such as, edematous & inflamed tissue appears bright and fat containing tissue appears dark. Short TE and long TRs yield spin-density or proton-density weighted images, where MR signal is generated solely from measuring changes in proton spins. The strength of the MR signal intensity in these proton density weighted images is very low and thus they are not commonly used.

MR Hardware: The MR scanner consists of three key hardware components that work in conjunction to produce the final neuroimages. These are as follows:

- The main magnet: This is a cylindrical shaped electromagnet that produces a homogenous static magnetic field (field strength B_o). This field serves to bring the body protons in their initial equilibrium state. Most scanners used have field strengths above 1.0 Tesla. 1.5 Tesla is the standard field strength of most scanners used for clinical purposes. 3 Tesla scanners, however, are becoming more popular, particularly in research.
- The gradient coil system: This consists of cylindrical gradient coils that produce the orthogonal gradient fields (G_x, G_y and G_z). These strong, fast and linear orthogonal fields are required for spatial localization of the MR signal.
- The radiofrequency transmitter Coil: This is a large cylindrical coil fitted within the main magnet tunnel. It produces the radiofrequency energy that flips the spin of the protons and alters their momentum.

Image Acquisition: Depending on the prerequisites of a particular application, the parameters of image acquisition are adjusted to best complement it. These

parameters form the MR protocol and determine the contrast, resolution and location of the final images. Typically an MR protocol includes defining the following set of MR sequence parameters:

- The specific MR sequence to be used: T1 or T2 or Proton density.
- Determining the timing of application of the RF pulses and signal acquisition: TR and TE.
- Flip Angle: The degree to which the RF alters the spin of the protons from their equilibrium state.
- Field of View: The image area (in mm square) that contains the region of interest to be scanned.
- Matrix Size: The number of data points collected in all directions.

Spatial resolution is determined by voxel size. Voxel size in turn is dependent on matrix size, field of view and slice thickness.

Image Pre and Post Processing: The images produced by the MR scanner show the anatomy in significant detail. To express this anatomy in meaningful quantitative terms, such as specific size and shape details of different regions of interest, the images need further morphometric analysis. This morphometric analysis refers to volumetric and shape quantification of brain anatomy. It consists of a series of pre and post processing steps (Paus et al. 2005).

Pre Processing Steps: The MR images generated are first corrected for any scanner induced inhomogenieties (eddy currents). Next the image is segmented into brain and non brain tissue; subsequently the brain tissue alone is extracted

(Paus et al. 2005). This completes the initial pre processing steps of image analysis. In order to normalize for inter-individual differences in brain size, the images in native space are registered with the standardized stereotaxic space of a template brain. The template brain could be chosen from one of the brain atlases available (MNI 305, ICBM 152, SYS333) or alternatively an average of a particular study's sample brains could be used to generate a template. Registration can be of two main types: 1) Linear registration: This is typically a nine-parameter registration (scaling × rotation × translation); and 2) Non-Linear Registration: This is a high resolution registration, which takes a voxel from image 1 to a voxel in image 2 and measures the displacement field between the two images (Paus et al. 2005). Following the registration of the images they are classified into grey matter, white matter and CSF, based on tissue intensities. This generates probabilistic density images of grey matter, white matter and CSF. Different morphometric techniques can be used for further processing of the data, depending on the requirements of the research study.

1.5.3 *In-Vivo* Magnetization Transfer Ratio

This section describes in detail the magnetization transfer (MT) technique that is employed by this thesis to evaluate the microstructure of the corpus callosum. This thesis examines the relationship of sex hormones with the microstructure of the corpus callosum, using this technique (Chapter 7). Understanding the biological properties of the callosal tissue that are used by MT to generate contrast is, therefore, important to account for the microstructural changes reflected by varying MTR values (Chapter 7).

Traditionally MRI produced brain images by generating contrast between tissues based on the MR sequence used (T1, T2) and its specific parameters. Magnetization transfer ratio (MTR) can be thought of as another MR sequence that generates contrast based on the distribution and relaxation times of macromolecular protons and the degree of water-macromolecular magnetization interaction (Mehta et al. 1996).

Body tissue consists of two separate populations of hydrogen protons: the freely mobile pool of water protons and the immobile pool of macromolecular-bound protons. The bound protons as compared with the mobile protons have very short T2 relaxation times. As a result the bound protons are not directly visible on MRI. These invisible protons are indirectly visualized by the MTR technique. In this technique an off-resonance excitation pulse is applied to saturate selectively the macromolecular-bound protons (Mehta et al. 1996). The macromolecular magnetization pool in turn influences the magnetic moment of neighbouring free protons. Thus there is an exchange of magnetization between the two pools of protons, which can be referred to as the 'cross-relaxation' phenomenon (Mehta et al. 1996). As a result of the longitudinal magnetization gained by the free protons via cross-relaxation they will take longer to return to their original equilibrium state. Their T1 relaxation time will increase and their NMR signal strength will decrease. The degree to which magnetization transfer affects the signal intensity of the free pool depends on the following factors: the relative population sizes of the two pools, the relaxation times of the two pools and the magnetization transfer exchange rate (Mehta et al. 1996). In order to measure

the signal reduction caused by the saturation pulse, images are acquired both in the MT on and off state. Signal variation is then calculated as the ratio of the percent signal change between the MT on (M_0) and MT off (M_t) acquisitions. This ratio is called the magnetization transfer ratio (MTR = $(M_0-M_t)/M_0$).

Biological Basis: The principle source of macromolecules in biological tissue that provides the MT signal is the cell membrane. Cell membrane is a complex structure made up of lipid bilayers and proteins. In case of the central nervous system the greatest MT effect is generally by white matter, followed by grey matter whereas the acellular CSF (lacking macromolecules) does not experience any MT. The myelinated axonal membranes of white matter have been shown to be the prime determinants of MTR (Schmierer et al. 2004). The myelin sheath is made up of multiple layers of oligodendroglial cell membrane, repeatedly coiled along the long axis of the axons. The lipoprotein structure of myelin is composed of cholesterol, phospholipids, glycospingolipids and various structural proteins. Kucharczyk et al. (1994) conducted a study to examine the effects of major lipid components of white matter on magnetization transfer of water protons. They observed that the major lipid component of myelin affecting MTR was galactocerebroside (a sphingolipid). Furthermore, various animal studies showed a significant difference in the MTR value of demyelinating tissue as compared with normal healthy tissue. Dousset et al. (1992) showed that the magnetization transfer ratio of experimentally induced demyelinating lesions in guinea pigs was significantly lower in comparison with that of normal healthy white matter. Similarly, Zaaraoui et al. (2008) demonstrated that MTR is a sensitive indicator of

myelin loss and repair in mice. Apart from the degree of myelination a few other factors have been shown to influence the MTR signal. These factors include axonal density, tissue hydration, vascularity and glial tissue. Mottershed et al. (2003) demonstrated that axonal density strongly correlated with MTR of patients with multiple sclerosis. Dousset et al. (1992) showed that the magnetization transfer ratio of edematous lesions was slightly but significantly lower than that of normal white matter in guinea pigs. Temiak et al. (1994) observed a significant difference in the MTR value of old versus new multiple sclerosis lesions. In addition to demyelination, this difference was attributed to gliosis and edema.

1.6 CORPUS CALLOSUM: ASSOCIATION WITH AGE AND SEX

In this section the existing literature on association of age and sex with the volume of the corpus callosum is discussed. This thesis explores sex differences in the volume of the corpus callosum during adolescence (Chapter 4). Hence, knowledge of previous studies examining sexual dimorphism in CC volume is vital to understand better the advantages of this study relative to previous studies. Furthermore, literature on sex differences in the volume of the corpus callosum is studied and accordingly described (in this section) in an age-wise fashion; beginning from the perinatal period, progressing to childhood, adolescence, adulthood and finally ending in the elderly stage of life.

1.6.1 Association of Age and Sex with the Corpus Callosum during the perinatal period

This section describes the association of age and sex with CC volume during the prenatal period up to the first two years of life. Sexual dimorphism in the volume of the corpus callosum is rarely reported during this period.

Total Corpus Callosum: Starting from the earliest stages of life, post-mortem studies investigated the effect of age on the CC of fetuses. Koshi et al. (1997) autopsied 27 fetal brains ranging from 20 to 42 weeks of gestation. They observed that the absolute length of the corpus callosum showed a significant increase with gestational age. No sex differences were seen in the absolute length of the corpus callosum. Similarly, Clarke et al. (1989) investigated the effects of age and sex on the corpus callosum of the autopsied brains of 32 fetuses (20 to 42 weeks of gestation), 23 infants (birth to 14 months) and 5 children (2 to 14 years). Like Koshi et al. (1997), they too observed that the absolute length and area of the corpus callosum increases rapidly in fetuses up to 33 weeks and again in infants between 2 and 12 months. Moreover their report demonstrated that the corpus callosum reached adult size at around five years of age. No sex differences were observed in the length and area of the corpus callosum of fetuses. In infants, however, males had significantly greater callosal length and area as compared with females. Hwang et al. (2004) conducted an ultrasound study on a large sample of 200 neonates (birth to 28 days). They investigated sex differences in the CC during the neonatal period, that is, the first

month following birth. No sex differences were observed in the absolute area of the total CC.

Corpus Callosum Segments: The study by Koshi et al. 1997 (described above) examined the effects of age and sex on the width of the genu, body and splenium of fetal brains. They observed that the width of the genu alone showed a significant steady increase with gestational age. No sex difference was reported in the width of any of the callosal segments investigated. The study by Hwang et al. 2004 (described above) investigated sex differences in the absolute area and thickness (width) of the callosal sub-regions of neonates. They observed no sex difference in the absolute area of any of the callosal sub-regions examined. The thickness (width) of the splenium, however, was seen to be greater in female neonates as compared with male neonates.

In summary, studies examining callosal growth during early stages of life reported rapid increases in its absolute size during the prenatal period up to infancy. No sex differences are evident in the absolute area of the CC during the prenatal and neonatal period. In infancy, there were reports of greater male versus female absolute area of the corpus callosum. Sex differences in relative (brain size-corrected) area of the CC during the perinatal period of development were rarely investigated. See Table 1.1.

Table 1.1 Summary of Studies on Sex differences in CC Area: Prenatal to Infancy								
AUTHOR	YEAR	STUDY TECHNIQUE	N	AGE	RELATIVE AREA		ABSOLUTE AREA	
					TOTAL	SEGMENTAL	TOTAL	SEGMENTAL
Koshi et al.	*1997*	*Post mortem*	*27*	*20 - 42 gestational weeks*	*NI*	*NI*	*M=F*	*M=F*
Clarke et al.	*1989*	*Post mortem*	*32*	*21 - 42 gestational weeks*	*NI*	*NI*	*M=F*	*NI*
Clarke et al.	*1989*	*Post mortem*	*23*	*Birth - 14 months*	*NI*	*NI*	*M>F*	*NI*
Hwang et al.	*2004*	*Ultrasonography*	*200*	*Birth - 28 days*	*NI*	*NI*	*M=F*	*M=F*

Note: NI refers to Not Investigated.

1.6.2 Association of Age and Sex with the Corpus Callosum of Children and Adolescents

This section describes the association of age and sex with CC area during childhood and adolescence. Literature on callosal sex differences during this period is inconsistent. These inconsistencies provide the impetus for re-examination of callosal sex differences during adolescence by this thesis.

Most of the studies reported sex differences in the absolute area of the corpus callosum, however, these do not represent true sex differences in CC area because they have not been corrected for total brain size. Total brain size, irrespective of sex, is known to be a major predictor of regional brain sizes, such as that of the CC. Correlations of up to 0.40 (Johnson et al. 1994) and 0.45 (Bermudez et al. 2000) have been reported between the area of the corpus callosum and total brain volume. These correlations highlight the importance of normalizing for brain size when examining sex differences in the CC. This is to ensure that the differences observed between the CC area of males and females

can be attributed to their sex rather than the presence of overall larger brains in males versus females. Therefore, studies that have examined true sex differences, that is, sex differences in brain-size corrected (relative) area of the corpus callosum have been given impetus in this thesis.

Total Corpus Callosum (Relative Area): Raunch and Jinkins (1994) investigated the association of age and sex with the relative area of the CC of 45 participants from birth to 20 years of age .They reported a strong correlation between age and relative CC in the first decade (0 to 10 years). This correlation represented an increase of 8% per year in the relative callosal area. During the second decade (10 to 20 years) they reported a weak correlation between relative area of the CC and age with only 2.8% increase in relative CC area per year. No sex differences were observed in the relative area during the first or second decade. Giedd et al. (1999) investigated the development of the corpus callosum in 139 children and adolescents aged 5 to 18 years. They observed a trend ($p=0.07$) for larger relative CC area in females. There was, however, no sex difference reported in the relative CC area versus age. Lenroot et al. (2007) reported results of the expanded Giedd et al. 1999 sample, which included 387 participants aged 3 to 27 years. They observed that the developmental trajectory of the relative CC area of females ($n=178$) was significantly higher than that of males ($n=209$), which suggests that the area of the corpus callosum was larger in females versus males. De Bellis et al. (2001), on the other hand, investigated age-related increases in the relative CC area of 138 subjects aged 7 to 17 years and observed that the relative CC area increased at faster rates in boys as compared with girls.

Corpus Callosum Segments (Relative Area): In the Giedd et al. 1999 study (see above), boys had significantly higher trajectories than girls for the anterior body, posterior body and splenium. This suggested that during development at certain points the absolute area of these specific segments was greater in boys versus girls, but the general growth pattern for the two sexes was the same. They did, however, observe a trend (p value between 0.05 and 0.10) for proportionately larger genu, posterior midbody and isthmus in females.

Sex differences in the absolute area of the total corpus callosum and its segments during childhood and adolescence, unlike its relative area, were not found to be significant by two studies (Giedd et al. 1999, Raunch and Jinkins 1994). Age associated changes in the absolute volume of the corpus callosum, however, were very similar to those of the relative volume; with a 13% increase during the first decade and a 3% increase in the second decade (Raunch and Jinkins 1994).

In summary, the results of studies investigating sexual dimorphism in the relative area of the total corpus callosum and its segments, during childhood and adolescents were mixed. Some researchers did not observe any sex differences in callosal area (Raunch and Jinkins 1994), while some observed a female versus male advantage (Giedd et al. 1999, Lenroot et al. 2007). Reports of sexually dimorphic growth patterns of the CC during this period were, however, more frequent. Multiple large-scale studies observed some evidence of sexually dimorphic volume or sexually dimorphic age-related growth of the CC, during adolescence (Giedd et al. 1999, Lenroot et al. 2007, De Bellis et al.). These sex

differences were limited to the anterior and central segment of the corpus callosum (Giedd et al. 1999). Regarding sex differences in absolute callosal area, most studies had no significant results. See Table 1.2.

Table 1.2 Summary of Studies on Sex Differences in CC Area: Childhood to Adolescence

AUTHOR	YEAR	STUDY TECHNIQUE	N	AGE (years)	RELATIVE AREA		ABSOLUTE AREA	
					TOTAL	SEGMENTAL	TOTAL	SEGMENTAL
Lenroot et al.	*2007*	*MRI*	*387*	*3 - 27*	*F>M*	*NI*	*M=F*	*NI*
Giedd et al.	*1999*	*MRI*	*139*	*5 - 18*	*F>M r=0.153*	*F>M: genu, posterior midbody and isthmus*	*M=F*	*M=F: all segments*
Raunch et al.	*1994*	*MRI*	*29*	*0 - 10*	*M=F r=0.179*	*NI*	*M=F*	*NI*
Raunch et al.	*1994*	*MRI*	*16*	*10 - 20*	*M=F r=0.243*	*NI*	*M=F*	*NI*
Hayakawa et al.	*1989*	*MRI*	*77*	*0 - 15*	*NI*	*NI*	*M=F*	*NI*
Allen et al.	*1991*	*MRI*	*24*	*2 - 15*	*NI*	*NI*	*M>F*	*M=F: all segments*

Note: NI refers to Not Investigated; Effect Size (r) for sex difference in relative volume of Total CC is reported, since this thesis examines sex difference in relative and not absolute volumes.

1.6.3 Association of Age and Sex with the Corpus Callosum of Adults

This section describes the association of age and sex with CC area of adults.

Once again, most studies consider the absolute area of the corpus callosum when examining sex differences, rather than its relative area. These sex differences are confounded by the total brain size. Studies that have examined sex differences in the brain-size corrected, that is, relative area of the corpus callosum of adults are described below.

Total Corpus Callosum (Relative Area): Most of the studies investigating the association of age and sex with the relative area of the CC in adults observed sex

differences (F>M). Johnson et al. (1994) conducted an MRI study on 200 healthy participants aged 16 to 55 years. They did not observe a significant correlation between the relative area of the corpus callosum and age in either of the two sexes. They also divided their sample into subgroups based on 10-year age blocks, and compared their means. No significant differences were observed in the relative CC area of any of the age subgroups, in either of the two sexes. In case of sex effects, they observed that the relative area of the corpus callosum was significantly greater in women versus men. When investigating specific age subgroups they observed that women had greater relative CC area as compared with men in all subgroups, except the 46 to 55 years range subgroup. Similarly Bermudez and Zatorre (2000) demonstrated that the relative area of the total corpus callosum was significantly greater in women (n=59) as compared with men (n=78) aged 25 ±5 years. Lee et al. (2009) compared the relative CC area of 68 healthy participants in their twenties with 91 healthy participants in their forties. They observed no significant difference between the two groups. Sullivan et al. (2001) also reported no significant correlation between relative area of the corpus callosum and age in either sex, in a sample of 92 participants aged 22 to 71 years. Sex differences, however, were reported in this study: the relative CC area was greater in men as compared with women. On the other hand, no sex differences in the relative area of the CC were reported by Allen et al. (2002; 46 adult participants aged 22 to 49 years) and Constant and Ruther (1996; 104 adult participants aged 19 to 65 years).

Corpus Callosum Segments (Relative Area): The study by Bermudez and Zatorre 2000 (described above) investigated the effects of sex on the anterior third, anterior midbody, posterior midbody, isthmus and splenium of adults. They observed that the relative area of all the callosal segments were significantly greater in women as compared with men. Witelson et al. (1989) conducted a post-mortem study to investigate the effects of sex on the corpus callosum of 50 adult autopsied brains. They divided the corpus callosum into seven segments which were as follows: rostrum (region 1), genu (region 2), anterior midbody (region 3 and 4), posterior midbody & isthmus (region 5 and 6) and splenium (region 7). They observed that the relative area of the isthmus alone was significantly greater in women as compared with men. Similarly, Steinmetz et al. (1992) observed that the relative area of the isthmus alone was significantly greater in women versus men (n=52). Dubb et al. (2003) via a large-scale study (n=189) demonstrated that age related contraction in the callosal area of men was mainly limited to their genu. Sullivan et al. (2001; study described above) examined the association of age and sex with the genu, body and splenium of the corpus callosum and observed opposing results. They observed a negative correlation between relative area of only the genu and age in women. The relative areas of all three segments were seen to be greater in men as compared with women. Allen et al. (2002; study described above) investigated sex differences in the area of the anterior third, middle third, isthmus and posterior fifth of the corpus callosum and observed no significant sex differences in the relative area of any of the callosal segments. Constant and Ruther (1996; study described above) investigated sex differences in the seven Witelson (1989)

defined segments of the corpus callosum and observed no sex differences in the relative areas of any callosal segment.

A unique feature observed by some studies investigating age associated changes in absolute CC area was the continued growth of the CC during adulthood. Pujol et al. (1992) investigated the effects of age and sex on the corpus callosum of 90 healthy participants aged 11 to 63 years. They had a longitudinal study design and re-scanned each participant after two years. Changes in the absolute area of the corpus callosum were prominent in the second decade, persisted in the twenties and were absent thereafter. Additionally the biennial growth rate of the corpus callosum was seen to be significantly greater in women as compared with men. Cowell et al. (1992) investigated the effects of age and sex on the corpus callosum of 146 participants aged 2 to 79 years. They observed that the growth curve for the corpus callosum followed a curved pattern for both males and females. The absolute area of the corpus callosum was seen to peak at around 20 years in men and between 40 and 50 years in women. The above-mentioned studies give additional support for the sexually dimorphic patterns of continued growth of the corpus callosum in adulthood. Thus the corpus callosum appeared to be one of the rare structures that exhibit limited increases in size with age during early adulthood. It is the sexually dimorphic nature of this continued growth of the corpus callosum that forms the basis of this thesis.

The absolute area of the total corpus callosum, unlike its relative area, was shown to be larger in men as compared with women by most studies; these sex differences were reported most frequently in the genu and midbody of the

corpus callosum (Johnson et al. 1994, Bermudez and Zatorre 2000, Allen et al. 2002, Clarke et al. 1994).

Table 1.3 Summary of Studies on Sex differences in CC Area: Adulthood

AUTHOR	YEAR	STUDY TECHNIQUE	N	AGE (years)	RELATIVE AREA		ABSOLUTE AREA	
					TOTAL	SEGMENTAL	TOTAL	SEGMENTAL
Bermudaz et al.	*2000*	*MRI*	*137*	*10 - 40*	*F>M r=0.214*	*F>M: genu, anterior and posterior midbody, isthmus and splenium*	*M>F*	*M>F: genu, posterior midbody*
Johnson et al.	*1994*	*MRI*	*42*	*15 - 25*	*F>M r=0.394*	*NI*	*M=F*	*NI*
Johnson et al.	*1994*	*MRI*	*41*	*25 - 35*	*F>M r=0.309*	*NI*	*M>F*	*NI*
Johnson et al.	*1994*	*MRI*	*34*	*35 - 45*	*F>M r=0.339*	*NI*	*M=F*	*NI*
Johnson et al.	*1994*	*MRI*	*39*	*45 - 55*	*M=F r=0.266*	*NI*	*M=F*	*NI*
Johnson et al.	*1994*	*MRI*	*31*	*55 - 65*	*F>M r=0.441*	*NI*	*M=F*	*NI*
Witelson et al.	*1989*	*Post mortem*	*50*	*25 - 70*	*NI*	*F>M: isthmus*	*NI*	*M>F: genu and anterior midbody*
Witelson et al.	*1991*	*Post mortem*	*62*	*25 - 70*	*NI*	*NI*	*M=F*	*NI*
Kertesz et al.	*1987*	*MRI*	*104*	*18 - 49*	*NI*	*NI*	*M=F*	*NI*
Hayakawa et al.	*1989*	*MRI*	*56*	*15 - 60*	*NI*	*NI*	*M=F*	*NI*
Clark et al.	*1994*	*MRI*	*60*	*21 - 43*	*NI*	*NI*	*M>F*	*NI*
Allen et al.	*2002*	*MRI*	*46*	*22 - 49*	*M=F r=0.001*	*M=F: all segments*	*M>F*	*M>F: splenium*
Constant et al.	*1996*	*MRI*	*104*	*19 - 65*	*M=F r=0.022*	*NI*	*M=F*	*NI*
Sullivan et al.	*2001*	*MRI*	*92*	*22 - 71*	*M>F*	*M>F: genu, body and splenium*	*M>F*	*M>F: genu, body and splenium*

Note: NI: Not Investigated; Effect Size (r) for sex difference in relative volume of Total CC is reported, since this thesis examines sex difference in relative and not absolute volume.

In summary, most studies examining sex differences in the relative area of the corpus callosum reported greater area in women as compared with men (Johnson et al. 1994, Bermudez and Zatorre 2000). Rarely no sex differences were observed in the relative area of the CC (Allen et al. 2002, Constant and

Ruther 1996). These sex differences were most frequently seen in the isthmus (Witelson et al. 1989, Steinmetz et al. 1992) and less commonly across all segments (Bermudez and Zatorre 2000). As expected given the sex differences in brain size, the absolute area of the CC was usually greater in men as compared with women (Bermudez and Zatorre 2000, Clarke et al. 1994). See Table 1.3.

1.6.4 Association of Age and Sex with Corpus Callosum of Elderly

This section describes the studies that have examined sex differences in the brain-size corrected, that is, relative area of the corpus callosum of the older population (above 55 years). Sex differences in the elderly population have been reported separately because women undergo menopause during late 40s to early 50s, which marks the cessation of the ovarian production of sex hormones (Guyton et al. 2000). Thus, examining sex differences during this period separately enables one to assess how sexual dimorphism of the corpus callosum changes with variation in sex hormonal milieus.

Total Corpus Callosum (Relative Area): Davatzikos et al. (1998) reported that the relative area of the corpus callosum correlated negatively with age in both sexes of an older population (n=114, 56 to 85 years). No sex difference was reported in the relative area of the corpus callosum. Salat et al. (1996) conducted an MRI study on 76 older participants aged 65 to 95 years. They observed that the relative area of the corpus callosum declined significantly with age in women but not in men. The relative area of the total corpus callosum was significantly greater in women as compared with men. Burke and Yeo (1994) observed no sex

difference in the relative area of the total corpus callosum of older individuals (n=97, 60 to 90 years). See Table 1.4.

Corpus Callosum Segments (Relative Area): Burke and Yeo (1994; study described above) investigated the effects of age and sex on the anterior and posterior half, and the seven Witelson (1989) defined segments of the corpus callosum. They observed that the relative areas of the rostrum and genu were significantly greater in women (n=59) as compared with men (n=38). Davatzikos et al. (1998; study described above) investigated the effects of age on relative area of callosal subregions and observed that age was negatively correlated with the relative area of the splenium. Alternatively, relative areas of the anterior and posterior extremes of the corpus callosum were positively correlated with age. In the case of sex effects, the relative area of the splenium was reported to be greater in women as compared with men. Salat et al. (1996; study described above) investigated the effect of age on the anterior, middle and posterior segments of the corpus callosum of older individuals. They observed that the relative area of the anterior and middle callosal segment declined significantly with age in women but not in men. The relative area of the posterior callosal segment was significantly greater in women as compared with men. See Table 1.4.

The absolute area of the total corpus callosum did not demonstrate a female versus male advantage, unlike its relative area, in the older population. It was found to be either greater in older males versus females (Davatzikos et al. 1998) or equal in size (Burke et al. 1994). The absolute area of the total corpus

callosum, however, did demonstrate an age associated decline similar to its relative area (Davatzikos et al. 1998, Burke et al. 1994). See Table 1.4.

Table 1.4 Summary of Studies on Sex Differences in CC Area: Elderly

AUTHOR	YEAR	STUDY TECHNIQUE	N	AGE (years)	RELATIVE AREA		ABSOLUTE AREA	
					TOTAL	SEGMENTAL	TOTAL	SEGMENTAL
Salat et al.	*1996*	*MRI*	*76*	*65 - 95*	*F>M r=0.194*	*F>M: splenium*	*NI*	*NI*
Davatzikos et al.	*1998*	*MRI*	*114*	*56 - 85*	*NI*	*F>M: splenium*	*M>F*	*F>M: splenium*
Burke et al.	*1994*	*MRI*	*97*	*60 - 90*	*M=F*	*F>M: rostrum and genu*	*M=F*	*F>M: rostrum*

Note: NI refers to Not Investigated; Effect Size (r) for sex difference in relative volume of Total CC is reported, since this thesis examines sex difference in relative and not absolute volumes.

1.7 HORMONES AND MENSTRUAL CYCLE: RELATION WITH THE BRAIN

As reviewed above, sexual dimorphism in the volume of the corpus callosum is extensively researched. These sex differences are rarely reported during the early years of life (Section 1.6.1: Koshi et al. 1997, Hwang et al. 2004, Clarke et al. 1989) and reports of a sexually dimorphic CC become more frequent during childhood and adolescence (Section 1.6.2: Giedd et al. 1999 and Lenroot et al. 2007). Sex differences in CC area are most robust during late adolescence and adulthood (Section 1.6.2 and 1.6.3: Giedd et al. 1999 and Lenroot et al. 2007, Bermudez and Zatorre 2000, Johnson et al. 1994, Sullivan et al. 2001, Witelson et al. 1989). Sex difference in the relative area of the corpus callosum is first reported during adolescence. Adolescence is in fact the period during which most of the physical differences between boys and girls manifest (Grumbach et al. 2003, Guyton et al. 2000). These include the development of secondary sexual

characteristics such as breasts, facial and pubic hair and voice changes. Sex hormones are known to play the most important role in the development of these sexually dimorphic physical attributes (Grumbach et al. 2003, Guyton et al. 2000). It is, therefore, possible that sex hormones also play a role in the sexually dimorphic development of the corpus callosum. This thesis for the first time poses a novel question regarding the possible role of sex hormones as one of the potential factors responsible for the development of sexual dimorphism in callosal volume (Chapter 6 and 7).

The physical differences between men and women include not just primary and secondary sexual characteristics but also other attributes, for example body muscle-fat ratio, blood viscosity and bone shape. Sex chromosomes and sex hormones direct the development of these differences. It is therefore not surprising that sex chromosomes and sex hormones also play an important role in generating the differences observed in the brains of men and women.

There are many sex hormones, of these the main sex steroids are estrogen, progesterone and testosterone. Sex hormones are lipid-soluble hormones and therefore cross the blood-brain barrier. Inside the brain, these hormones penetrate the neuronal membrane and bind to intracellular steroid receptors to exert their influence (reviewed by Beyer 1999). Multiple areas of the brain express receptors for sex hormones. These include not only the pre-optic area of the hypothalamus that is responsible for feedback regulation of the sex hormones, but also other regions such as, hippocampus, amygdala and midbrain (reviewed by Beyer 1999).

Animal and human studies demonstrate that sex steroid hormones correlate with various aspects of brain structure and function (human: Perrin et al. 2008, Peper et al. 2009, Neufang et al. 2009; rat: Bimonte et al. 2000a, Fitch et al. 1989b). Various human studies have reported correlations between the level of sex hormones and macroscopic features of the brain such as the volume of grey matter and white matter (Perrin et al. 2008, Peper et al. 2009, Neufang et al. 2009). There are no human studies demonstrating the role of sex steroids on the CC specifically. There are, however, animal (rat) studies describing the influence of sex steroid hormones on the CC. The rat's corpus callosum, like that of humans, is sexually dimorphic with the absolute mid-sagittal area of the CC being greater in males versus females (Berrebi et al. 1988, Fitch et al. 1991a). Research in rat brains has demonstrated that sex steroids are responsible for the development of these sex differences. Specifically, testosterone has a masculinising effect on the CC of both male and female rats. Testosterone exerts this organizational effect on male rats prenatally and on female rats during the early postnatal period. This is evidenced by the fact that the CC of male rats exposed to an androgen blocker (flutamide) prenatally was smaller than that of controls (Fitch et al. 1991b) and that of male rats castrated in the early postnatal period (day 1) was similar in size to that of controls (Fitch et al. 1989b), whereas the CC of female rats given testosterone in the early postnatal period (day 4) was greater than that of controls (Fitch et al. 1990). Ovarian hormones, unlike testosterone, were seen to exert a feminizing influence on the CC of female rats only. Male rats that were castrated and given synthetic estrogen (Diethylstilbestrol) or estrogen antagonist (tamoxifen) in the early postnatal

period (day 4) developed a CC of comparable size to that of control rats, which had undergone sham procedures (sham surgeries and injections). In case of female rats neonatal exposure to ovarian hormones was seen to influence the response of the CC to ovarian hormones produced later in life (Bimonte et al. 2000a). The CC of female rats upon exposure to adult estrogen (via ovary transfer or subcutaneous estrogen pellet day 70 till 184) decreased in size when the female rats had prior neonatal exposure to estrogen (day 1 till 25) but increased in size when they did not have any prior exposure to neonatal estrogen (Bimonte et al. 2000a). Thus, neonatal exposure to ovarian hormones primed the female rat CC to respond to the ovarian hormones produced later in life in a characteristic female manner. Moreover, female rats that were ovariectomized (day 8 till 25) had larger CC than control females, which had undergone sham surgeries (Fitch et al. 1991a, Bimonte et al. 2000a); and adult exposure to estrogen (via ovary transfer or subcutaneous estrogen pellet insertion on day 70 till 184) counteracted this enlarging effect of ovariectomy (Bimonte et al. 2000b). It is important to note that ovariectomy after day 78 was seen to have no effect on callosal size, suggesting that after the initial priming 'organizational' effect of neonatal estrogen the influence of subsequent ovarian hormone exposure on CC is established prior to adulthood (day 78; Mack et al. 1994). These studies examine the effects of sex steroids on the absolute size (area and width) of the CC. The absolute CC size was not adjusted for total brain size because consistent correlations were not observed between the CC and the brain size (Fitch et al. 1991a). There are also rodent studies describing the influence of sex steroid hormones on the cellular development of the brain such as the degree of

dendritic branching, synapse formation, myelination, cell death and survival (Baulieu et al. 2000, Marin-Husstege et al. 2004, Levy et al. 1996, review paper by Beyer et al. 1999). Sex hormones have been shown to influence not only brain structure but also various aspects of brain function such as cognitive abilities, mood and performance on motor tasks (reviewed by Ankney et al. 1996, Kimura et al. 1994, Fink et al. 1996, Maki et al. 2002). These studies suggest that the difference in the milieu of sex hormones of men and women may be responsible for some of the differences in the structure and function of their brain.

Males and females are exposed to varying levels of sex hormones from a very early stage of development. During the prenatal period, male fetuses are exposed to a high level of testosterone produced by their developing gonads. On the other hand, female fetuses do not experience such hormonal influences because their gonads are not functional until puberty. Jacobson and Gorski (1981) observed effects of sex hormones on brain structure in rats; they showed that androgen (following aromatization to estrogen) is responsible for the sexual dimorphism of the brain displayed in the immediate postnatal period. Specifically, they observed a sub-region of the hypothalamic pre-optic nucleus called the sexually dimorphic nucleus to be approximately five times larger in newborn male rats as compared with their female counterparts. This structural difference was shown to depend on the perinatal steroid hormone milieu (Jacobson and Gorski 1981, Dohler et al. 1984). Similarly, prenatal exposure to male testosterone has also been shown to be responsible for sex differences observed in the structure of the CC (Fitch et al. 1991b). Several subsequent

studies support the role of sex hormones in organizing sex-specific neuronal architecture (reviewed by Beyer 1999). Thornton et al. (2009) observed effects of sex hormones on behaviour. They examined the effects of prenatal androgens on rhesus monkeys and observed that testosterone and dihydrotestosterone (directly without aromatization) organize neural mechanisms that effect sexually dimorphic behaviour (such as non-sexual play behaviour).

The level of sex hormones in both boys and girls remains low during early childhood. It is upon reaching puberty that the gonads begin producing increasing quantities of sex hormones. Boys are exposed to increasing levels of testosterone and girls to increasing levels of estrogen and progesterone. This surge in the level of sex hormones may drive some of the sex differences in the brain that develop in children following puberty. Only a handful of studies address this question. Peper et al. (2009) conducted an *in-vivo* magnetic resonance study on 80 prepubertal children, aged 10 to 15 years and observed that the absolute volume of grey matter correlated negatively with estradiol levels in girls and positively with testosterone levels in boys. Perrin et al. (2008) reported a positive correlation between testosterone level and the relative volume of white matter in boys (aged 12 to 18 years). Similarly, Neufang et al. (2009) observed a positive correlation between testosterone and the absolute volume of amygdala, hippocampus, and diencephalon in both boys and girls, aged 8 to 15 years. They also observed a negative correlation between estrogen and absolute volume of grey matter in the parahippocampus. Overall,

testosterone appears to have a greater growth-inducing influence on the brain as compared with estrogen.

Post-puberty and continuing into adult life, males are exposed to relatively constant levels of testosterone. Females on the other hand, experience monthly cyclic variations in the level of estrogen and progesterone. The hormone levels vary significantly between different phases of the menstrual cycle. Keeping in mind the relationship between sex hormones and brain exhibited by the above-mentioned studies, it is important to review the possible effects of hormonal variation during menstrual cycle on the brain structure and function.

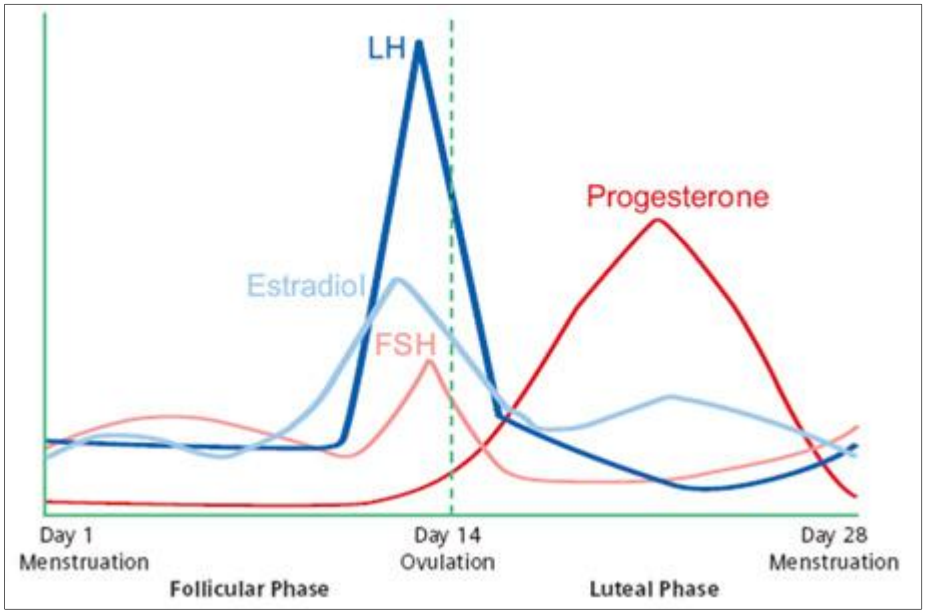

Figure 1.7: Variation in gonadal and pituitary hormones across the menstrual cycle phases. LH: Luteinizing hormone, FSH: Follicle stimulating hormone. Chart from Siemens website http://www.medical.siemens.com

The menstrual cycle can be classified into four phases (see Figure 1.7). These are the menstrual, follicular, ovulatory and luteal phases. The menstrual phase has

low levels of sex hormones. The follicular and ovulatory phases have high levels of estrogen. Lastly, the luteal phase has high levels of progesterone.

There is dearth of literature investigating the effects of menstrual cycle-phase on brain structure and function. Most of the research on this topic has been done on animals. In one such study, Lagnub and Watson (1994) investigated the effect of estrous cycle-phase on the morphology of the anteroventral paraventricular nucleus of the rat hypothalamus. They observed a 39% increase in axosomatic synapses in the estrus (ovulatory) versus proestrus (early follicular) phase and a 22% decrease in axosomatic synapses in the metestrus (early luteal) versus estrus phase. This finding does not directly show the potential effects of cycle-phase on the brain because the anteroventral paraventricular nucleus of the hypothalamus is responsible for stimulating the secretion and feedback regulation of the sex hormones. It is therefore the classic target area for estrogen and has the highest number of estrogen and progesterone receptors in the brain. But sex hormones have been shown to affect other areas of the brain as well. Carillo and Collado (2007) observed cycle-phase to effect the morphology of the anteroventral medial amygdala in the rat. They showed an increase in the number of Nissl stained neurons and NADPH diaphorese positive neurons in this region during the estrus (follicular) phase as compared with the diestrus (ovulatory) phase. This clearly demonstrates that the neuronal architecture within the brain, even in regions other than those directly involved in regulating the menstrual cycle, are influenced by the cyclic variation in sex hormones over a short period (4-5 days for rats).

Human studies reporting the effects of menstrual cycle-phase on brain volume and morphology are few. In one such report Grant et al. (1988) studied the effect of menstrual cycle-phase on total intracranial CSF volume. The CSF volume was shown to be significantly increased in luteal phase as compared with the ovulatory phase. Grant also investigated the effect of oral contraceptives on CSF volume. Oral contraceptives inhibit the natural cyclic production of sex hormones and the exogenous supply via the contraceptive pills themselves form the main source of sex hormones that the body and brain are exposed to. This exogenous source accounts for a much lower and more constant level of estrogen and progesterone. Grant et al. (1988) did not observe any significant effect of oral contraceptives on CSF volume. The use of oral contraceptives, however, is an important factor that needs to be considered when investigating the effect of female sex hormones on the brain. Protopopescu et al. (2008) studied the structural changes in the hippocampus and basal ganglia across the menstrual cycle. They observed the absolute volume of grey matter in the right anterior hippocampus to be greater in late follicular phase (day 10 to day 12) as compared with the luteal phase. On the other hand, the absolute volume of grey matter in the right dorsal basal ganglia was seen to be greater in luteal phase as compared with the late follicular phase. Similarly Pletzer et al. (2010) observed that women had significantly larger right fusiform/parahippocampal gyrus during the early follicular phase as compared with the mid-luteal cycle phase. They also observed significantly larger prefrontal cortices, pre- and postcentral gyri, parahippocampal and fusiform gyri and temporal regions, in women using oral contraceptives as compared with those not using oral contraceptives.

Overall, menstrual cycle changes in the brain are not just limited to the microscopic architectural variations seen in synaptic plasticity, dendritic and glial morphology but also extend to encompass larger macroscopic changes in regional volumes. These cyclic variations in brain volume may be responsible for the conflicting findings of studies investigating sexual dimorphism in brain volume.

1.8 AIMS OF THE THESIS

Evaluation of the existing literature revealed adolescence to be a vital period with regards to the presence of sexual dimorphism in the size of the corpus callosum. Reports of sex differences in the volume of the CC first begin to appear during adolescence. The first goal of this thesis is to investigate sex differences in the relative volume of the corpus callosum during adolescence. While many researchers have examined these differences the results of their studies were often conflicting, making it difficult to reach a consensus. This thesis examines these sex differences in two large samples of typically developing adolescents, namely the Saguenay Youth Study (n=737) and the IMAGEN Study (n=1,979). Moreover, this thesis is the first to examine sex differences in a multi-slice, mid-sagittal slab of the corpus callosum instead of a single-slice, using validated FreeSurfer based analysis tools. This multi-slice, mid-sagittal slab of the corpus callosum provides a 3D measure versus the 2D surface area used conventionally. Normalizing 2D area of the CC with 3D volume of the brain causes disproportionate reduction of CC size in individuals with larger heads.

Furthermore, this thesis examined CC sex differences using strict statistical parameters that corrected for inter-segmental dependence of the CC segments.

The next goal is to determine the functional correlates of the corpus callosum and sexual dimorphism in them. Once again, this is an area that has been researched previously. The literature, however, comprises mostly of small-scale studies conducted on individuals with agenesis or transection of the corpus callosum, with coexisting neurodevelopmental disorders. These studies demonstrate that the corpus callosum was associated with improved performance in many cognitive, emotional and motor brain functions such as verbal fluency, language comprehension, working memory, abstract reasoning and problem solving, processing speed, finger dexterity, bimanual coordination and emotional processing (Section 1.4). This thesis uses previous knowledge on functional correlates of the corpus callosum to explore the contribution of the CC towards specific brain functions in more detail; with particular emphasis on identifying the specific segments of the CC responsible for these functions and sexual dimorphism in structure-function relationships. This aim is achieved by conducting a study on the Saguenay Youth sample that examines the association of the corpus callosum with cognitive performance on the WISC-III intelligence test for the following domains: 1) processing speed; 2) perceptual reasoning; 3) verbal comprehension; and 4) working memory in 737 boys and girls. Additionally the association of the corpus callosum with bimanual coordination performance in the Thurston altered finger tapping task (Leonard et al. 1987) is also investigated in the same sample. For the functions seen to be associated

with the CC, further investigation is done to explore the specific segment that contributes to this relation.

These studies, by virtue of their large sample size (versus small acallosal samples), healthy participant population (versus acallosal participants), well-controlled statistical methods and modern freesurfer based volume measurement technique, are able to accurately and reliably determine the relationship of the corpus callosum with intelligence subsets, motor coordination and affective (emotional) processing of language.

The final goal is to determine the role of sex hormones in the development of sex differences in the structure and function of the corpus callosum. Previous literature has examined how sex hormone levels correlate with grey-matter volume, white-matter volume and the volume of certain brain regions such as the hippocampus, amygdala and diencephalon. The relationship between sex hormones and corpus callosum remains largely unexplored. This thesis is the first to investigate the relationship of sex hormones with the structure and function of the corpus callosum. The relationship of sex hormones with the volume of the corpus callosum is investigated using two main approaches:

- Observational Approach: Using the observational approach, this thesis examines the differences in relative CC volume associated with differences in the level of endogenous sex hormones (bioavailable testosterone) in adolescent boys (n≈350) and their exposure period (exposure to gonadal estrogen and progesterone since menarche in

years) in adolescent girls (n≈325), of the large scale Saguenay Youth study. Additionally, callosal volume and function (bimanual coordination) changes associated with natural variation in female sex hormone levels across four phases of the menstrual cycle, is investigated in a smaller longitudinal study sample called Menstrual Cycle study (n=26 adult women, scanned at four time points).

- Pseudo-experimental Approach: Using the experimental approach, this thesis examines the changes in callosal volume associated with exogenous suppression of female sex hormone production, caused by the use of oral contraceptive pills in adolescent girls. This is done in three separate samples, the Saguenay study sample (n=106), IMAGEN study sample (n=104) and a smaller sample belonging to the Menstrual Cycle study (n=26).

By means of these studies, this thesis for the first time sheds some light on the role of sex hormones in the development of sexual dimorphism in the structure and function of the human corpus callosum.

The volume and microstructural properties of the corpus callosum are measured via high resolution T1 weighted and MTR images, respectively. The volume and microstructural properties of the corpus callosum are always measured first for the total corpus callosum as a whole and then across its five segments (anterior, mid-anterior, central, mid-posterior and posterior). When examining callosal volumes for sex differences, relative volumes of the total corpus callosum and its segments are always considered. Relative volume refers to the brain size-

corrected volume of the corpus callosum. Relative volumes of the total corpus callosum and its five segments are calculated by dividing their absolute volumes with the absolute volume of the total brain. Relative instead of absolute volumes are used because the size of the brain can influence the size of the corpus callosum.

2 METHODOLOGICAL DETAILS OF THE SAGUENAY YOUTH STUDY AND THE IMAGEN STUDY

2A SAGUENAY YOUTH STUDY: METHODOLOGY

2A.1 Introduction

The Saguenay Youth Study (SYS) is a large-scale (n≈1,000) study that evaluates the consequences of prenatal exposure to maternal cigarette smoking (PEMCS) on brain and behaviour and cardiovascular and metabolic health in adolescence. Prenatal exposure to maternal cigarette smoking is assessed retrospectively. Brain and behaviour and cardiovascular and metabolic phenotypes are assessed quantitatively over five sessions (telephone interview, home, hospital, laboratory and school) in a prospective fashion. To facilitate the study of gene-environment interaction, the SYS study has a family-based design with DNA obtained in both biological parents and adolescent siblings. Additionally, these siblings are recruited in a population with known genetic founder effect (Bouchard et al. 1983, Moreau et al. 2011) living in the Saguenay Lac Saint-Jean (SLSJ) region of Quebec, Canada.

2A.2 SLSJ Population

French colonization of the SLSJ region began in 1838 from the Baie-Saint-Paul region (Bouchard et al. 1983). Currently, the SLSJ region has a population of approximately 300,000 inhabitants who originated from a much smaller

settlement of these French settlers (Bouchard et al. 1983, Gauvreau et al. 1991). There has been low immigration to the SLSJ region since; as a result the current SLSJ population is the largest North American population with known genetic founder effect (reviewed by Moreau et al. 2011).

2A.3 Descriptive Statistics

The studies of this thesis are conducted on 737 participants of the Saguenay youth study. This sample consists of 356 boys and 381 girls. The mean age of the boys is 180 (SD: 22) months and that of girls is 181 (23) months. There is no significant difference between the mean age of boys and girls (p=0.444). The mean puberty stage, measured with puberty development scale (PDS; see Section 2A.7) of boys is 3.38 (0.87) and that of girls is 4.08 (0.74). The mean puberty stage of boys is significantly lower than that of girls (p≤0.001). Of the total sample of girls 56 are using oral contraceptive pills. For detail see Table 2A.1.

Table 2A.1 SYS: Demographics of the Sample				
	Boys		Girls	
	n	Mean (SD)	n	Mean (SD)
Age (months)	*355*	*180 (22)*	*379*	*181 (23)*
Puberty Stage	*342*	*3.38 (0.87)*	*377*	*4.08 (0.74)*
OCP Using Girls	-	-	*56*	-
Percentage with no PEMCS (%)	*196*	*55*	*190*	*50*

Table 2A.1 *SYS: The sample size (n) along with the mean and standard deviation (SD, in brackets) for the following variables in both boys and girls: age (in months), pubertal stage, and prenatal exposure to maternal cigarette smoking (PEMCS).*

2A.4 Selection Criteria

Approximately half the participants selected are exposed to PEMCS and the other half are unexposed. The unexposed participants are matched to the exposed participants for level of maternal education and school attended. The inclusion criteria for all participants are as follows: 1) Adolescent within 12 to 18 year age range with a sibling (from the same biological parents) within the same age range; 2) Both biological parents of French Canadian ancestry (at least two generations); and 3) Both biological parents alive and available for DNA sampling. Inclusion criteria for exposed participants are as follows: Prenatal exposure to maternal cigarette smoking during the second trimester (≥1 cigarette/day). Inclusion criteria for non-exposed participants are as follows: No maternal exposure to cigarette smoking prenatally for 12 months before pregnancy and throughout pregnancy. Exclusion criteria for all participants are as follows: 1) Prenatal: Alcohol abuse during pregnancy, gestational diabetes; 2) Birth complications: premature birth (<35 weeks), low birth weight (<2,500 g), multiple birth; 3) Medical conditions: diabetes Type 1, rheumatologic disorders, congenital heart defects and aneurysm; 4) Neurodevelopmental conditions: epilepsy, brain tumours or infection, hearing and vision deficits, low IQ (<70) and other major neurodevelopmental disorders (e.g. autism); and 5) MR contraindications: Metal implants, claustrophobia. For details see Table 2A.2.

2A.5 Recruitment

All adolescent participants are recruited from local high schools. School authorities are contacted by the SYS team to gain permission for recruitment. Following approval by relevant school authorities, the visiting team attended classrooms where they made presentations introducing students to the basic purpose and procedures of the SYS study. Prior to the school visit, detailed information brochures of the study had been mailed to the parents of all the school students, along with a self-addressed and stamped response card to contact the SYS team if they were interested. The families of interested candidates who returned the response card are then interviewed over the telephone by a research nurse to assess their basic eligibility criteria. The telephone interview includes questions on parent and child demographics, pregnancy and birth and child's medical and neurodevelopment health. This telephone interview is conducted with the biological mother wherever possible and all responses are recorded on a laptop computer by the nurse. Eligible families are then visited by the SYS team at home, where the consent (parents) and assent (participants) forms were signed and recruitment completed. The home visit is followed by a laboratory visit for neuropsychological testing and a hospital visit for cardiovascular & metabolic phenotyping and MRI testing and concludes with a school visit for blood sampling (fasting blood sample taken between 8:00 and 9:00 am). Further details of testing and recruitment procedures are provided by Pausova et al. (2007). The study is approved by the Research Ethics Committee of the Chicoutimi Hospital.

Table 2A.2 SYS: Selection Criteria		
Demographics	*Both biological parents are French Canadians*	Inclusion
	Both parents available for Blood Sampling	Inclusion
	There are two siblings in eligible age range 12-18 yrs	Inclusion
	Child, parent or grandparent is adopted	Exclusion
Pregnancy and birth	*NonExposed: No cigarette smoking during*	Inclusion
	12 months before pregnancy	
	During pregnancy	
	During breast feeding	
	Exposed: Prenatal exposure to maternal cigarette	Inclusion
	smoking during 2nd Trimester (≥1/day)	
	Prenatal alcohol abuse during pregnancy	Exclusion
	Gestational diabetes	Exclusion
	Low Birth Weight (<2,500 g)	Exclusion
	Premature birth (<35 weeks)	Exclusion
	Multiple Birth (e.g. twins)	Exclusion
	Detached placenta during birth	Exclusion
	Hyperbilirubinemia requiring transfusion	Exclusion
Child's Medical History	*Type 1 diabetes*	Exclusion
	Systematic rheumatologic disorders	Exclusion
	Malignant tumours requiring chemotherapy	Exclusion
	Congenital heart defects or heart surgery	Exclusion
	Aneurysm	Exclusion
Neurological Conditions	*Epilepsy*	Exclusion
	Bacterial Infection of CNS	Exclusion
	Brain Tumour	Exclusion
	Head trauma with loss of consciousness >30 minutes	Exclusion
	Muscular dystrophy, myotonic dystrophy	Exclusion
Developmental Conditions	*Nutritional and metabolic diseases*	Exclusion
	Neurodevelopmental Disorders (autism etc)	Exclusion
	Hearing Deficits	Exclusion
	Vision Problems (e.g. strabismus)	Exclusion
Mental Health	*Treatment for Schizophrenia, bipolar disorder*	Exclusion
	IQ < 70	
	Reading ability < 2 SD	Exclusion
MR Contraindications	*Metal implants*	Exclusion
	Electronic implants (e.g. Pacemakers)	Exclusion
	Severe Claustrophobia	Exclusion

Table 2A.2 *SYS: The inclusion and exclusion criteria for recruitment of participants.*

2A.6 Demographics

Age and Sex: Sex and date of birth of the participant are recorded during the telephone interview. The date of birth is then used to calculate the age of the participants in months at the time of the hospital visit.

2A.7 Puberty Development Scale

There are several ways of measuring sexual maturation that can be grouped, broadly, into two categories. The first category is constituted by hormonal indices. Sex hormones are responsible for inducing sexual development of the body, both externally and internally (Grumbach et al. 2003, Guyton et al. 2000). Sex hormones advance the external secondary sexual characteristics and mature the internal reproductive organs. Therefore, they represent an objective estimate of the state of the endocrine system responsible to a large extent for the sexual maturation of the body, at both an external and internal level. The second category in formed by multiple means of measuring pubertal stage based on development of secondary sexual characteristics. These means include physical examination, picture-based self-reports, and description-based self-reports. Tanner (1962) was the first to describe five stages of pubertal development (1=no sexual development to 5 =adult sexual development) based on physical examination of the secondary sexual characteristics, such as breasts and genitals. These pubertal stages are referred to as the five Tanner stages. Physical examination by a trained clinician to determine the pubertal stage, as described by Tanner, is considered to be the gold standard means of measuring

external sexual development. There are, however, limitations to determining pubertal stage by physical examination that restrict its use. Physical examination requires trained personnel, permission to examine physically children is difficult to obtain and a suitable place to conduct the examination. Picture based written interview is a suitable alternative where children are provided photographs or drawings to examine and asked to indicate the picture that represents them most accurately. Alternatively, children are asked to answer questions about their secondary sexual development using a questionnaire. One of such questionnaires was introduced by Peterson et al. (1988).

Puberty Development Scale (PDS) is a self-report questionnaire on sexual development that has been adapted from the original questionnaire developed by Peterson et al. (1988). Puberty Development Scale is an eight item questionnaire. The first three questions are meant for both boys and girls and are relating to: 1) growth of height; 2) growth of body hair; and 3) skin changes (especially pimples). The next two questions are for boys alone, regarding: 4) growth of facial hair; and 5) voice changes (deepening). The last three questions are for girls alone, regarding: 6) growth of breasts; 7) menstruation; and 8) menarche (age at which menstruation began). For questions 1 till 6 there are four responses: not started, barely started, definitely started and almost complete. These responses are scored as 1, 2, 3 and 4 points, respectively. For the menstruation question (7) there are two options, no and yes. These responses are scored as 1 and 4 points, respectively. Based on the total points scored, the adolescents are categorized as one of five pubertal stages:

prepubertal (boys=3, girls=2 and no menarche), early pubertal (boys=4-5, girl=3 and no menarche), midpubertal (boys=6-8, girls=4-6 and no menarche), late pubertal (boys=9-11, girls=7 and menarche) and postpubertal (boys=12, girls=8 and menarche).

Pubertal development scale ratings of puberty have been compared with puberty ratings from the gold standard means of physical examination, to assess its accuracy. Pearson's correlation between physical exam and PDS puberty stage was reported to be high, between 0.60 and 0.90 (ShirtCliff et al. 2009, Brooks-Gunn et al. 1987). The kappa concordance reported between physical examination and PDS puberty stage, however, was modest, between 0.24 and 0.36 (ShirtCliff et al. 2009, Brooks-Gunn et al. 1987), with no difference between sexes. On average, approximately 50 percent of children rated their puberty stage identical to that assessed by the doctor on physical examination (ShirtCliff et al. 2009). In one report Carskadon et al. (1993) compared self-reported PDS ratings of adolescents with parental PDS ratings of the same adolescents. They demonstrated that boys overestimated their pubertal stage, whereas girls did not (Carskadon et al. 1993). The association of PDS puberty stage with hormones via structural equation modelling revealed that PDS puberty stage significantly predicted testosterone level in boys and estradiol level in girls (ShirtCliff et al. 2009). In fact, PDS captured basal hormone levels in parallel with physical exam (ShirtCliff et al. 2009). Although PDS has modest associations with physical exam it appears to represent the basal level of hormones comparable to physical exam. Overall, there is evidence to suggest that PDS puberty stage is a reliable measure

of the Tanner stage (based on physical examination) and it is closely associated with the level of sex hormones. Therefore, in situations where physical exam and picture based interviews for assessing pubertal development are not possible, PDS would provide a non-invasive and reliable alternative.

2A.8 Oral Contraceptive Pill Use

The use of oral contraceptive pill in girls is assessed via a medical questionnaire filled out by the mother during the initial home visit. In this questionnaire, mothers are asked to specify which medicines (if any) are taken by their children. Based on the answer to this question, use of oral contraceptive pills in girls is assessed.

2A.9 Neuropsychological Testing

2A.9.1 General Intelligence

General Intelligence broadly refers to the ability of abstract and non-abstract thought, reasoning and problem solving, learning and comprehension, understanding, communication and planning. Charles Spearman (1904) originally described the theory on intelligence, in which he explained intelligence as the basic mental ability that underlies cognitive performance in multiple domains. This theory was further developed by Raymond Catell who recognized two discrete factors or domains of general intelligence called fluid and crystallized intelligence (Catell 1963). Fluid intelligence refers to the ability of reasoning and problem solving in the face of novel challenges, without using acquired skills and

knowledge (Niesser et al. 1996). Crystallized intelligence, on the other hand, refers to reasoning abilities based on acquired knowledge and skills (Niesser et al. 1996). Fluid and crystallized intelligence are well correlated, since individuals with good reasoning and problem solving skills usually acquire more knowledge over time (Niesser et al. 1996). The current hierarchical models of intelligence further sub-divide fluid and crystallized intelligence into many different categories. General intelligence, in fact, has been shown to include as many as 70 sub-domains of cognitive abilities (Niesser et al. 1996).

Many different tests have been designed to study intelligence. Stanford-Binet intelligence scale was one of the first test batteries designed to assess intelligence. It has been revised several times since its inception and currently the Stanford-Binet test measures intelligence of 2 to 23 years old individuals in multiple cognitive domains of both fluid and crystallized intelligence. Wechsler Scales of intelligence were a group of intelligence tests introduced later that gained significant popularity over time and are currently the most common tests of intelligence employed in psychological research. In comparison with the Stanford-Binet test, the Wechsler test has multiple versions that have been tailored for different ages. These include Wechsler intelligence scale for preschool and primary school going children (WPPSI: for children aged between 4 to 6 years), Wechsler intelligence scale for children (WISC: for children aged between 7 to 16 years) and Wechsler adult intelligence scale (WAIS: for adults from 17 to 74 years). Additionally, the Wechsler tests were developed carefully to distinguish between different types of intelligence. There are four main

domains of intelligence that are investigated by the Wechsler scale, namely: 1) verbal memory and reasoning; 2) mathematic memory and reasoning; 3) abstract reasoning and problem solving; and 4) speed of abstract processing. The first two verbal and mathematic subscales of Wescler test measure crystallized intelligence. This is because performance on these tests is based on learned general knowledge, vocabulary and mathematic skills. The last two abstract subscales of Wechsler test measure fluid intelligence; since they are dependent on ability to solve new abstract problems, independent of any learned knowledge. Other standardized tests of intelligence include the Kaufman assessment battery for children (KABC). This test measures intelligence in 3 to 18 year old children and was designed especially for use with mentally disabled children. The Woodcock-Johnson test of cognitive abilities is a standardized intelligence test that can be administered to individuals from 2 years onwards. The above-mentioned standardized tests of intelligence are scored based on performance of a large sample of the general population. The mean of the general population is standardized to a score of 100 and one standard deviation to 15. Hence 95% of the general population is expected to have an intelligence score between 70 and 130 (mean ± 2 standard deviations = 100 ±30). The intelligence score derived in this fashion is referred to as intelligence quotient (IQ).

This thesis examines the association of the corpus callosum with intelligence in children and adolescents. In view of the age range of the study sample and the fact that the sample consists of healthy participants, the Wechsler scale of

intelligence for children (WISC-III) is used. This scale consists of multiple tests, conducted over a 70 to 80 minute period that evaluate general cognitive ability over four distinguished domains of intelligence. Details of these four domains of intelligence and the WISC-III tests that are used to examine them are given in Figure 2A.1.

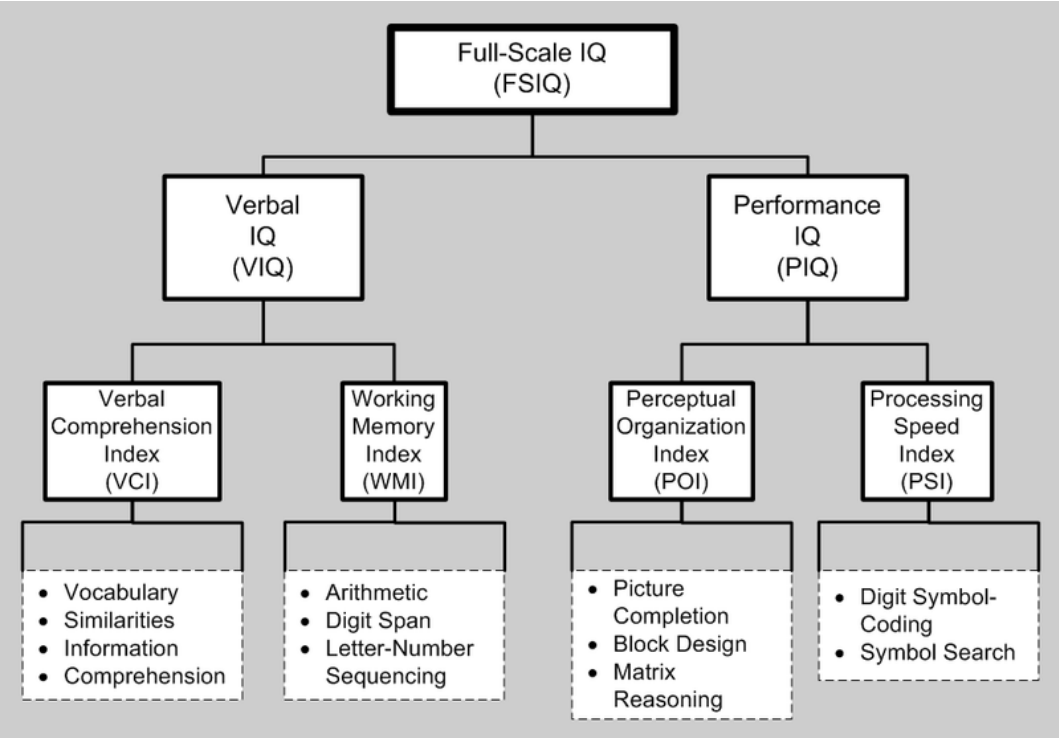

Figure 2A.1: Table of WISC-III tests and their IQ scores. Schematic from http://en.wikipedia.org/wiki/Wechsler_Adult_Intelligence_Scale

Verbal Comprehension

Verbal comprehension tests measure cognitive abilities of verbal comprehension, verbal reasoning and problem solving based on general knowledge and vocabulary. The total IQ score for performance in the verbal comprehension tests is called verbal comprehension index (VCI). The verbal comprehension tests are as follows:

Vocabulary: In this test, the researcher asks the participant to define a given word. Performance in this test depends on the degree of one's learned vocabulary and his/her ability to comprehend and verbally express vocabulary.

Similarities: In this test, the researcher asks the participant how two words are alike or similar in meaning. Performance in this test is dependent on one's abstract verbal reasoning skills.

Comprehension: In this test, the researcher asks the participant questions about social situations or common concepts. Performance in this test is dependent on one's ability to deal with abstract social conventions, rules and expressions.

Information: In this test, the researcher asks the participant general knowledge questions. Performance in this test is dependent on the degree of general knowledge acquired from environment.

Working Memory (also called Freedom from Distractibility)

Working memory tests measure cognitive abilities of mathematical problem solving and reasoning based on calculation skills and memory. The total IQ score for performance in the working memory tests is called working memory index (WMI). The working memory tests are as follows:

Digit Span: In this test, the researcher recites a given sequence of numbers and then asks the participant to repeat the sequence, either as heard or in reverse

order. Performance in this test is dependent on attention, concentration and short term memory.

Arithmetic: In this test, the researcher asks the participant to solve some orally administered maths questions within a fixed time interval. Performance in this test is dependent on attention, calculation abilities and numerical reasoning skills.

Letter Number Sequencing: In this test, the researcher recites a mixed series of alternating alphabets and numbers (such as, w-7-t-4). The participant is then asked to repeat first the numbers in ascending order (4-7) and then the alphabets (t-w) in ascending order. The series keeps getting longer until the participant fails four trials of one string length.

Perceptual Reasoning

Perceptual reasoning tests measure cognitive abilities of abstract reasoning to solve non-verbal problems involving designs and patterns based on logical thinking. The total IQ score for performance in the perceptual reasoning tests is called perceptual reasoning index (PRI). The perceptual reasoning tests are as follows:

Block Design: In this test, the researcher asks the participant to assemble red and white blocks such that they create a pattern similar to that displayed in a picture within a fixed time interval. Performance in this test is dependent on visuospatial perception and analysis and abstract problem solving skills.

Matrix Reasoning: In this test, the researcher shows the participant an assortment of pictures with one missing picture. The participant is then asked to select a picture from five given options that best fits the assortment. Performance in this test is dependent on non-verbal abstract reasoning and problem solving.

Picture Completion: In this test, the researcher shows the participant pictures of common objects with a missing part. The participant is then asked to identify the missing part. Performance in this test is dependent on ability to quickly perceive visual details.

Processing Speed

Processing speed tests measure speed of solving abstract non-verbal problems involving designs and patterns based on logical thinking. The total IQ score for performance in the perceptual reasoning tests is called processing speed index (PSI). The processing speed tests are as follows:

Coding: In this test the participants are provided with a digit-symbol code and asked to go through a grid of numbers and copy the correct symbol above each number, within a fixed time interval. Performance in this test is dependent on mental speed and visual-motor coordination.

Symbol Search: In this test the researcher identifies certain target symbols to the participant. The participant is then asked to scan rows of symbols and mark

whether each row contains the target symbol or not. Performance in this test is dependent on concentration and visual motor quickness.

Sum of the verbal comprehension index (VCI) and working memory index (WMI) comprises the **Verbal IQ (VIQ).** Sum of the perceptual reasoning index (PRI) and processing speed index (PSI) comprises the **Performance IQ (PIQ)**. Sum of the verbal IQ and performance IQ score comprise the **full scale IQ (FSIQ)**.

2A.10 Thurston Bimanual Tapping Task

Finger tapping tasks are one of the most common paradigms used to study bimanual motor coordination. There are many different kinds of finger tapping tasks that vary in the use of a pacing stimulus, specific motor movements involved and task complexity. One of the simplest bimanual motor coordination tasks was that introduced by Petellier et al. (1993). This task involves performing as many asynchronous index finger-thumb opposition movements of the two hands as possible in a period of 15-seconds. Stephen et al. (1999) introduced a modified version of this simple altered finger-tapping task; it involved pacing the bimanual finger taps to an auditory stimulus (rhythm of beeps at increasing frequency). This was a more complex version of the altered finger tapping task that measured the accuracy of bimanual motor coordination. Thurston (1944) examined bimanual motor coordination by means of a task that involved tapping a stylus on a labelled plate in a specific order, simultaneously with both hands (Leonard et al. 1987). The number of taps made over a 30-second period was measured. The Thurston task required both accurate and fast performance of

coordinated bimanual movements. Preilowski (1972, 1975) assessed bimanual motor coordination based on how well individuals can draw lines to trace specific pathways, using two separate levers on an apparatus that moved the pen horizontally and vertically, respectively. Angled pathways required simultaneous vertical and horizontal pen movements and therefore, demanded coordinated bimanual visuomotor responding. The Preilowski task was a complex visuomotor task that assessed accuracy of coordinated bimanual movements. Modified versions of the Preilowski task with improvements in the controlling apparatus (Brown et al. 1991) and computerized administration (Marrion et al. 2003) have also been introduced. Many different tasks have, therefore, been designed over the years to study bimanual motor coordination. Certain tasks provide better measures of the speed of coordinated bimanual movement (Petelliers task, Thurston task), while others provide better measure of the accuracy of bimanual motor movement (Preilowski task, Stephens task).

Bimanual motor coordination skills of the SYS study participants were examined based on their performance on the Thurston finger tapping task. This task is conducted on an apparatus specially designed for this task which consists of two brass plates mounted on a wooden board, with a stylus next to each plate (Leonard et al. 1988). Each plate is divided into four equal pie-shaped sections labelled 1, 2, 3 and 4, respectively. The styli are wired such that each time both the styli touched corresponding sections of the two plates an electric circuit is completed, activating a mechanical counter. The participant is asked to hold a stylus in each hand and tap corresponding sections of the two brass plates in

ascending order as quickly as possible. The total number of taps made in a 30-second interval is recorded. Prior to the actual trial, a test trial is run to familiarize the participant with the task. Two trials are conducted and their mean calculated to more accurately assess bimanual coordination skills of the participant.

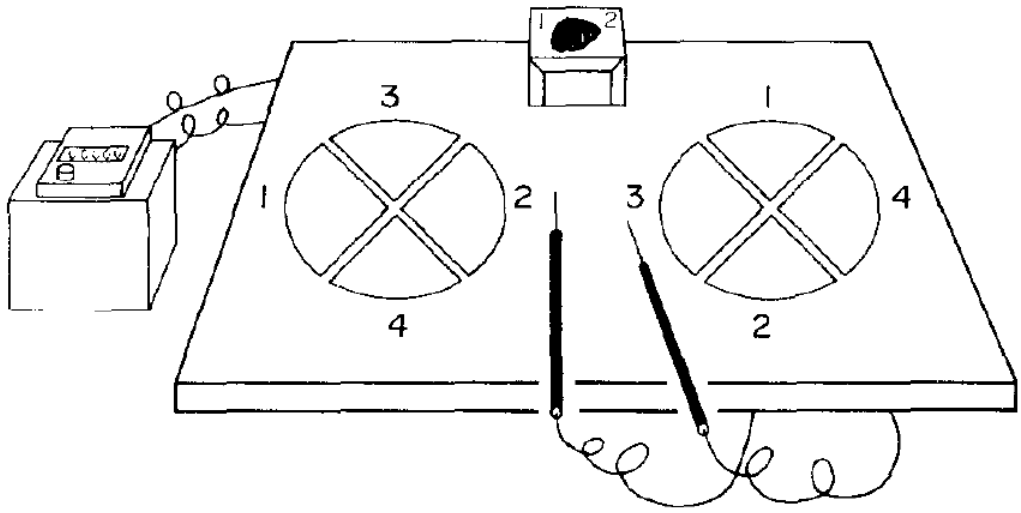

Figure 2A.2: Apparatus used for Thurston tapping task. A wooden board with two round brass plates, which are divided into four sections and two styli connected to an electronic ticker. Schematic from Leonard et al. (1987).

The Thurston tapping task is a more complex form of the simple altered finger tapping task (Petellier et al. 1993). This task required accurate and fast performance of bimanual movements in a spatially ordered sequence. Compared with simple tapping tasks, performance of Thurston task is dependent on more aspects of bimanual control. Some evidence of callosal association has been demonstrated with performance on all other bimanual tasks but not with the Thurston task. This thesis is the first to investigate callosal association with Thurston Tapping task. It is important to note that the choice of behavioural

tasks investigated in this thesis is opportunistic and limited to the tasks available in the existing SYS dataset.

2A.11 MRI Acquisition

All scanning is performed on a Philips 1.0 T superconducting magnet. For each participant the T1 weighted image is acquired with the following acquisition parameters: 3D RF spoiled gradient-echo scan, 140-160 high resolution T1-weighted images, with TR=25 ms, TE=5 ms, flip angle=30^0 and 1 mm isotropic resolution.

2A.12 Serum Bioavailable Testosterone Level (cnmol/L)

The participants are asked to fast over night, starting 8:00 p.m. Fasting blood samples are taken in the morning between 8:00 and 9:00 a.m. Testosterone is quantified using serum derived from blood collected in 5 ml BD Vacutainers (Becton, Dickinson and Company Vacutainers; additive: clot activator and gel for serum separation; silicone coated interior; http://catalog.bd.com/bdCat/viewProduct.doCustomer?productNumber=367986). Following collection, the blood is kept at room temperature for 30-120 minutes and then centrifuged for 15 minutes. The separated serum is then aliquoted into 2 x 2 ml polypropylene tubes and 8 x 1.2 ml tubes and stored at -70 degrees until required for assays.

The serum level of testosterone (nanomoles per litre) and sex hormone binding globulin (nanomoles per litre) are analyzed using radioimmunoassay (Testosterone RIA DSL-4000; Diagnostic Systems Laboratory) at the Hospital

Hôtel-Dieu in Montreal. Radioimmunoassay is a technique that measures the level of testosterone in participant serum by adding the serum to a mixture of radiolabelled testosterone and antibodies, bound together. The free testosterone molecules of the participant serum displace the antibody bound-radiolabelled testosterone molecules. The ratio of participant to radiolabelled testosterone in the total free testosterone molecules is measured and used to calculate the total level of testosterone in patient serum. It is important to note that most of the testosterone in serum is bound to sex hormone binding globulin (SHBG) and a small amount to albumin (Cumming et al. 1985). Only a 1 to 2 percent of the total testosterone in the serum is free (Cumming et al. 1985). The free testosterone is referred to as bioavialable testosterone and is responsible for acting on the testosterone receptors in the body. Sexual development of the body is, therefore, induced by bioavialable testosterone and not total testosterone. Bioavailable level of testosterone (nanomoles per litre) is estimated using an equation generated by Sodergard et al. (1982), which calculates the balance between total, bound and free (bioavialable) testosterone level in the body.

2B IMAGEN STUDY: METHODOLOGY

2B.1 Introduction

IMAGEN is a large-scale (n≈2,000), multicentre, European study that explores cognitive and behavioural traits, such as impulsivity, reinforcer sensitivity and emotional reactivity, relevant for substance use in adolescence (Schumann et al.

2010). It is conducted at eight different centres across England, Ireland, France and Germany. Data are acquired in 13- to 15 year old participants on multiple quantitative phenotypes through cognitive and behavioural testing, structural and functional neuroimaging and blood sampling. IMAGEN is a prospective study; all participants are intended to undergo re-assessment 2 years later in order to re-evaluate their mental health in general and substance use in particular. This will allow the investigators to assess the predictive value of behavioural and imaging phenotypes obtained during the initial visit.

2B.2 IMAGEN Population

As mentioned previously the IMAGEN study is a Europe-wide study, with all participants recruited from multiple centres across England, Ireland, France and Germany. There are two characteristic features of the population sample. Firstly, the sample is mainly of Caucasian ethnicity in order to minimize possible difficulties related to population stratification (heterogeneous genetic background of participants) in the genetic arm of the study (Cardon et al. 2003). Secondly, the sample is diverse in terms of socioeconomic status, academic achievement and behavioural functioning. This diversity ensures that the influence of socioeconomic, academic and behavioural factors on the development of psychiatric traits, particularly substance abuse, can be explored.

2B.3 Descriptive Statistics

The studies of this thesis are conducted on 1,979 participants of the IMAGEN study. This sample consists of 964 boys and 1,015 girls. The mean age of the boys is 172 (SD: ±15) months and that of girls is 173 (±12) months. There is no significant difference between the mean age of boys and girls (p=0.276). The mean puberty stage (based on PDS) of boys is 3.29 (±0.69) and that of girls is 3.99 (±0.46); this difference is significant (p≤0.001). Of the total sample of girls 54 girls use oral contraceptive pills. For details see Table 2B.1.

Table 2B.1 IMAGEN: Demographics of the Sample				
	Boys		Girls	
	n	Mean (SD)	n	Mean (SD)
Age (months)	*964*	*172 (15)*	*1015*	*173 (12)*
Puberty Stage	*955*	*3.29 (0.68)*	*1005*	*3.99 (0.46)*
OCP Using Girls	-	-	*54*	-

Table 2B.1 *IMAGEN: The sample size (n) along with the mean and SD (in brackets) for the following variables in both boys and girls: age (in months) and pubertal stage.*

2B.4 Selection Criteria

The inclusion and exclusion criteria are similar to those used in the Saguenay Youth Study. The Inclusion criterion for participants is as follows: 1) Adolescent within 13 to 15 year age range. Exclusion criteria are as follows: 1) Prenatal Alcohol abuse during pregnancy, gestational diabetes; 2) Birth complications: premature birth (<35 weeks), low birth weight (<2,500 g), multiple birth, jaundice requiring transfusion; 3) Medical conditions: diabetes Type 1, systemic rheumatologic disorders, tumours requiring chemotherapy, congenital heart defects and aneurysm; 4) Neurodevelopmental conditions: epilepsy, brain

tumours or infection, muscular dystrophy, hearing and vision deficits, low IQ and other major neurodevelopmental disorders; and 6) MR contraindications: Metal implants, claustrophobia. For more details on selection criteria refer to Table 2B.2.

Table 2B.2 IMAGEN: Selection Criteria		
Demographics	*Child in target age (14 yrs)*	Inclusion
Pregnancy and birth	*Prenatal alcohol abuse during pregnancy*	Exclusion
	Gestational diabetes	Exclusion
	Low Birth Weight (<2,500 g)	Exclusion
	Premature birth (<35 weeks)	Exclusion
	Detached placenta during birth	Exclusion
	Hyperbilirubinemia requiring transfusion	Exclusion
Child's Medical History	*Type 1 diabetes*	Exclusion
	Systematic rheumatologic disorders	Exclusion
	Malignant tumours requiring chemotherapy	Exclusion
	Congenital heart defects or heart surgery	Exclusion
	Aneurysm	Exclusion
Neurological Conditions	*Epilepsy*	Exclusion
	Bacterial Infection of CNS	Exclusion
	Brain Tumour	Exclusion
	Head trauma with loss of consciousness >30 minutes	Exclusion
	Muscular dystrophy, myotonic dystrophy	Exclusion
Developmental Conditions	*Nutritional and metabolic diseases*	Exclusion
	Neurodevelopmental Disorders (autism etc)	Exclusion
	Hearing Deficits	Exclusion
	Vision Problems (e.g. strabismus)	Exclusion
Mental Health	*Treatment for Schizophrenia, bipolar disorder*	Exclusion
	IQ < 70	Exclusion
MR Contraindications	*Metal implants*	Exclusion
	Electronic implants (e.g. Pacemakers)	Exclusion
	Severe Claustrophobia	Exclusion

Table 2B.2 *IMAGEN: The inclusion and exclusion criteria for recruitment of participants.*

2B.5 Recruitment

All adolescent participants are recruited in high schools. In order to ensure sample diversity in terms of socioeconomic status, both private and state funded high-schools are approached. Additionally, in order to maximize ethnic homogeneity of the sample, care is taken to target high-schools in areas with the least ethnic diversity. The IMAGEN research team of each centre contacted multiple local high schools to introduce the study and gain assent for recruitment. Following approval by the relevant school authorities, the research team visits the schools where they inform the children about the aims and assessment procedures of the study. Different approaches are used by different acquisition centres to inform the children, such as power point presentation before assemblies or talks in classrooms. During the school visit, contact details are sought from all interested candidates aged 14 years, who are subsequently mailed detailed information packs. These information packs include: 1) brochures that inform parents of interested candidates about the IMAGEN project; 2) letters addressed to the parent and child inviting them to participate; 3) medical questionnaire to assess inclusion and exclusion criteria; 4) consent forms to be signed by both parent and child; and 5) self-addressed and stamped envelope to return the relevant forms to the IMAGEN team if their child wishes to participate. The information packs received are reviewed by the IMAGEN team to identify children who fulfil the inclusion/exclusion criteria. Telephone interviews are then conducted with interested parents of respondents who fulfilled the eligibility criteria. During this telephone interview, a convenient date for the parent and

child to visit the testing centre is arranged. All testing including, blood sampling, cognitive assessment and MRI examination are conducted in the centre laboratory, in a single day or in two days. At the time of data acquisition participants also underwent fMRI examination and neuropsychological testing. The methodological details of these tests, however, will not be discussed because they have not been used by any study of this thesis.

For more detailed information on the methods and implementation procedures employed, supplementary materials and Standard Operation Procedures (SOPs) are provided (http://www.IMAGENeurope.com/en/Publications_and_SOP.php).

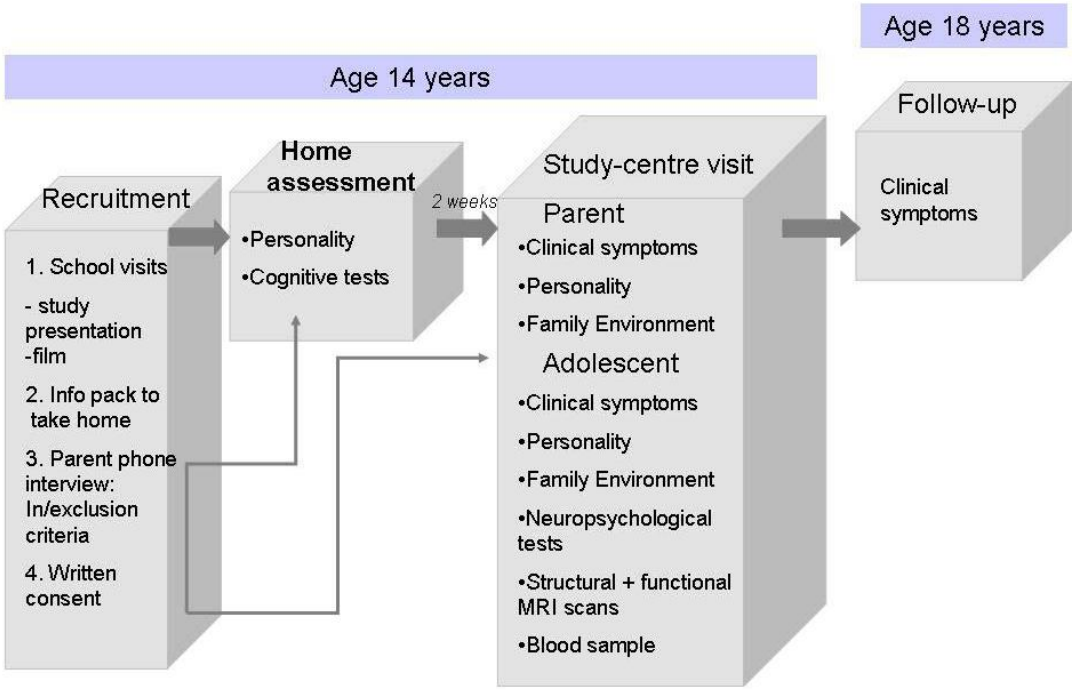

Figure 2B.1 Overview of recruitment and assessment procedures of the IMAGEN study. Schematic taken from Schumann et al. 2010.

2B.6 Demographics

Age and Sex: Sex and date of birth of the participants are documented on forms included in the information packs returned by interested parents to the IMAGEN centre (see Section 2B.4). The date of birth is then used to calculate the age of the participants in months at the time of the centre visit.

2B.7 Puberty Development Scale

Pubertal stage is measured using the same Puberty development scale (PDS) used in the Saguenay Youth study (Section 2A.6). Briefly, PDS is a self-report questionnaire that includes questions on development of height, pubic hair, facial hair, voice and skin changes, breast development, menarche and menstruation. Boys are rated between pubertal stages 1 to 5, based on the development of pubic and facial hair and voice changes. Girls are rated between pubertal stages 1 to 5 based on the development of pubic hair, breasts and menarche. Puberty development scale stage is well correlated with the clinical exam based Tanner stage; moreover, it is well related with the level of sex hormones (ShirtCliff et al. 2009, Brooks-Gunn et al. 1987). For more details on PDS, its reliability and other means of assessing sexual development, refer to Section 2A.6.

2B.8 Oral Contraceptive Pill Use

Oral contraceptive pill use in girls is assessed via a time line follow back (TLFB) questionnaire on drug use filled out by a researcher interviewing the child. In this

questionnaire, the researcher asks the participants to specify which prescription medicines (if any) are taken by them in the last month. Based on the answer to this question, use of oral contraceptive pills in girls is assessed.

2B.9 MRI Acquisition

Scanning across all centres is performed on 3.0 T superconducting magnets from a number of manufacturers (Siemens, Philips, General Electric and Bruker). In order to ensure similar image contrast and signal to noise ratio across all centres the parameters of the image acquisition techniques employed by all scanners are kept constant. The high resolution anatomical MR images acquired and their acquisition parameters are as follows: 3D T1 weighted (T1W) magnetization prepared gradient echo sequence (MPRAGE) based on the ADNI protocol (http://www.loni.ucla.edu/ADNI/Cores/index.shtml). As per ADNI, the sequence parameters are 160-170 high resolution T1W images, with TR=2300 ms, TE=2.8 ms, flip angle=8-9^0 and 1.1 mm isotropic resolution.

For quality control an American College of Radiology (ACR) phantom and healthy volunteers are scanned at each centre, twice a year and before and after every scanner upgrade. This provides information about geometric distortions and signal uniformity related to hardware differences in radiofrequency coils and gradient systems at each centre and across multiple sites to help determine inter-site variability in structural measures.

Table 2C.1 Comparison of Saguenay and IMAGEN Study Methodology		
	Saguenay Youth Study	**IMAGEN Study**
Participant Ethnicity	*French Canadian*	*European*
Sample Size (n)	*737*	*1,979*
All Boys	*356*	*964*
All Girls	*381*	*1,015*
OCP Using Girls	*56*	*54*
Age Range (years)	*12-18*	*13-15*
Mean Age (months; SD in brackets)		
All Boys	*180 (22)*	*172 (15)*
All Girls	*181 (23)*	*173 (12)*
OCP Using Girls	*203 (15)*	*176 (5)*
Puberty stage (SD)		
All Boys	*3.38 (0.87)*	*3.29 (0.68)*
All Girls	*4.08 (0.74)*	*3.99 (0.46)*
OCP Using Girls	*4.70 (0.46)*	*4.15 (0.36)*
MRI Magnet Strength (Tesla)	*1.0*	*3.0*
MRI acquisition Parameters for high resolution T1W Images		
Slices	*140-160*	*160-170*
TR (ms)	*25*	*2300*
TE (ms)	*5*	*2.8*
Flip Angle	30^0	$8\text{-}9^0$
Resolution (mm)	*1×1×1*	*1.1×1.1×1.1*
Neuropsychological Testing		
WISC-III IQ scores	*Measured*	*Not Measured*
Bimanual Motor Coordination (via Thurston Bimanual Task)	*Measured*	*Not Measured*

Table 2C.1 *Comparison of the SYS and IMAGEN studies across the following features: participant ethnicity, sample size, demographics (age and puberty), MR scanner strength, MRI acquisition parameters (number of slices, TE: echo time, TR: repetition time, flip angle and resolution) and neuropsychological testing (Wechsler intelligence tests and Thurston bimanual task).*

3 VALIDATION OF FULLY AUTOMATED, FREESURFER ESTIMATES OF THE CORPUS CALLOSUM AREA AND VOLUME WITH SEMI-AUTOMATED, DISPLAY-ASSISTED MANUAL ESTIMATES

3.1 INTRODUCTION

The majority of previous morphometric studies of the corpus callosum used an operator-driven approach for outlining the mid-sagittal area (Giedd et al. 1999 and 1996, De Bellis et al. 2001, Lenroot et al. 2007, Good et al. 2001, Luders et al. 2002 and Gur et al. 2002). Some of the earlier approaches used photographs of the autopsied and formalin-fixed mid-sagittal sections of the CC (Lamantia and Rakic et al. 1990, Witelson et al. 1991, Koshi et al. 1997, Clarke et al. 1989) or printed its mid-sagittal MR images (Burke and Yeo et al. 1994, Cowell et al. 1992, Allen et al. 1991, Pujol et al. 1993). The CC outline was then manually traced on these photographs or prints (Lamantia and Rakic et al. 1990, Koshi et al. 1997, Burke and Yeo et al. 1994, Cowell et al. 1992, Witelson et al. 1991). Today, modern techniques are available that enable this entire process to be conducted digitally using semi-automated ways. Magnetic resonance imaging (MRI) is an *in-vivo* technique that generates grey-scale images of the brain utilizing the magnetic property of body protons. Once digital images of the brain are obtained, the corpus callosum can be outlined on them directly with the aid of various visualization tools. Visualization tools such as Display (detailed in MNI BCI website below), Image 1.46 (detailed in Rashband et al. 1993), Performance Plus

(program of the General Electric MR scanner software), BRAINS (detailed in Andreasen et al. 1993) are available which enable manual tracing of the corpus callosum outline directly on the computer screen. Moreover they also aid this process in various ways. Display, for example, is an MRI visualization and analysis tool developed by MNI BIC (http://www.bic.mni.mcgill.ca/ServicesSoftware/HomePage) that makes the task of outlining the CC much easier by enabling one to select an intensity range of voxels to be highlighted. Since the white matter of the CC has a significantly higher intensity that those of its neighbouring voxels, it is easy to outline the CC. In MRI brain volumetric analysis the gold standard for calculating the size of the corpus callosum is the manual delineation of its mid-sagittal area by trained experts. There are, however, limitations to this approach. Manual delineation of each participant's CC is a time consuming process, which will be less efficient when dealing with large sample sizes (Jack et al. 1990, Dewey et al. 2010). Furthermore it requires an expert who is knowledgeable about the anatomy of the CC, experienced in selecting specific mid-sagittal slices and skilled in outlining the CC. The results may vary depending on rater skills, acquisition parameters, image quality and computer tracing tools employed (Jack et al. 1990, Dewey et al. 2010).

More recently, there has been increasing use of new techniques that provide fully automated methods to calculate total and regional brain volumes. FreeSurfer is one such tool. FreeSurfer segments the brain into specific regions on the spatially normalized or standardized images based on the segmentation patterns of a specific brain atlas provided (Fischl et al. 2002, FreeSurfer Wiki). In

the corpus callosum, it not only segments it in the mid-sagittal slice but continues laterally into both the hemispheres, thus producing a multi-slice, three dimensional slab (FreeSurfer Wiki, Francis et al. 2011). This provides a comprehensive three dimensional segmentation of the corpus callosum. Several recent studies have used FreeSurfer to segment and calculate the volume of the corpus callosum. These include the study by Francis et al. (2011) who examined the volume of the corpus callosum in high-risk offspring of patients with schizophrenia. Tartaglia et al. (2009) studied the influence of primary lateral sclerosis on CC volume and Vatta et al. (2011) examined the influence of autism on CC volume. Both used FreeSurfer for volumetric analysis of the corpus callosum.

Despite the above use of FreeSurfer to quantify the size of the CC, there has been no validation of the CC size estimates calculated by these new, fully automated techniques with those calculated by standard, auto-assisted techniques classically used. This has been done, for example, for other structures, such as the hippocampus and amygdala (Dewey et al. 2010, Shen et al. 2010, Pardoe et al. 2009). The Pearson correlation between the manually delineated and FreeSurfer delineated volume of the hippocampus has been reported between 0.75 and 0.85 (Dewey et al. 2010, Shen et al. 2010, Pardoe et al. 2009, Morey et al. 2009). The Pearson correlation between the manually delineated and FreeSurfer delineated volume of the amygdala and caudate nucleus were found to be 0.80 on the right and 0.90 on the left (Dewey et al. 2010). These correlation values demonstrate that FreeSurfer volumetric measures closely resemble the

standard manually delineated volumetric measures, suggesting that FreeSurfer is a reliable segmenting tool for these brain structures.

Before using FreeSurfer as the main tool in subsequent studies on corpus callosum, this chapter validates the FreeSurfer estimates of callosal size with the manual tracing of the CC carried out by an expert. This is done by correlating the manually delineated mid-sagittal areas of the CC with the corresponding FreeSurfer calculated ones in a large number of MRI scans acquired in the SYS study.

3.2 METHODOLOGY

The measures of callosal size that this study calculates include the following: 1) manual CC Area: the mid-sagittal area of the corpus callosum, manually delineated by a trained expert (INK) using Display program; 2) FreeSurfer CC Volume: volume of the multi-slice FreeSurfer segmentation of the corpus callosum calculated automatically; and 3) FreeSurfer CC Area: mid-sagittal area of the FreeSurfer CC slice registered to (or corresponding to) the slice on which the manual delineation is performed. These measures are then used to estimate the following correlations: 1) the intra-class correlation between the manual CC area and FreeSurfer CC area; and 2) the Pearson correlation between the FreeSurfer CC area and FreeSurfer CC volume. By comparing the manually delineated mid-sagittal areas of the CC with the corresponding FreeSurfer calculated ones, this study is able to determine how accurate FreeSurfer is in segmenting the CC relative to the trained expert. Additionally, correlating the FreeSurfer calculated

mid-sagittal area of the CC with its FreeSurfer calculated volume demonstrates how the volume measures of the CC vary with the area measures that are typically used to estimate CC size.

3.2.1 Participants

This study is conducted on 737 participants (356 boys and 381 girls) of the Saguenay Youth Study. All participants are French-Canadian adolescents aged between 12 and 18 years belonging to the Saguenay Lac Saint-Jean (SLSJ) region of Quebec, Canada. Inclusion criteria consist of having an age between 12 and 18 years and maternal and paternal grandparents of French-Canadian ancestry. The exclusion criteria consist of positive medical history for meningitis, malignancy or heart disease requiring surgery, severe mental illness or mental retardation and MR contraindications. Details of testing and recruitment procedures are provided in Chapter 2, Section 2A.

3.2.2 MRI Acquisition

All scanning is performed on a Philips 1.0 T superconducting magnet. For each participant the T1 weighted images acquired and their acquisition parameters are as follows: 3D RF spoiled gradient-echo scan, 140-160 high-resolution T1-weighted images, with TR=25 ms, TE=5 ms, flip angle=30^0 and 1 mm isotropic resolution.

3.2.3 MRI Analysis

3.2.3A Manual CC Area: The mid-sagittal surface area of the CC is calculated by a trained expert (INK) on the high resolution, native T1-weighted images using Display program (http://www.bic.mni.mcgill.ca/ServicesSoftware/HomePage). Once a T1-weighted scan is opened via Display program, mid-sagittal slice is selected based on the expertise of the tracer, who used her knowledge of callosal anatomy to identify the mid-sagittal slice. The tracer was blind to the sex of each case. Mid-sagittal location was determined by anatomical references to the origin of the fornix, falx cerebri, septum pellucidum and third ventricle. Mid-sagittal slice is typically selected as the one with the septum pellucidum lying below the CC and the fornix originating from the body (or isthmus) of the CC. Furthermore, the presence of additional midline structures, such as falx cerebri and third ventricle aid in the selection of the mid-sagittal slice. After selection of the mid-sagittal slice the "brush" size for outlining the corpus callosum is defined. Thereafter, a specific MR intensity range is defined based on the MR intensities observed across the length of the corpus callosum; these intensity values are displayed for each voxel on the left bottom corner of the screen by simply pointing the cursor on that voxel. MR intensity of the Tracing is then initiated and as a cursor (brush) is moved along the callosal border, voxels within the specified intensity range are automatically highlighted in red. Special care is taken when outlining the site where the fornix originated from the corpus callosum to ensure that the ventral curvature is smooth, without including any fornix tissue. Subsequently, the region within the highlighted red border is filled

to yield a red coloured CC (two dimensional) mask. Erase and undo commands are available during this process to correct any errors in the delineation. Once the tracer is satisfied with the final red CC trace, volume is calculated via a Display command. Area of the corpus callosum is calculated as the sum of all the voxels constituting the red CC trace. It is important to note that the volume and the surface area of the CC traced on this single mid-sagittal slice are the same because voxel thickness is 1 mm (Volume = Surface area × thickness).The red CC trace is then saved as a binarized two dimensional CC mask, referred to as the manual CC mask. In this way Display program enables a computer-aided manual delineation of the corpus callosum, calculation of CC mid-sagittal area and creation of the manual CC masks.

3.2.3B FreeSurfer CC Volume: FreeSurfer version 5.0.0 is used for the automated volumetric analysis, performed on native, high-resolution T1-weighted images. These images are first corrected for non uniformities caused by motion artifacts, scanner induced inhomogenieties and intensity inhomogenieties. Then they are normalized for intensity. Next, brain extraction is performed on these motion- and intensity-corrected and normalized images. This step strips away the skull and any background noise, leaving behind only brain tissue. Following this preliminary processing, the native images undergo non-linear registration to the MNI305 template (Evans et al. 2003), which is aligned with the Talairach and Tournoux atlas (1988). FreeSurfer automatically segments the corpus callosum in the standardized T1 weighted images by utilizing a probabilistic atlas based approach. This probabilistic atlas is created by

FreeSurfer and it represents the probability that each voxel will belong to a specific brain region based on class labels, functions of neighbourhood patterns and profiles of intensity values. Each voxel in the individual standardized T1 weighted images is then labelled by FreeSurfer based on the probabilities shown by the atlas at that point, and on its intensity. The corpus callosum is segmented in multiple slices, on either side of the mid-sagittal plane. The lateral extent of segmentation refers to the distance off the midline and this is set at two slices off the midline on either side; thus, the thickness of segmentation is 5 slices (one mid-sagittal slice plus two adjacent slices on each side) and from 1-mm thick slices – the CC "slab" is 5-mm thick. Once the segmentation of the corpus callosum is complete, the standardized T1 weighted images are converted back to their native form by applying the inverse of the transformation matrix previously used. The volume of the corpus callosum is then calculated as the total sum of all the voxels included in the multi-slice, 3D segmentation of the corpus callosum. The entire FreeSurfer analysis pipeline described above is an automatic one. In order to conduct a quality check on segmentation of the images, multiple slices in all three orientations (coronal, axial and sagittal) of each segmented brain scan are displayed and visually inspected by a trained expert (INK). All scans where the corpus callosum is overestimated (including fornix) or underestimated are excluded; this quality-control procedure is carried out before the comparison with the manually segmented CC. Additionally outliers whose mean FreeSurfer CC volume is above or below three standard deviations of the mean volume are also excluded.

3.2.3C FreeSurfer CC Area: In order to compare the FreeSurfer and manual tracing results, a 2D slice is identified that corresponds to the mid-sagittal slice selected manually by the expert while outlining the CC using Display. To do so, the native FreeSurfer segmented images are registered via a rigid, six-parameter linear transformation to the manual CC masks. This produces a FreeSurfer CC mask of the same slice as that on which manual delineation is performed. Each FreeSurfer CC mask is visually inspected, and those that fail to classify the corpus callosum correctly are excluded from the study. Mid-sagittal area of each FreeSurfer CC mask is calculated using a simple minc command (http://www.bic.mni.mcgill.ca/ServicesSoftware/HomePage).

For 107 of the participants, the FreeSurfer pipeline is unable to generate the FreeSurfer masks. Of the total 107 participants, 45 percent were boys and 55 percent were girls. This is because the alignment (the rotation and translation) of the native segmented FreeSurfer images is not compatible with that of the manual CC masks, even though both were generated from the same native T1 weighted images. The reason for this is an error occurring in the projection of the segmented FreeSurfer images from their standardized form back to their native form.

There are a total of 737 T1 weighted scans. For 107 scans FreeSurfer CC masks could not be generated due to technical limitations described above. Overall, a total of 630 FreeSurfer CC masks are generated and their areas calculated. Of these masks the following are excluded: 1) 10 failed quality control; and 2) 2 outliers. Following the exclusion, a total of 618 FreeSurfer CC masks (304 boys

and 314 girls) are left. Final analysis is conducted using these 618 FreeSurfer CC masks.

3.2.4 Statistical Analysis

All statistical analysis is performed using the SPSS software, PASW Statistics 18.0. The first step is to assess how the fully automatic, FreeSurfer technique compares with the semi-automatic, manual technique in segmenting the corpus callosum. For this purpose intra-class correlation between the manual CC area and FreeSurfer CC area is calculated. The intra-class correlation coefficient is calculated for the total sample and then separately for boys and girls. Next, the effect of volumetric technique used (manual versus Freesurfer) and sex on the mid-sagittal area of the corpus callosum is assessed via a 2×2 repeated measures ANOVA. Post-hoc analysis is subsequently performed via: 1) paired sample T-test between the manual CC area and FreeSurfer CC area, for the total sample and then separately for boys and girls; and 2) independent sample T-test for comparison of sex differences in the manually estimated CC areas and Freesurfer estimated CC areas. These analyses are meant to assess if FreeSurfer demonstrates a consistent overestimation or underestimation of the CC areas with respect to the manual tracing, in boys and girls; they also assess if sex differences observed in the CC area are influenced by the volumetric technique used. Lastly, Pearson correlation between the FreeSurfer CC area and FreeSurfer CC volume is calculated. Estimating the agreement between these two measures will ensure that CC volume can be used confidently as the main measure of CC size in future studies.

3.3 RESULTS

3.3.1 Demographics

The mean age of boys (n=304) and girls (n=314) included in this study is 207 (SD: ±33) months and 208 (±34) months, respectively. There is no significant difference in the mean age of the two sexes ($t_{(616)}$=0.486, p=0.627). For details see Table 3.1

Table 3.1 SYS: Sample Demographics and Descriptive Statistics for CC Area			
	All Mean (SD)	**Boys Mean (SD)**	**Girls Mean (SD)**
N (sample size)	*622*	*304*	*318*
Age (months)	*208 (34)*	*207 (33)*	*208 (34)*
Manual CC Area (mm^2)	*602 (83)*	*628 (80)*	*582 (80)*
FreeSurfer CC Area (mm^2)	*613 (83)*	*621 (80)*	*604 (86)*

Table 3.1 *SYS: Sample size (n) along with the mean and SD (in brackets) for the following variables in both boys and girls: age (months), manual CC area (mm^2) and freesurfer CC area (mm^2).*

3.3.2 Intra-Class Correlation between FreeSurfer CC area and Manual CC area

The intra-class correlation coefficient between FreeSurfer CC area and Manual CC area is r=0.904, p=0.001, df=617. The manual and FreeSurfer estimates of CC area are, therefore, very close to each other. The intra-class correlation is also calculated separately for boys and girls. The correlation values of both the sexes are very similar. For details see Table 3.2 and Figure 3.1A and 3.1B.

Table 3.2 SYS: Intra-Class Correlation between Manual and FreeSurfer CC Area (mm^2)			
	r	p	df
All	*0.904*	*0.001*	*617*
Boys	*0.902*	*0.001*	*303*
Girls	*0.917*	*0.001*	*313*

Table 3.2 *SYS: The intra-class correlation (r: intra-class correlation coefficient, p: level of significance, df: degree of freedom) between manual and freesurfer estimates of the CC area (mm^2), in both boys and girls.*

Figure 3.1A SYS: Correlation between FreeSurfer and Manual CC Area (mm^2) in Boys ICC=0.902, p=0.001, df=303

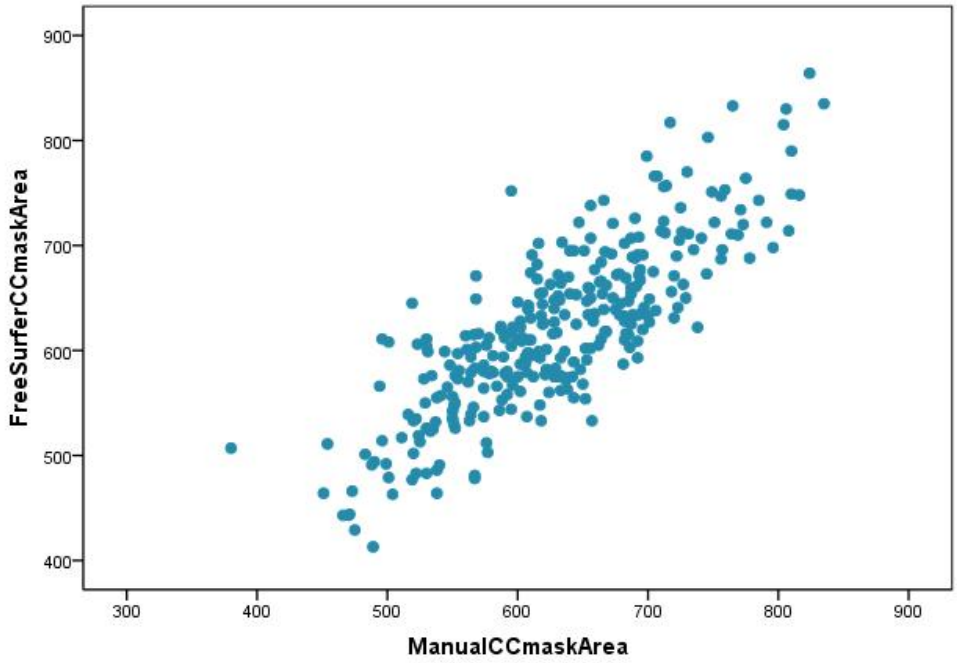

Figure 3.1A *SYS: The intra-class correlation (r: intra-class correlation coefficient, p: level of significance, df: degree of freedom) between freesurfer CC area (mm^2; y axis) and manual CC area (mm^2; x axis) in boys.*

Figure 3.1B SYS: Correlation between FreeSurfer and Manual CC Area (mm^2) in Girls ICC=0.917, p=0.001, df=313

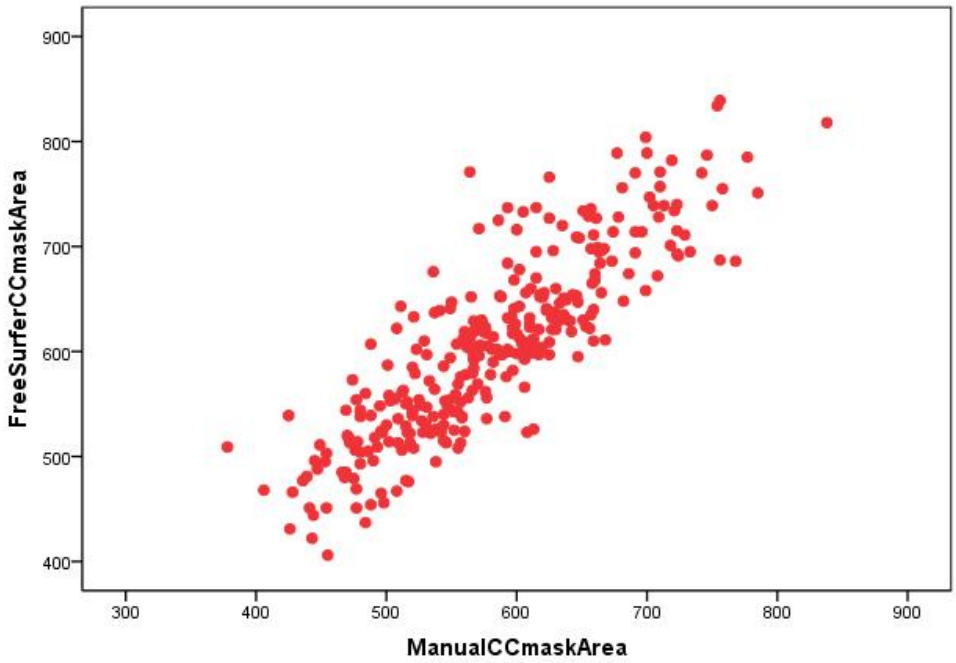

Figure 3.1B *SYS: The intra-class correlation (r: intra-class correlation coefficient, p: level of significance, df: degree of freedom) between freesurfer CC area (mm^2; y axis) and manual CC area (mm^2; x axis) in girls.*

3.3.3 Effect of Volumetric technique and Sex on CC area: Repeated Measure ANOVA

There is a significant interactive effect of both volumetric technique used and sex on the area of the corpus callosum ($F_{1,616}$=67.73, p=0.001) (Table 3.3A and 3.3B). Post-hoc analysis is conducted to further investigate this interactive effect. Results of the post-hoc analysis are described below.

Table 3.3A SYS: Descriptive Statistics for Manual and FreeSurfer CC Area in Boys and Girls		
	Boys	**Girls**
Manual CC Area (mm^2)	*628 (80)*	*582 (80)*
FreeSurfer CC Area (mm^2)	*621 (80)*	*604 (86)*

Table 3.3A *SYS: The mean and SD (in brackets) for the following variables in both boys and girls: manual CC area (mm^2) and freesurfer CC area (mm^2).*

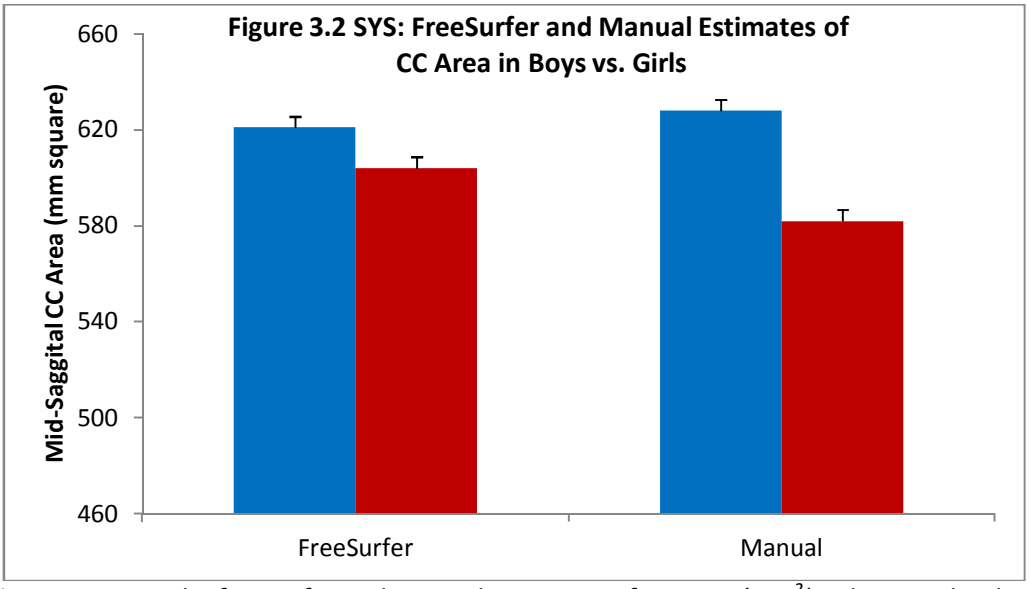

Figure 3.2 *SYS: The freesurfer and manual estimates of CC area (mm^2) in boys and girls. Boys are shown in blue and girls in red. Error bars represent standard error of mean.*

Table 3.3B SYS: Results for Association of Volumetric Technique and Sex with CC Area (Repeated measure ANOVA)			
	Sex	**Volumetric Technique**	**Interactive**
CC Area (mm^2)	***$F_{1,616}$=25.06, p=0.001***	***$F_{1,616}$=14.27, p=0.001***	***$F_{1,616}$=67.73, p=0.001***

Table 3.3b *SYS: The results for association of volumetric technique and sex with CC area (mm^2). These results are obtained using repeated measure anova with sex as between and volumetric technique (manual vs. freesurfer) as within subject variables. Statistics quoted include: f ratio for between and within group variance (f) and level of significance (p).*

3.3.3A Pairwise T-test between FreeSurfer CC area and Manual CC area

Pairwise comparison between the FreeSurfer CC area and manual CC area reveals a significant difference between the two ($t_{(617)}$=-3.715, p=0.001). The mean CC area calculated by FreeSurfer (613 ±83 mm^2) is slightly but significantly greater than that calculated manually (602 ±83 mm^2). The percentage area difference between the two is 1.7%.

This comparison is also carried out separately for boys and girls. For both sexes the difference between the FreeSurfer and manual CC area is significant (Boys: $t_{(303)}$=3.065, p=0.002 and Girls: $t_{(313)}$= -8.723, p=0.001). In case of boys, the mean CC area calculated by FreeSurfer (621 ±80 mm^2) is slightly lower than that calculated manually (628 ±80 mm^2). The percentage area difference between the two is 1.1%. In case of girls, the mean CC area calculated by FreeSurfer (604 ±86 mm^2) is slightly greater than that calculated manually (582 ±80 mm^2). The percentage area difference between the two is 3.7%. For details see Table 3.4 and Figure 3.2.

Table 3.4 SYS: Results for Association of Volumetric Technique with CC Area (mm^2) (Post-Hoc Analysis via Paired T-test between Manual and FreeSurfer CC Area)			
	t	**p**	**df**
All	***3.715***	***0.001***	***617***
Boys	***3.065***	***0.002***	***303***
Girls	***8.723***	***0.001***	***313***

Table 3.4 *SYS: The results for association of volumetric technique with CC area. This post-hoc analysis is carried out by conducting a paired t-test between manual and freesurfer CC area (mm^2), done separately in boys and girls. Statistics quoted include: t value (t), level of significance (p) and degree of freedom (df).*

3.3.3B T-test for Sex Differences in FreeSurfer CC area and Manual CC area

Here this study tests whether the same conclusions would be reached vis-à-vis sex differences in CC area independent of the segmentation technique. On both the FreeSurfer and manual CC areas, a T-test comparison between the CC area values of boys and girls is performed. In case of both the FreeSurfer and manual measures, the CC area of boys is significantly greater than that of girls. This sex

difference is slightly stronger in manual measures ($t_{(616)}$=2.384, p=0.001) as compared with FreeSurfer measures ($t_{(616)}$=2.384, p=0.015). For details see Table 3.3A and 3.5 and Figure 3.2.

Table 3.5 SYS: Results for Association of Sex with CC Area (mm^2) (Post-Hoc Analysis via Independent T-test between Boys and Girls)			
	t	**p**	**df**
Manual	***7.295***	***0.001***	***616***
FreeSurfer	***2.384***	***0.015***	***616***

Table 3.5 *SYS: The results for association of sex with CC area. This post-hoc analysis is carried out by conducting an independent t-test between CC area (mm^2) of boys and girls, done separately for the manual and freesurfer estimates. Result statistics quoted include: t statistic (t), level of significance (p) and degree of freedom (df).*

3.3.4 Correlation between FreeSurfer CC area and FreeSurfer CC volume

The Pearson correlation coefficient between the FreeSurfer CC area and FreeSurfer CC volume is very high (r=0.972, p=0.001, n=611). This correlation is also calculated separately for boys and girls. The correlation values of both the sexes are very similar. For details see Table 3.6 and Figure 3.3A and 3.3B.

Table 3.6 SYS: Pearson's Correlation between FreeSurfer CC Area (mm^2) and Volume (mm^3)			
	r	**p**	**df**
All	***0.972***	***0.001***	***611***
Boys	***0.969***	***0.001***	***302***
Girls	***0.975***	***0.001***	***309***

Table 3.6 *SYS: The results for the Pearson's correlation (r: pearson's correlation coefficient, p: level of significance, df: degree of freedom) between freesurfer CC area (mm^2) and volume (mm^3), in both boys and girls.*

Figure 3.3A SYS: Correlation between FreeSurfer CC Area (mm^2) and Volume (mm^3) in Boys; r=0.969, p=0.001, df=302

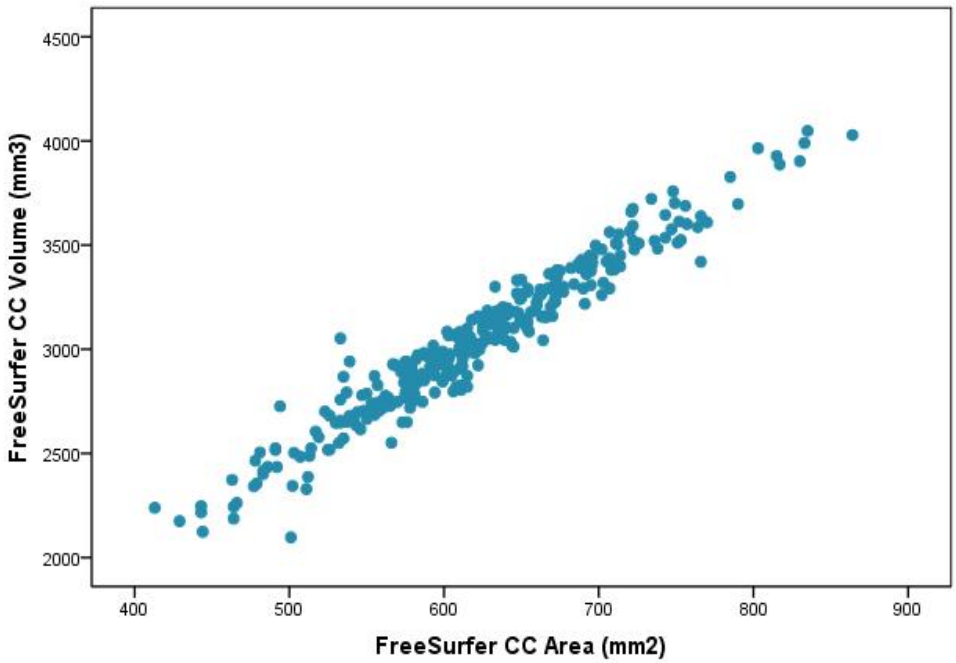

Figure 3.3A *SYS: The Pearson's correlation (r: pearson's correlation coefficient, p: level of significance, df: degree of freedom) between freesurfer CC area (mm^2; x axis) and freesurfer CC volume (mm^3; y axis) in boys.*

Figure 3.3B SYS: Correlation between FreeSurfer CC Area (mm^2) and Volume (mm^3) in Girls; r=0.975, p=0.001, df=309

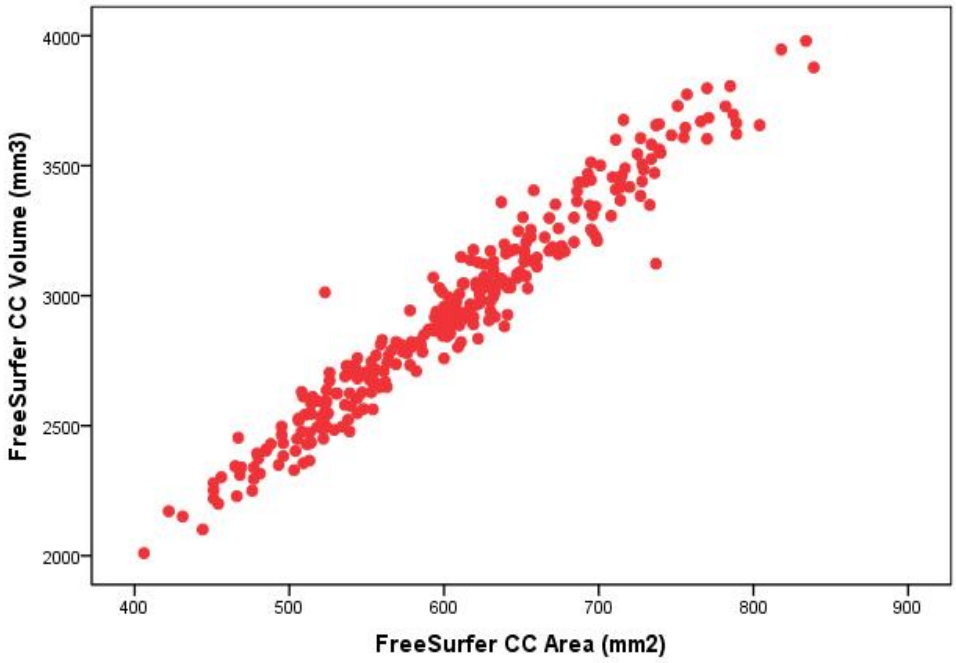

Figure 3.3B *SYS: The Pearson's correlation (r: pearson's correlation coefficient, p: level of significance, df: degree of freedom) between freesurfer CC area (mm^2; x axis) and freesurfer CC volume (mm^3; y axis) in girls.*

3.4 DISCUSSION

The increasing use of FreeSurfer as a tool for brain volumetry mandates the comparison and validation of its results against the results of standard manual tracing procedures. FreeSurfer segmentation of many subcortical structures, such as the hippocampus, putamen, amygdala and caudate nucleus, have been validated in this manner by previous researchers (Dewey et al. 2010, Shen et al. 2010, Pardoe et al. 2009, Morey et al. 2009). In case of the corpus callosum, several researchers have used FreeSurfer as a means to segment it and to study the effects of various pathologies on its size (Francis et al. 2011, Tartaglia et al. 2009, Vatta et al. 2011). None of these researchers, however, have validated the use of this automated method against the gold standard, namely the manual tracing method. This has been done by correlating the mid-sagittal surface area of the corpus callosum calculated by manual tracing with that calculated by FreeSurfer (on the same slice) in over 600 typically developing adolescents. Intra-class correlation coefficient (ICC) is the appropriate measure for correlation in this case, since an intra-individual comparison of the same measure is being carried out. The ICC is high ($r=0.904$, $p=0.001$, $df=617$). The ICC is, in fact, higher than that observed for other subcortical structures, which are rarely found to be above 0.80 (Dewey et al. 2010, Shen et al. 2010, Pardoe et al. 2009, Morey et al. 2009). This is expected considering the relative ease for defining the border of the corpus callosum in comparison with the different grey-matter subcortical structures. One of the few challenges in segmenting the corpus callosum, however, is observed at its junction with the fornix. Fornix is seen as a narrow

white matter bundle, of similar MR intensity as the CC, emerging from the ventral surface of the corpus callosum in the mid-sagittal plane. In manual tracing, the trained expert smoothly demarcated the ventral border of the CC separating it from the fornix. In contrast, FreeSurfer segmentation of this specific region of the CC is, in some cases, not as smooth. A slight overestimation at the junction of the fornix is noted in these cases, often in the form of a slight protrusion. Visual inspection of the FreeSurfer tracings by a skilled person allows a quality check of the segmentations, ensuring that they were comparable to the manual tracings.

Pairwise analysis of the FreeSurfer and manual mid-sagittal CC areas reveal that FreeSurfer overestimates the CC area. There was a small but significant difference (11 mm^2, which equals 2%) between the FreeSurfer CC area (613 ±83 mm^2) and manual CC area (602 ±83 mm^2). One of the reasons for this overestimation may be the inclusion of a few extra voxels at the junction of the fornix, as mentioned above. It is important to note, however, that small differences of up to 27 mm^2 (3.5% volume difference) have in fact been observed between CC mid-sagittal areas measured manually by two different experienced tracers. This inter-rater reliability was investigated in a subsample of scans from the Cycle study (n=41) and demonstrated high intraclass correlations (ICC=0.940, $p \leq 0.001$). Previous studies examining volume differences between the FreeSurfer and manual measures of brain structures such as hippocampus, amygdala, putamen and caudate nucleus also found the FreeSurfer estimates to be significantly greater than the manual ones (Dewey et al. 2010, Shen et al. 2010,

Pardoe et al. 2009, Morey et al. 2009). These volume differences, however, were found to be much greater in relative magnitude than those of the corpus callosum. Dewey et al. (2010) found the percentage volume difference between FreeSurfer and manual measure of the hippocampus to be ≈30%, amygdala to be ≈20%, putamen ≈14% and caudate nucleus to be ≈7%. It is possible that the distinctive appearance and intensity of the CC from its surrounding tissue enables FreeSurfer to segment the corpus callosum more accurately than other structures (i.e. closer to manual estimates).

Sex differences are observed in the mid-sagittal CC area estimated by both FreeSurfer and manual means. In both cases, the mid-sagittal area of boys is significantly greater than that of girls. The magnitude of the sex difference in CC area, however, is smaller in FreeSurfer measures (2.7% sex difference in area) as compared with manual measures (5.7% sex difference in area). This is because in case of boys, FreeSurfer in comparison to manual approach underestimates the CC area by 1% (7 mm^2) whereas in case of girls, FreeSurfer in comparison to manual approach overestimates CC area by 3.5% (22 mm^2). One of the factors why FreeSurfer overestimates mid-sagittal area in girls and underestimates it in boys might be differences in the shape and size of their CCs. The corpus callosum of boys is larger and more tubular in shape, whereas that of girls is smaller and has a bulbous splenium (Allen et al. 1991, Hayakawa et al 1989). It may be speculated that having a smaller CC with a narrower central versus splenial region may be associated with poorer FreeSurfer segmentation at the junction of the fornix, resulting in overestimation of CC area in girls; and having a larger

circumference is associated with removal of more voxels from the boundary between the CC and the callosal gyrus, resulting in underestimation of CC area in boys. Further investigation, however, is required to determine specifically where and why did FreeSurfer overestimate mid-sagittal area of girls and underestimate it in boys, as compared with their manual tracings.

The standard output generated by FreeSurfer is CC volume. This is a three dimensional measure of CC segmentation in multiple slices extending laterally on each side of the mid-sagittal plane. Conventionally, researchers have used mid-sagittal area as the measure for CC size. Whether CC volume presents a more appropriate measure for CC size is an important issue to be explored. The corpus callosum is essentially a wide concave shaped bundle of nerve fibres. It is, therefore, a three dimensional structure which has a length (along the y axis), a height (along z axis) and a width (along the x axis). Although FreeSurfer generates the volume of the corpus callosum by segmenting it along relatively arbitrary lateral boundaries, it enables us to deal with the CC as a 3D structure. A major advantage of considering the CC in terms of a 3D structure is more appropriate normalization of the CC with total brain size. When studying the effects of various factors on the size of the corpus callosum, most researchers consider the relative (brain volume corrected) rather than the absolute area of the CC. This is to correct for the influence of brain volume on the CC, which is a potential confound. Comparing the 2D area of the corpus callosum with 3D volume of the total brain causes a disproportionate reduction of the observed ratio for larger head sizes. FreeSurfer helps overcome this limitation by

comparing the 3D volume of the CC with the 3D volume of the total brain. The area and volume of the corpus callosum measured by FreeSurfer are very well correlated with each other (r=0.972, p=0.001, n=611); thus, validating the volumetric measures against the area ones. It can now be confidently claimed that FreeSurfer based CC volume is a reliable measure of callosal size.

Manual tracing of the CC is considered to be the gold standard because it ensures that a trained expert outlines the corpus callosum precisely along its natural border. Visual inspection of the FreeSurfer segmentation by a trained expert, however, helps achieve comparable results. This quality check removes all poorly segmented CCs, ensuring all segmentations included are accurate. This study proves that FreeSurfer segmentations are very similar to manual ones. In addition, FreeSurfer has the added advantage of being faster, simpler, less tracer-dependant and having fewer pre-requisites. Moreover the prime FreeSurfer output is CC volume, which enables more appropriate normalization of CC size with the total brain volume.

3.5 CONCLUSION

To our knowledge, this is the first study to have addressed the issue of validity of automated callosal volumetry. A large sample of over 600 participants is used to explore this topic, ensuring the significance of its results. Based on the results of this study it is concluded that automatic FreeSurfer based CC segmentation is consistent with the manual Display-assisted segmentation. Quality check by visual inspection of FreeSurfer segmentation, however, is very important to

ensure consistency and accurate analysis. The standard output of FreeSurfer, namely the CC volume, relates closely to the traditionally used measure of CC area. Employing either of the two to study CC size will, therefore, be justified.

4 ASSOCIATION OF AGE AND SEX WITH THE RELATIVE VOLUME OF THE TOTAL CORPUS CALLOSUM AND ITS SEGMENTS DURING ADOLESCENCE

4.1 INTRODUCTION

Men and women differ in various physical attributes and mental abilities. Sexual dimorphism of the human body extends beyond secondary sexual characteristics. There are many differences between men and women that range from the relative number of red blood cells (M>F; Kumar and Clark, 1998), a muscle-fat ratio (M>F; Maughan et al. 1983, Millner et al. 1983) to the shapes of bones (Churchchill et al. 2007, Moore et al. 2006). Similarly, the two sexes also differ in brain structure (Akney et al. 1992, Good et al. 2001 and Luders et al. 2002, reviewed in Paus 2011) and functions (calculation abilities: Spelke et al. 2005; memory: Bleecker et al. 1988; emotions: Kring et al. 1998).

Sex differences in the corpus callosum vary at different stages of its development or maturation (see Table 1.1). A sex difference in the relative area of the corpus callosum is first reported in samples comprised of children and adolescents (Giedd et al. 1999, Lenroot et al. 2007). Most of the physical differences between boys and girls also begin to manifest during adolescence (Grumbach et al. 2003, Guyton et al. 2000). In view of the fact that sexual dimorphism develops over time, age is an important factor to consider when investigating sex differences in the corpus callosum. Literature examining the Influence of age and sex on the

shape, size and structure of the corpus callosum during adolescence was extensive. The findings of most of the studies exploring this topic, however, were inconsistent.

One of the earliest and most widely referenced reports on sexual dimorphism of the corpus callosum dates back to 1982. DeLacoste-Utamsing & Holloway (1982) were the first to report robust differences in the corpus callosum of adult men and women. In this postmortem study, they observed that the absolute surface area of the total corpus callosum and all its segments were greater in men, except for the splenium of the corpus callosum that was found to be greater in women (DeLacoste-Utamsing & Holloway 1982). Brain-corrected surface area of the corpus callosum, on the other hand, and all its segments was greater in women (brain-size corrected surface area of the corpus callosum will be referred to as relative surface area of the corpus callosum from here onwards). Since then many researchers have investigated the influence of age and sex on the corpus callosum during different growth periods with varying results.

Before introducing this study, this paragraph discusses briefly the literature examining sexual dimorphism in corpus callosum. This highlights inconsistencies in the results of current studies and provides the background needed to understand better the advantages of this study relative to previous studies. Giedd et al. (1999) conducted one of the largest studies examining sex differences in the relative area of the corpus callosum of 139 children and adolescents aged 5 to 18 years. They demonstrated a trend ($p=0.07$) for greater relative area of the corpus callosum in girls as compared with boys. In addition

they also examined the specific sub-regions of the corpus callosum; for this purpose, seven segments of the CC, namely rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium, were examined. They observed a trend (p=0.05 to 0.10) for greater relative area of the genu, posterior midbody and isthmus in girls as compared with boys. Few researchers, other than Giedd et al. (1999), have examined sex differences in callosal segments of adolescents. Raunch and Jinkins (1994) reported no sex differences in the relative area of the corpus callosum of 45 participants from birth to 20 years. Studies examining age-associated changes in the area of the corpus callosum have more consistent results. The (absolute and relative) area of the total corpus callosum increases rapidly during the first decade of life (Hayakawa et al. 1989, Raunch and Jinkins 1994); thereafter, the growth rate slows down. Up to a 3% increase in relative area (and 2% in absolute area) of the total corpus callosum is reported during the second decade (Hayakawa et al. 1989, Raunch and Jinkins 1994). Some researchers studied sex differences in the developmental trajectories of the corpus callosum. De Bellis et al. (2001), for example, showed that the relative area of the corpus callosum increased at a faster rate in boys (n=61) as compared with girls (n=57) during childhood and adolescence (from 6 to 17 years). Lenroot et al. (2007) conducted a large sample of 387 participants aged 3 to 27 years, which was mainly an expansion of the Giedd et al. (1999) sample. In contrast to De Bellis et al. (2001), they observed that the developmental trajectory of the relative area of the corpus callosum in females (n=178) was significantly higher than that of males (n=209). In summary, the results of studies investigating sexual dimorphism in the relative area of the total

corpus callosum and its segments, during childhood and adolescents were mixed (see Table 1.2). Some researchers did not observe any sex differences in callosal area (Raunch and Jinkins 1994), while some observed a female versus male advantage (Giedd et al. 1999). These sex differences were mainly observed in the anterior and central segment of the CC (Giedd et al. 1999). Reports of sexually dimorphic area of the CC during this period were, however, more frequent. Age-associated increases in the relative area of the corpus callosum were observed during adolescence. Sex differences in these age associated increases, that is, developmental trajectories demonstrate a female versus male advantage during childhood and adolescence (Lenroot et al. 2007, DeBellis et al. 2001).

Multiple factors relating to the sample size, age and methodological approaches employed could be responsible for the contradictory results of existing studies examining sex differences in the volume of the corpus callosum. These factors include:

- Sample Size: Studies examining the association of age and sex with the corpus callosum vary considerably in sample size (from n=20 to 390; refer to Section 1.6). In order to reveal the sex differences in the surface area of the corpus callosum and its segments a relatively large sample size is required. This indicates that these sex differences are relatively small in magnitude (effect sizes between 0.1 and 0.3; refer to Section 1.6).

- Sample Age: One of the reasons for the disparity in the results quoted above pertains to the variation in the age range. For example, one study

could be describing results for 5 to 18 year old individuals while another for 3 to 27 year old individuals. Corpus callosum develops more rapidly during the earlier years of childhood as compared with late adolescence. Thus, sex differences observed in samples with more individuals in the younger age range will be different from those observed in samples with more participants in the older age range.

- Methodology: Principally, two techniques are used to assess the surface area of the corpus callosum. These include postmortem examination of the brain and *in-vivo* magnetic resonance imaging. The results of these two types of studies may vary due to the changes that take place in brain anatomy and morphology following death. These changes include swelling of certain areas of the brain or shrinkage of brain tissue due to neuronal cell death and breakdown of the cell membrane (Raunch & Jenkins 1994). Additionally in postmortem studies formalin fixation is carried out in order to preserve the brain. This may also alter brain morphology and can cause minor increases in brain weight (Witelson & Goldsmith 1991). Moreover postmortem studies usually differ from *in-vivo* magnetic resonance studies in terms of sample size, age range and disease. They usually comprise of a limited number of subjects due to scarcity of people volunteering their body for scientific research. Most people presented for autopsy are elderly and/or died of disease or trauma.

The findings of postmortem studies could vary from each other due to a number of confounding factors. These include variation in timing between death and tissue fixation and size measurement. Similarly, it has also been suggested that the results of *in-vivo* magnetic resonance studies could vary from each other due to various factors. Field strength of the scanner, acquisition parameters, image resolution, computational tools used for delineation and classification, are all examples of parameters that may vary across imaging studies and be responsible for the inconsistencies observed in their results.

- Brain Size: Brain size is known to be a major confounding factor that affects the size of the corpus callosum and its segments. Correlations of up to 0.40 (Johnson et al. 1994) and 0.45 (Bermudez et al. 2000) have been reported between the corpus callosum and total brain volume. Head size is, therefore, a strong predictor of differences between male and female CC. Men on average have larger brains as compared with women, thus the absolute measures of CC size are also more likely to be larger in them. This undue male advantage, however, is overcome by considering relative area of the CC that has been normalized for brain size.
- Normalization for Brain Size: Strategies used for normalizing the surface area of the corpus callosum for total brain size are equally variable. The indices used for estmation of brain size vary from brain weight to brain volume to cross-sectional brain area. Bermudez et al. (2000)

demonstrated that using different indices of brain size for normalization produce discordant results for sex differences in CC area. CC areas normalized for brain volume were significantly greater in females versus males for all segments, whereas those normalized for brain area were significantly greater in females versus males for the anterior mid-body and splenium only (remaining segments, that is, genu, posterior mid-body and isthmus showed no sex difference). Similarly, Johnson et al. (1994) showed that CC area normalized for brain volume was significantly ($P<0.0001$) greater in females versus males, whereas that normalized for mid-saggital brain area showed no sex difference ($p>0.05$). Both means of normalization have certain limitations. Correcting the 2D mid-sagittal area of the CC with a 3D measure of brain size causes disproportionate reduction in the relative area of the CC for larger head sizes. At the same time, using a single slice 2D measure of brain size such as mid-saggital area does not account for as much of the variation in the size of the brain as its 3D volume.

- Segmentation: The techniques used for the segmentation of the corpus callosum vary throughout literature. The corpus callosum is divided into arbitrary segments and sex differences in the surface area of these segments then measured. This segmentation could be done along the curvature of the corpus callosum or along a straight line running from anterior to posterior extent of the corpus callosum. The number of divisions could vary from 3 to 5 (Bermudez & Zatorre 2001) to 7 (Clarke et al. 1989) or even up to 20 (Allen et al. 1991). The segments could be

equal or unequal in size; for example in a study by Allen et al. (2003) the corpus callosum was divided into 4 segments, the third segment being much smaller than the rest. Most studies segment the CC arbitrarily as described above, but rarely subdivisions have been created based on cluster analysis of regional widths (Denenberg et al. 1991).

- Inter-individual Variability: There is considerable degree of inter-individual variability in both size and shape of the corpus callosum. The surface area of the corpus callosum varies more in men as compared with women (Allen et al. 2002). This could be one of the factors affecting the sex differences reported in the corpus callosum.

This chapter aims to examine the association of age and sex with the relative volume of the corpus callosum during adolescence. This is done in two large-scale samples of adolescents: 1) the Saguenay Youth Study sample (n=737, 12 to 18 years); and 2) the IMAGEN sample (n=1,979, 13 to 15 years). There are multiple areas of improvement in these studies as compared with previous studies. One of the reasons for the inconsistent results of previous studies may be limitations in their sample size, which were not large enough to encompass the population variation in CC size. Another drawback of previous studies was the sample age-range. Sexual dimorphism of the corpus callosum has not been widely detected prior to childhood, reports of sexual dimorphism in callosal size were more frequent during childhood and adolescence and sex differences were well established by adulthood (see Section 1.6). When studying association of age and sex with the corpus callosum it is, therefore, essential to investigate

them separately in children and adolescents, as the results of the two groups are bound to differ. The SYS and IMAGEN studies are two of the largest known studies to date that examine the association of age and sex with the corpus callosum of adolescents alone. By virtue of their large sample size and strict age range (only adolescents) these studies are able to provide some reliable and well powered results about sexual dimorphism in the corpus callosum of adolescents. Another limitation of previous MR studies investigating sex differences in the size of the corpus callosum is that they normalize for brain size by relating the 2D area of the CC with the 3D volume of the total brain, which causes a disproportionate reduction in the observed ratio for larger head sizes. The SYS and IMAGEN studies measures the 3D volume, rather than 2D area, of the corpus callosum across multiple mid-sagittal slices, which is then correlated with the volume of the total brain; hence ensuring that two 3D measures are compared. Some studies have related the CC area with the midline area of the brain to overcome this problem, however, doing so accounts for lesser variance in brain size. Finally, the corpus callosum is examined not just as whole but also as five separate segments (anterior, mid-anterior, central, mid-posterior and posterior), so as to determine which specific sub-regions of the corpus callosum experience the age and sex associations observed (see Section 4A.2.2). This is important since there is a topographic representation of cortical areas along the length of the CC (see Section 1.3 and 1.4). Most studies divide the CC into arbitaray segments along a straight line connecting the anterior-most to posterior-most points of the CC (Bermudez et al. 2000, Witelson et al. 1991, Giedd et al. 1999, Sullivan et al. 2000). These arbitrary segments may cut across axon bundles

having homogenous topographic origins. Unlike previous studies, the SYS and IMAGEN studies examine sex differences in CC segments both before and after correcting for the co-relatedness between such arbitararily drawn segments of the CC.

Examining the association of age and sex with the relative volume of the corpus callosum and its segments in two distinct samples is another major advantage of this research. This enables validation of the results of the two studies against each other, thus substantiating the significance of their findings. When comparing the two studies, however, it is important to keep in mind the differences between them. In comparison to the Saguenay Youth Study, the IMAGEN study has a narrower age range. By the age of 13 years most boys and girls have reached puberty and begun to display sexual dimorphism in their bodies (Guyton 2000, Grumbach 2003). We, therefore, expect to observe sex differences in the relative volume of the corpus callosum of both the IMAGEN and SYS adolescents. On the other hand, the association of age with the relative volume of the total corpus callosum is not expected to be similar in the two studies. This is because the corpus callosum demonstrates limited increases in volume during the second decade (Hayakawa et al. 1989, Raunch and Jinkins 1994), that is, it grows slowly during adolescence. Hence, the age associated changes in the relative volume of the corpus callosum are expected be more significant over the six year duration (12 to 18 years) of the SYS study than the age associated changes across the two year duration (13 to 15 years) of the IMAGEN study.

In summary, this chapter re-examines sexual dimorphism in the corpus callosum during adolescence using improved study samples, design and analytical techniques.

4A.2 METHODOLOGY: SYS STUDY

This study is conducted by analyzing data from 737 adolescents of the Saguenay Youth Study. Methodological details of the study design and participants, their recruitment and testing procedures have been provided in an earlier chapter (Chapter 2, Section 2A) of this thesis. Here the methodological approaches relevant to this study are briefly discussed.

4A.2.1 Participants

This dataset consists of a total of 737 adolescents (356 boys and 381 girls) of French-Canadian origin belonging to the population with the known genetic founder effect, namely the Saguenay Lac Saint-Jean (SLSJ) region of Quebec, Canada. All participants are aged between 12 to 18 years.

4A.2.2 MRI Acquisition

For each participant T1 weighted (T1W) MRI scans are obtained on a Philips 1.0 T superconducting magnet. Acquisition parameters of these structural scans are as follows: 3D RF spoiled gradient-echo scan, 140-160 high resolution T1W images, with TR=25 ms, TE=5 ms, flip angle=30^0 and 1 mm isotropic resolution.

4A.2.3 MRI Volumetric Analysis

FreeSurfer version 5.0.0 is used for the automated volumetric analysis that is performed on native, high resolution T1-weighted (T1W) images. These images are first corrected for non-uniformities caused by motion artifacts, scanner induced inhomogenieties and intensity inhomogenieties. Then they are normalized for intensity. Next, brain extraction is performed on these motion- and intensity-corrected and normalized images. This step strips away the skull and any background noise, leaving only the brain tissue. Following this pre-processing, the native images undergo non-linear registration to the MNI305 template (Evans et al. 2003), which is aligned with the Talairach and Tournoux atlas (1988). FreeSurfer automatically segments the corpus callosum in the standardized T1W images by utilizing a probabilistic atlas-based approach. This probabilistic atlas is created by FreeSurfer and it represented the probability that each voxel would belong to a specific brain region based on class labels, functions of neighbourhood patterns and profiles of intensity values. Each voxel in the individual standardized T1W images is then labelled by FreeSurfer based on the probabilities shown by the atlas at that point, and on its intensity. The corpus callosum is segmented in five slices (one mid-sagittal slice plus two adjacent slices on either side). The thickness of the CC “slab” segmented is 5 mm, since each slice is 1 mm thick; callosal fibres at the lateral extremes are still bundled together displaying the same basic anatomic shape and structure as that in the middle. Once the segmentation of the corpus callosum is complete, the standardized T1W images are converted back to their native form by applying

the inverse of the transformation matrix previously used. The absolute volume of the total corpus callosum is then calculated as the total sum of all the voxels included in the multi-slice, 3D segmentation of the corpus callosum. The total corpus callosum is then parcellated into five segments, namely the posterior, mid-posterior, central, mid-anterior and anterior segments. The divisions of the CC are equally spaced in terms of distance along the long axis of the CC (see Figure 1.2; http://surfer.nmr.mgh.harvard.edu/fswiki/mri_cc#Description). The absolute volume of all five segments is calculated automatically. The relative volumes of the total corpus callosum and its five segments are then calculated as a percentage by dividing their absolute volumes with the total volume of the brain and multiplying the result with 100. The total volume of the brain is estimated using an equation that calculates the total grey-matter, white-matter and CSF volume of the cerebrum and cerebellum. This total brain volume variable is referred to as "BrainSegVolEq" and it is equivalent to the "BrainSegVol" variable of FreeSurfer 4.0.

The entire FreeSurfer analysis pipeline described above is an automatic one. In order to conduct a quality check on segmentation of the images, multiple slices in all three orientations (coronal, axial and sagittal) of each segmented brain scan are displayed and visually inspected by a trained expert (INK). All scans where the corpus callosum is overestimated (including fornix) or underestimated are excluded (n=6). Additionally, outliers whose mean FreeSurfer CC volume is above or below three standard deviations (SD) of the mean volume are also excluded (n=5). Following the quality check and removal of outliers volumetric data is left

for a total of 702 adolescents, 342 boys and 360 girls. For more details on precise number of participants for whom volumetric data for the total corpus callosum and each of its segment is available refer to Table 4A.1.

4A.2.4 Statistical Analysis

All statistical analysis is performed using the SPSS software, PASW Statistics 18.0. The main and interactive association of age and sex with the relative volume of the total corpus callosum and its segments is assessed via linear regression. Sex is coded as +1 for girls and -1 for boys. Mean-centred age of all participants is calculated by subtracting their age with the total sample's mean age. The linear regression model is run with the volume measures specified as the dependent variables. There are three blocks of independent variables. The first block consists of all the potential confounding variables that are as follows: 1) prenatal exposure to maternal cigarette smoking (PEMCS); and 2) use of oral contraceptive pill (OCP) in girls. Sex and mean-centred age are specified as the independent variables in the second block, in order to determine the main effect of each. The product of sex and mean-centred age is specified as the independent variable in the third block, to determine any interactive sex × age effect on the callosal volumes. The callosal segmental volumes are seen to be well correlated. Therefore, in order to control for inter-segmental dependence when examining the correlation of age and sex on a specific segment, all the remaining segments are specified as independent variables in a preliminary block (see below for more details).

For each linear regression model the adjusted R^2, standardized regression coefficient (β), t value for the regression coefficient and the significance of the t value are calculated. The standardized regression coefficient (β) represented the standard deviation units by which the dependent variable changed for each standard unit change in the independent variable. Thus explaining how well the dependent and independent variables are correlated with each other. Results of a two-tailed T-test for the significance of the regression coefficient (t value and p value) are also calculated to demonstrate if the correlation (β) observed is significant or not. Adjusted R^2 explains the percentage variance in the dependent variable contributed by the independent variables. SPSS calculates and displays adjusted R^2 in a stepwise sequence, based on the blocks of independent variables entered. SPSS displays the adjusted R^2 for each block in the model summary, adding on the variance contributed by the independent variables of the previous blocks. Thus, by calculating the change in adjusted R^2 of the first and second block, the percentage variance in the dependent CC volume contributed specifically by age and sex is estimated.

This study considers only the relative volumes (brain-size corrected volumes) of the corpus callosum and its segments, when examining sex and age related differences. As previously explained, brain size is a major predictor of CC size with high correlations (Pearson's *r*: 0.4 to 0.5) between the two (Bermudez et al. 2000, Johnson et al. 2000). Moreover, total brain volume is also observed to be highly correlated with the volume of the CC in the SYS study (Pearson's r=0.382, $p<0.0001$, df=704; for more details see Appendix A). It is, therefore, essential to

normalize for total brain volume since this is one of the most evident confounds when examining sex differences. Total brain volume is normalized for by dividing the absolute volume of the total corpus callosum and all its segments with the total brain volume. These brain-corrected volumes of the total CC and its segments are referred to as 'relative volumes'.

This study examines the association of age and sex with the relative volumes of a specific callosal segment after co-varying for the volume of the remaining segments. This is done because the volumes of the callosal segments correlate with one another; thus if one segment is large the other segments are also likely to be proportionately larger. Moreover, this co-dependency between CC segments may also be because the segments cut across functionally homogenous bundles of callosal axons. In order to elucidate sex differences in the relative volume of each callosal segment that is not confounded by the influence of other segmental volumes it is important to control for inter-segmental dependence. It is important to note that correcting for inter-segmental dependence covaries for the inter-relatedness of the segments but does not "correct" for the actual size of these segments. Most previous studies do not control for this confound. To understand the impact of this procedure the results are calculated both before and after correcting for inter-segmental dependence.

4B.2 METHODOLOGY: IMAGEN STUDY

This study is carried out on 1,979 13 to 15 year old adolescents belonging to the IMAGEN study sample. Methodological details of the IMAGEN study design

including recruitment and testing procedures are explained in detail in an earlier chapter (Chapter 2, Section 2B) of this thesis. Here the methodological approaches relevant to this study are briefly discussed.

4B.2.1 Participants

This dataset consists of a total of 1,979 adolescents (964 boys and 1,015 girls), recruited from eight centres across England, Ireland, France and Germany. All participants are aged between 13 to 15 years.

4B.2.2 MRI Acquisition

The T1W MRI scans of the participants are obtained on 3.0 T superconducting magnets, from a number of manufacturers (Siemens, Philips, General Electric and Bruker). Acquisition parameters of these structural volume scans are as follows: 160-170 high resolution T1W sagittal slices, with TR=2300 ms, TE=2.8 ms, flip angle=8-9^0 and 1.1 mm isotropic resolution.

4B.2.3 MRI Volumetric Analysis

FreeSurfer version 5.0.0 is used for the automated volumetric analysis, performed on native, high resolution T1-weighted (T1W) images. The procedure of volumetric analysis is identical to that explained for the Saguenay Youth Study in section 4A.2.3. The slice thickness of the T1W scans of the IMAGEN study is 1.1 mm; considering that the CC is segmented in five slices (one mid-sagittal slice

plus two adjacent slices on either side) the final thickness of the CC "slab" segmented is 5.5 mm.

A quality check is performed on all scans by visual inspection of multiple slices of the scans in all three orientations, by a trained expert (INK). All scans where the corpus callosum is overestimated (including fornix) or underestimated are excluded (n=97). Outliers whose mean FreeSurfer CC volume is above or below three standard deviations of the mean volume are also excluded (n=10). Following the quality check and removal of outliers a total of 1,866 adolescents, 896 boys and 970 girls are left. For more details on precise number of participants for whom volumetric data for the total CC and each of its segment is available refer to Table 4B.1.1.

4B.2.4 Statistical Analysis

All statistical analysis is performed using SPSS 18.0. The statistical tests used are similar to that used in the Saguenay Youth study to examine the association of sex and age with the relative volume of the total corpus callosum and its segments (for details refer to Section 4A.2.4). Briefly, the main and interactive association of age and sex with callosal volumes is calculated via linear regression. Sex is coded as +1 for girls and -1 for boys. Mean centred age of all participants is calculated by subtracting their age with the total sample's mean age. The linear regression model is run with the volume measures specified as the dependant variables. There are four blocks of independent variables. The first block corrects for the possible confounding effect of acquisition site and is

comprised of seven variables, which act as dummy variables for acquisition site. Use of oral contraceptive pills is specified in the second block, to correct for the potential effect of OCP use on CC volumes. Sex and mean-centred age are specified as the independent variables in the third block, in order to determine the main effect of each. The product of sex and mean-centred age is specified as the independent variable in the final block, to determine any interactive sex × age effect on the callosal volumes. In order to control for inter-segmental dependence when examining the correlation of age and sex with a specific segment, all the remaining segments are specified as independent variables in a preliminary block. For each linear regression model the variance is estimated as the change in adjusted R^2; whereas correlation between age or sex and relative CC volumes is estimated via the standardized regression coefficient and T-test for its significance. All analysis is conducted on relative volumes of the total corpus callosum and its segments, in view of the high correlations between the absolute volume of the total brain volume and the corpus callosum in the IMAGEN study (Pearson's *r*=0.407, p<0.0001, df=1879; for more details see Appendix A). For detailed explanation of these adjustments and their purpose refer to Section 4A.2.4.

4A.3 RESULTS: SYS STUDY

4A.3.1 Descriptive Statistics

There are a total of 356 boys with mean age of 180 (SD: ±22) months and mean puberty stage of 3.38 (±0.87). The percentage of boys who have no prenatal

exposure to maternal cigarette smoking (PEMCS) is 55. There are a total of 381 girls with mean age of 181 (±23) months and mean puberty stage of 4.08 (±0.74). The percentage of girls who have no prenatal exposure to maternal cigarette smoking (PEMCS) is 50. Of the total 381 girls, 56 are using oral contraceptives. Descriptive statistics of the demographics and mean relative volume of the total corpus callosum and its segments in both sexes is shown in Table 4A.1.1 and Figure 4A.1.1 and 4A.1.2.

Pearson's correlation between the variables employed in the linear regression analysis to estimate the association of age and sex with the relative volumes of the corpus callosum and its segments is provided in Table 4A.1.2.

Table 4A.1.1 SYS: Sample Demographics and Descriptive Statistics for CC Volume				
	Boys		**Girls**	
	n	**Mean (SD)**	**n**	**Mean (SD)**
Age (months)	*355*	*180 (22)*	*379*	*181 (23)*
Puberty Stage	*342*	*3.38 (0.87)*	*377*	*4.08 (0.74)*
Girls Using OCP (n)	*-*	*-*	*56*	*-*
Percentage with no PEMCS (%)	*196*	*55*	*190*	*50*
Relative CC Volume (%)				
Total	***342***	***0.236 (0.028)***	***360***	***0.254 (0.029)***
Anterior	***344***	***0.067 (0.009)***	***360***	***0.071 (0.009)***
MidAnterior	***343***	***0.035 (0.005)***	***357***	***0.037 (0.006)***
Central	***342***	***0.034 (0.006)***	***361***	***0.038 (0.007)***
MidPosterior	*343*	*0.033 (0.006)*	*363*	*0.037 (0.006)*
Posterior	***341***	***0.066 (0.010)***	***363***	***0.071 (0.010)***

Table 4A.1.1 *SYS: The sample size (n) along with the mean and SD (in brackets) for the following variables in both boys and girls: age (months), puberty, pemcs and relative volume (%) for the total CC and its five segments (anterior, mid-anterior, central, mid-posterior and posterior).*

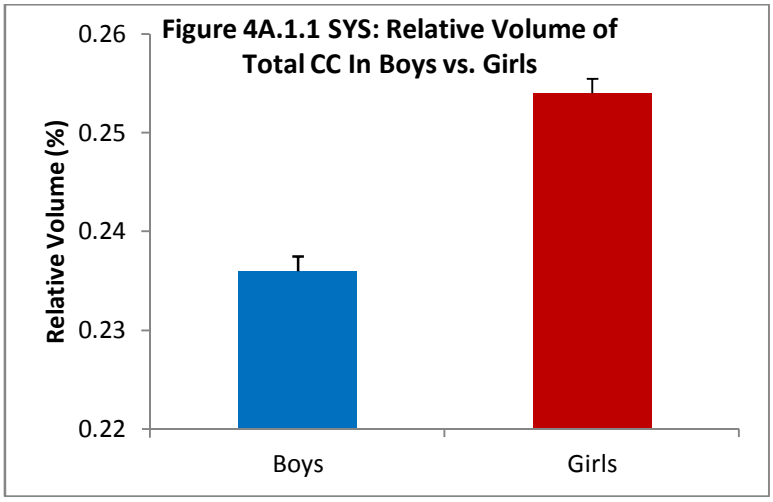

Figure 4A.1.1 *SYS: Bar chart for the relative volume (%) of the total corpus callosum in boys versus girls. Boys are shown in blue and girls in red. Error bars represent standard error of mean.*

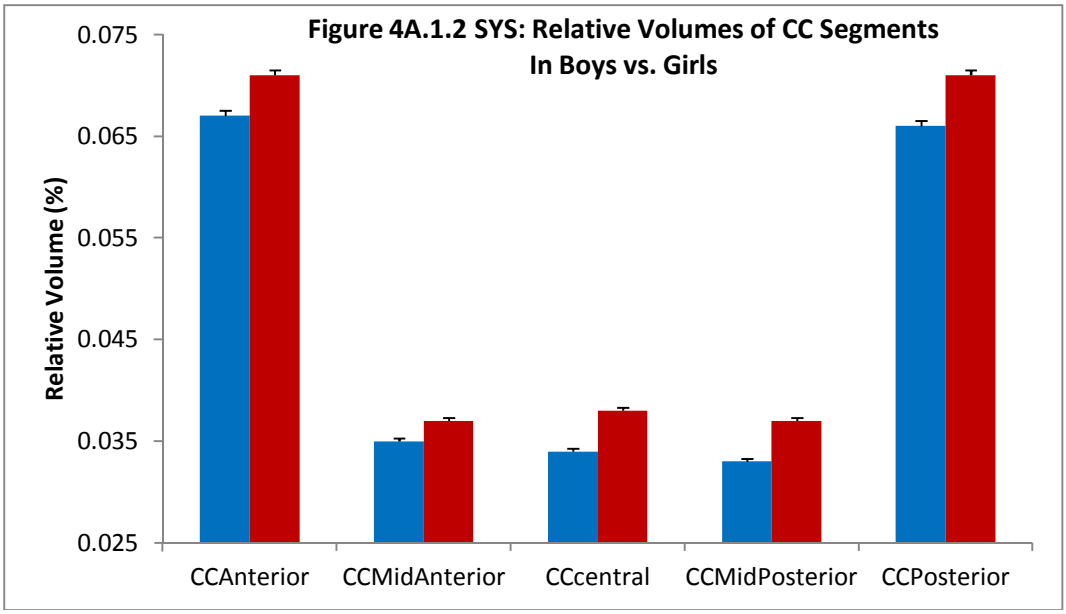

Figure 4A.1.2 *SYS: Bar chart for the relative volumes (%) of CC segments in boys versus girls. Boys are shown in blue and girls in red. Error bars represent standard error of mean. It is important to note that after removal of regional interdependencies the mid-anterior segment becomes larger in males versus females and the sex difference in the mid-posterior segment becomes non significant (see Table 4A.2.1).*

Table 4A.1.2 SYS: Correlation Matrix (Pearson's Correlation Coefficient)								
	Sex	**Age**	**OCP use**	**PEMCS**	**CC Anterior**	**CC Mid Anterior**	**CC Central**	**CC Mid Posterior**
Age	*0.021*							
OCP Use		*0.292***						
PEMCS	*0.056**	*0.034*	*-0.030*					
CC Volume								
Total	*0.302***	*0.196***	*0.131***	*-0.024*				
Anterior	*0.223***	*0.149***	*0.095**	*-0.015*				
MidAnterior	*0.186***	*0.161***	*0.064*	*0.004*	*0.516***			
Central	*0.280***	*0.079**	*0.080**	*0.035*	*0.310***	*0.645***		
MidPosterior	*0.263***	*0.167***	*0.091**	*-0.047*	*0.469***	*0.587***	*0.607***	
Posterior	*0.238***	*0.209***	*0.103**	*-0.054*	*0.588***	*0.450***	*0.301***	*0.526***

Table 4A.1.2 *SYS: The Pearson's correlation coefficient (r) between the following variables: sex, age (months), oral contraceptive pill (OCP) use, prenatal exposure to maternal cigarette smoking (PEMCS) and relative CC volumes (%; total and segmental). These variables are employed in the statistical model used to analyze the association of sex and age with relative CC volumes. The results of this analysis do not correct for puberty, and the association of puberty with the corpus callosum has been assessed in Chapter 6 of this thesis. * refers to p<0.05 and ** refers to p<0.001.*

4A.3.2 Relative Volume of the Total Corpus Callosum: Association with Age and Sex

There is a significant positive correlation between sex and the relative volume of the total corpus callosum (β=0.304, p=0.001). This correlation demonstrated that the relative volume of the total corpus callosum is significantly greater in girls (Mean ±SD: 0.254 ±0.029) as compared with boys (Mean ±SD: 0.236 ±0.028). There is also a significant positive correlation between age and the relative volume of the total corpus callosum (β=0.195, p=0.001). Together, sex and age account for 11% of the total variance in the relative volume of the total corpus callosum. For details see Table 4A.2.1 and 4A.2.2.

Table 4A.2.1 SYS: Results for Association of Sex and Age with Relative CC Volumes (Linear Regression Analysis with inter-Segmental Dependence Corrected)			
Relative CC Volume (%)	**Variance**	**Sex**	**Age**
Total	***ΔR^2=0.112, df=2,699, ΔF=45.0***	***β=0.304, p=0.001, t=8.25***	***β=0.195, p=0.001, t=5.23***
Anterior	***ΔR^2=0.003, df=2,684, ΔF=2.1***	***β=0.064, p=0.045, t=2.01***	*β=-0.003, p=0.921, t=-0.10*
MidAnterior	***ΔR^2=0.005, df=2,684, ΔF=4.1***	***β=-0.056, p=0.047, t=-1.99***	*β=0.049, p=0.076, t=1.78*
Central	***ΔR^2=0.016, df=2,684, ΔF=11.3***	***β=0.125, p=0.001, t=4.36***	*β=-0.037, p=0.192, t=-1.31*
MidPosterior	*ΔR^2=0.003, df=2,684, ΔF=2.5*	*β=0.049, p=0.094, t=1.68*	*β=0.048, p=0.094, t=1.68*
Posterior	***ΔR^2=0.012, df=2,684, ΔF=7.6***	***β=0.084, p=0.008, t=2.67***	***β=0.095, p=0.002, t=3.12***

Table 4A.2.1 *SYS: The results for association of sex and age (months) with relative volumes (%) of the total CC and its segments. These results are obtained using linear regression models (that correct for inter-segmental dependence). For each linear regression model the adjusted R^2 (ΔR^2; percentage variance in CC volumes explained by sex and age together), standardized regression coefficient (β; representing correlation), and results of a two-tailed T-test for significance of the regression coefficient (t: t value and p: level of significance) is quoted.*

Table 4A.2.2 SYS: Effect Sizes For Association of Sex and Age with CC Volumes (Linear Regression Analysis with inter-Segmental Dependence Corrected)				
Relative CC Volume (%)	**Sex**		**Age**	
	Cohen's d	**Effect Size r**	**Cohen's d**	**Effect Size r**
Total	*0.624*	*0.298*	*0.396*	*0.194*
Anterior	*0.154*	*0.077*	*0.008*	*0.004*
MidAnterior	*-0.152*	*0.076*	*0.136*	*0.068*
Central	*0.333*	*0.163*	*-0.100*	*0.050*
MidPosterior	*0.128*	*0.064*	*0.128*	*0.064*
Posterior	*0.204*	*0.102*	*0.239*	*0.118*

Table 4A.2.2 *SYS: The effect sizes for association of sex and age (months) with relative volumes (%) of the total CC and its segments (shown in Table 4A.2.1). The values for both effect size (r) and Cohen's d are demonstrated.*

4A.3.3 Relative Volume of CC segments: Association with Age and sex

There is a significant positive correlation of sex with the relative volume of the anterior (β=0.064, p=0.045), central (β=0.125, p=0.001) and posterior (β=0.084, p=0.008) segments of the corpus callosum, demonstrating that these segments are significantly larger in girls as compared with boys. In contrast, there is a

significant negative correlation of sex with the relative volume of the mid-anterior segment (β=-0.056, p=0.047) of the corpus callosum; demonstrating that this segment is significantly larger in boys as compared with girls. The mid-posterior segment of the CC is the only segment that demonstrates no sex difference. With regards to the relation of age with CC segments, there is a significant positive correlation of age with only the relative volume of the posterior segment of the corpus callosum (β=0.095, p=0.002). Together, sex and age account for 0.3%, 0.5% 1.6% and 1.2% of the total variance in the relative volume of the anterior, mid-anterior, central and posterior segment of the corpus callosum, respectively. For details see Table 4A.2.1 and 4A.2.2.

It is important to note that the results of the correlation of age and sex with the relative volume of callosal segments vary considerably when the dependence of the segments on each other is not corrected for. Briefly, the results for uncontrolled segmental volumes are as follows: 1) sex is positively correlated with the relative volume of all segments of the corpus callosum; and 2) age is positively correlated with the relative volume of the anterior, central, mid-posterior and posterior segment of the corpus callosum. Highlighting the impact of controlling for inter-segmental dependence on the effects of age and sex on the CC is a major contribution of this thesis. The inter-dependency between the arbitrarily segmented divisions of the corpus callosum is evidenced by the correlations between their volumes. It is important to note that these inter-segmental correlations are similar in both males and females (see Table 6A.1.2 and 6A.1.3) without any significant differences (p>0.05). This inter-segmental

relatedness may be due to the fact that these segments share axons having similar topographic origins. These results suggest that inter-segmental correlation influences the effects of age and sex on the CC and should, therefore, be controlled for when examining these effects. For details see Table 4A.3.1 and 4A.3.2.

Table 4A.3.1 SYS: Results for Association of Sex and Age with Relative CC Volumes (Linear Regression Analysis with inter-Segmental Dependence Not Corrected)			
Relative CC Volume (%)	**Variance**	**Sex**	**Age**
Anterior	***ΔR^2=0.067, df=2,701, ΔF=25.2***	***β=0.210, p=0.001, t=4.84***	***β=0.120, p=0.001, t=2.34***
MidAnterior	***ΔR^2=0.055, df=2,696, ΔF=20.3***	***β=0.193, p=0.001, t=5.05***	*β=0.163, p=0.070, t=4.22*
Central	***ΔR^2=0.080, df=2,700, ΔF=30.9***	***β=0.287, p=0.001, t=7.65***	***β=0.086, p=0.024, t=2.26***
MidPosterior	***ΔR^2=0.094, df=2,701, ΔF=36.8***	***β=0.278, p=0.001, t=7.48***	***β=0.175, p=0.001, t=4.67***
Posterior	***ΔR^2=0.093, df=2,701, ΔF=36.4***	***β=0.250, p=0.001, t=6.71***	***β=0.215, p=0.001, t=5.71***

Table 4A.3.1 *SYS: The results for association of sex and age (months) with relative volumes (%) of the total CC and its segments. These results are obtained using linear regression models (that **do not** correct for inter-segmental dependence). For each linear regression model the adjusted R^2 (ΔR^2; percentage variance in CC volumes explained by sex and age together), standardized regression coefficient (β; representing correlation), and results of a two-tailed T-test for significance of the regression coefficient (t: t value and p: level of significance) is quoted.*

Table 4A.3.2 SYS: Effect Sizes for Association of Sex and Age with CC Volumes (Linear Regression Analysis with inter-Segmental Dependence Not Corrected)				
Relative CC Volume (%)	**Sex**		**Age**	
	Cohen's d	**Effect Size r**	**Cohen's d**	**Effect Size r**
Anterior	*0.366*	*0.180*	*0.177*	*0.088*
MidAnterior	*0.383*	*0.188*	*0.320*	*0.158*
Central	*0.578*	*0.278*	*0.171*	*0.085*
MidPosterior	*0.565*	*0.272*	*0.353*	*0.174*
Posterior	*0.507*	*0.246*	*0.431*	*0.211*

Table 4A.3.2 *SYS: The effect sizes for association of sex and age (months) with relative volumes (%) of the total CC and its segments (shown in Table 4A.3.1). The values for both effect size (r) and Cohen's d are demonstrated.*

4B.3 RESULTS: IMAGEN STUDY

4B.3.1 Descriptive Statistics

There are a total of 964 boys with mean age of 172 (SD: ±15) months and mean puberty stage of 3.29 (±0.68). There are a total of 1,015 girls with mean age of 173 (±12) months and mean puberty stage of 3.99 (±0.46). Of the total 381 girls, 54 girls are using oral contraceptives. Descriptive statistics of the demographics and mean relative volumes of the total CC and its segments for both sexes are shown in Table 4B.1.1 and Figure 4B.1.1 and 4B.1.2.

Pearson's correlation between the variables employed in the linear regression analysis to estimate the association of age and sex with the relative volumes of the corpus callosum and its segments is provided in Table 4B.1.2.

Table 4B.1.1 IMAGEN: Sample Demographics and Descriptive Statistics for CC Volume

	Boys		Girls	
	n	**Mean (SD)**	**n**	**Mean (SD)**
Age (months)	*964*	*172 (15)*	*1015*	*173 (12)*
Puberty Stage	*955*	*3.29 (0.68)*	*1005*	*3.99 (0.46)*
OCP using Girls	-	-	*54*	-
Relative CC Volume (%)				
Total	*896*	*0.255 (0.034)*	*970*	*0.267 (0.035)*
Anterior	***894***	***0.070 (0.010)***	***972***	***0.074 (0.011)***
MidAnterior	***886***	***0.038 (0.007)***	***967***	***0.039 (0.007)***
Central	***895***	***0.039 (0.009)***	***972***	***0.041 (0.009)***
MidPosterior	*896*	*0.038 (0.007)*	*971*	*0.039 (0.007)*
Posterior	***897***	***0.070 (0.011)***	***971***	***0.074 (0.011)***

Table 4B.1.1 *IMAGEN: The sample size (n) along with the mean and SD (in brackets) for the following variables in both boys and girls: age (months), puberty, PEMCS and relative volume (%) of the total CC and its five segments (anterior, mid-anterior, central, mid-posterior and posterior).*

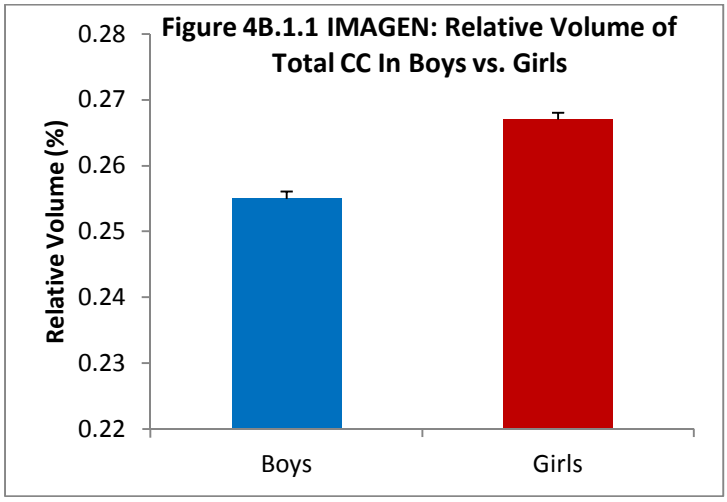

Figure 4B.1.1 *IMAGEN: Bar chart for the relative volume (%) of the total corpus callosum in boys versus girls. Boys are shown in blue and girls in red. Error bars represent standard error of mean.*

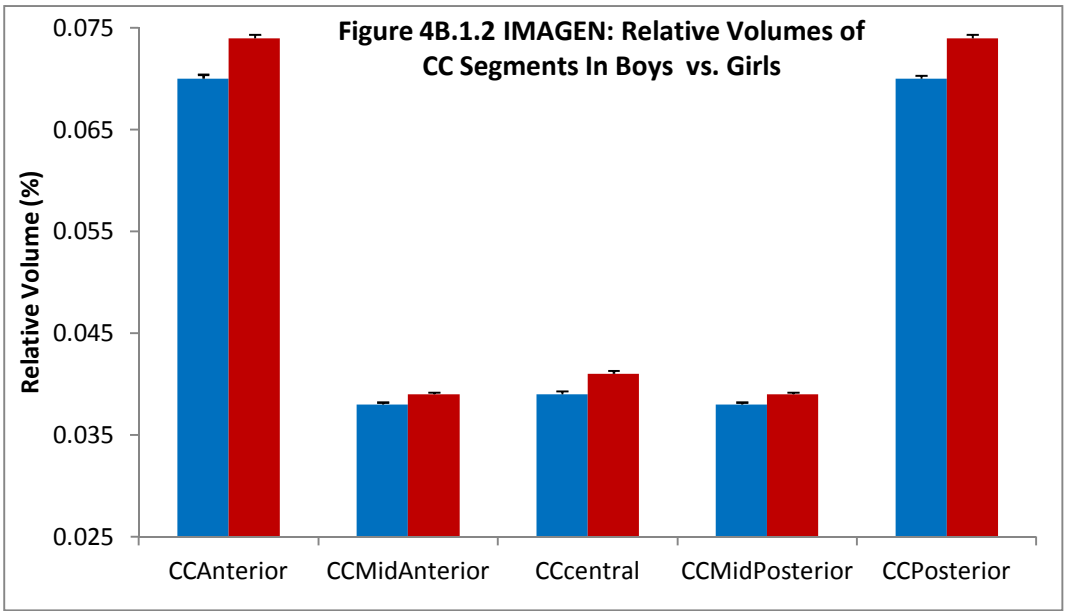

Figure 4B.1.2 *IMAGEN: Bar chart for the relative volumes (%) of CC segments in boys versus girls. Boys are shown in blue and girls in red. Error bars represent standard error of mean. It is important to note that after removal of regional interdependencies the mid-anterior segment becomes larger in males versus females and the sex difference in the mid-posterior segment becomes non significant (see Table 4B.2.1).*

Table 4B.1.2 IMAGEN: Correlation Matrix (Pearson's Correlation Coefficient)							
	Sex	**Age**	**OCP use**	**CC Anterior**	**CC Mid Anterior**	**CC Central**	**CC Mid Posterior**
Sex							
Age	*0.006*						
OCP Use	*0.158***	*0.109***					
CC Volume							
Total	*0.184***	*0.040*	*0.028*				
Anterior	*0.169***	*0.012*	*0.040**				
MidAnterior	*0.055**	*0.012*	*-0.044**	*0.462***			
Central	*0.133***	*0.005*	*-0.017*	*0.326***	*0.661***		
MidPosterior	*0.112***	*0.039**	*0.009*	*0.458***	*0.530***	*0.523***	
Posterior	*0.196***	*0.052**	*0.049**	*0.536***	*0.446***	*0.308***	*0.555***

Table 4B.1.2 *IMAGEN: The Pearson's correlation coefficient (r) between the following variables: sex, age (months), OCP use and relative CC volumes (%; total and segmental). These variables are employed in the statistical model used to analyze the association of sex and age with relative CC volumes. The results of this analysis do not correct for puberty and the association of puberty with the corpus callosum has been assessed in Chapter 6 of this thesis. * refers to p<0.05; ** refers to p<0.001;*

4B.3.2 Relative Volume of the Total Corpus Callosum: Association with Age and Sex

There is a significant positive correlation between sex and the relative volume of the total corpus callosum (β=0.196, p=0.001). This correlation demonstrates that the relative volume of the total corpus callosum is significantly larger in girls (Mean ±SD: 0.267 ±0.035) as compared with boys (Mean ±SD: 0.255 ±0.034). There is no significant correlation between age and the relative volume of the total corpus callosum, although a trend is observed (p=0.09). Together, sex and age account for 4% of the total variance in the relative volume of the total corpus callosum. For details see Table 4B.2.1 and 4B.2.2.

Table 4B.2.1 IMAGEN: Results for Association of Sex and Age with Relative CC Volumes (Linear Regression Analysis with inter-Segmental Dependence Corrected)			
Relative CC Volume (%)	**Variance**	**Sex**	**Age**
Total	***ΔR^2=0.039, df=2,1849, ΔF=40.2***	***β=0.196, p=0.001, t=8.81***	*β=0.038, p=0.091, t=1.69*
Anterior	***ΔR^2=0.007, df=2,1814, ΔF=9.9***	***β=0.080, p=0.001, t=4.15***	*β=-0.029, p=0.130, t=-1.51*
MidAnterior	***ΔR^2=0.005, df=2,1814, ΔF=10.1***	***β=-0.073, p=0.001, t=-4.46***	*β=-0.007, p=0.644, t=-0.46*
Central	***ΔR^2=0.010, df=2,1814, ΔF=17.4***	***β=0.101, p=0.001, t=5.88***	*β=-0.007, p=0.695, t=-0.39*
MidPosterior	*ΔR^2=0.001, df=2,1814, ΔF=0.4*	*β=-0.015, p=0.393, t=-0.85*	*β=0.006, p=0.720, t=0.36*
Posterior	***ΔR^2=0.009, df=2,1814, ΔF=15.5***	***β=0.093, p=0.001, t=5.12***	***β=0.040, p=0.028, t=2.20***

Table 4B.2.1 *IMAGEN: The results for association of sex and age (months) with relative volumes (%) of the total CC and its segments. These results are obtained using linear regression models (that correct for inter-segmental dependence). For each linear regression model the adjusted R^2 (ΔR^2; change in variance explained by sex and age), standardized regression coefficient (β; correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

Table 4B.2.2 IMAGEN: Effect Sizes for Association of Sex and Age with CC Volumes (Linear Regression Analysis with inter-Segmental Dependence Corrected)				
Relative CC Volume (%)	**Sex**		**Age**	
	Cohen's d	**Effect Size (r)**	**Cohen's d**	**Effect Size (r)**
Total	*0.409*	*0.201*	*0.079*	*0.039*
Anterior	*0.195*	*0.097*	*-0.071*	*0.035*
MidAnterior	*-0.209*	*0.104*	*-0.022*	*0.011*
Central	*0.276*	*0.137*	*-0.018*	*0.009*
MidPosterior	*0.040*	*0.020*	*0.016*	*0.008*
Posterior	*0.240*	*0.119*	*0.103*	*0.051*

Table 4B.2.2 *IMAGEN: The effect sizes for association of sex and age (months) with relative volumes (%) of the total CC and its segments (shown in Table 4B.2.1). The values for both effect size (r) and Cohen's d are demonstrated.*

4B.3.3 Relative Volume of CC segments: Association with Age and sex

There is a significant positive correlation of sex with the relative volume of the anterior (β=0.080, p=0.001), central (β=0.101, p=0.001) and posterior segment (β=0.093, p=0.001) of the corpus callosum. These correlations demonstrate that the relative volumes of the anterior, central and posterior segments of the corpus callosum are significantly larger in girls as compared with boys. In contrast, there is a significant negative correlation of sex with the relative volume of the mid-anterior segment (β=-0.073, p=0.001) of the corpus callosum. This correlation demonstrates that relative volume of the mid-anterior segment of the CC is significantly larger in boys as compared with girls. The mid-posterior segment of the corpus callosum is the only segment not associated with sex. With regards to the relation of age with CC segments, there is a significant positive correlation of age with only the relative volume of the posterior segment of the corpus callosum (β=0.040, p=0.028). Together, sex and age account for 0.7%, 0.5%, 1.0% and 0.9% of the total variance in the relative volume of the anterior, mid-anterior, central and posterior segments of the corpus callosum, respectively. For details see Table 4B.2.1.

As in the case of the SYS study, the results of the correlation of age and sex with the relative volume of CC segments, not controlled for inter-segmental dependence, are different: 1) sex is positively correlated with the relative volume of all segments of the corpus callosum; and 2) age is positively correlated with the relative volume of the mid-posterior segment, only. This further validates that controlling for inter-segmental dependence influences the association of age

and sex with the relative CC volumes. For details see Table 4B.3.1 and 4B.3.2. It is important to note that these inter-segmental correlations are similar in both males and females, except for the anterior-midanterior and anterior-central segment correlations which are significantly ($p<0.05$) higher in girls as compared with boys (for details see Appendix B). As mentioned previously, this inter-segmental relatedness may be due to the fact that these segments share axons having similar topographic origins.

Table 4B.3.1 IMAGEN: Results for Association of Sex and Age with Relative CC Volumes (Linear Regression Analysis with inter-Segmental Dependence Not Corrected)

Relative CC Volume (%)	Variance	Sex	Age
Anterior	***ΔR^2=0.032, df=2,1850, ΔF=32.3***	***β=0.182, p=0.001, t=8.02***	*β=0.010, p=0.661, t=0.44*
MidAnterior	***ΔR^2=0.007, df=2,1850, ΔF=7.2***	***β=0.081, p=0.001, t=3.59***	*β=0.028, p=0.223, t=1.22*
Central	***ΔR^2=0.021, df=2,1850, ΔF=21.7***	***β=0.146, p=0.001, t=6.56***	*β=0.014, p=0.527, t=0.63*
MidPosterior	***ΔR^2=0.017, df=2,1850, ΔF=16.7***	***β=0.127, p=0.001, t=5.59***	*β=0.034, p=0.141, t=1.47*
Posterior	***ΔR^2=0.040, df=2,1851, ΔF=39.2***	***β=0.195, p=0.001, t=8.46***	***β=0.061, p=0.009, t=2.62***

Table 4B.3.1 *IMAGEN: The results for association of sex and age (months) with relative volumes (%) of the total CC and its segments. These results are obtained using linear regression models (that **do not** correct for inter-segmental dependence). For each linear regression model the adjusted R^2 (ΔR^2; change in variance explained by sex and age), standardized regression coefficient (β; correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

Table 4B.3.2 IMAGEN: Effect Sizes for Association of Sex and Age with CC Volumes (Linear Regression Analysis with inter-Segmental Dependence Not Corrected)

Relative CC Volume (%)	Sex		Age	
	Cohen's d	Effect Size (r)	Cohen's d	Effect Size (r)
Anterior	*0.373*	*0.183*	*0.020*	*0.010*
MidAnterior	*0.167*	*0.083*	*0.057*	*0.028*
Central	*0.305*	*0.151*	*0.029*	*0.015*
MidPosterior	*0.260*	*0.129*	*0.068*	*0.034*
Posterior	*0.393*	*0.193*	*0.122*	*0.061*

Table 4B.3.2 *IMAGEN: The effect sizes for association of sex and age (months) with relative volumes (%) of the total CC and its segments (shown in Table 4B.3.1). The values for both effect size (r) and Cohen's d are demonstrated.*

4.4 DISCUSSION

The results based on the analysis of both the SYS and IMAGEN studies demonstrate that the relative volume of the corpus callosum is sexually dimorphic during adolescence. This finding is an important addition to the existing literature on sexual dimorphism in the volume of the corpus callosum. To date, researchers have examined relative volume of the corpus callosum in samples consisting of both children and adolescents and observed inconsistent results (Giedd et al. 1999, Lenroot et al. 2007, Raunch et al. 1994). The findings of the existing literature on sexual dimorphism in relative CC volume are, therefore, inconclusive. The SYS and IMAGEN are the largest known studies to date that demonstrate a significantly greater relative volume of the CC in girls as compared with boys; with the difference lying specifically in the anterior, central and posterior segment of the CC. Moreover, these sex differences are investigated and demonstrated specifically during adolescence, rather than childhood and adolescence as shown previously (Giedd et al. 1999, Lenroot et al. 2007).The consistent nature of the results of both the SYS and IMAGEN study provide valid and strong evidence of sexual dimorphism, albeit it is quite subtle, in the relative volume of the corpus callosum, during adolescence.

The relative volume of the total corpus callosum is significantly greater in girls as compared with boys, in both the SYS and IMAGEN study. The consistency of this finding across both samples validates the results and confirms the hypothesis that the volume of the corpus callosum displays sexual dimorphism during adolescence. Previous studies that examined sex differences in the relative area

of the corpus callosum of children and adolescents had mixed results. Some studies demonstrated no sex difference in the relative area of the corpus callosum (Raunch and Jinkins 1994), whereas some demonstrated a trend ($p=0.07$) for sexually dimorphic CC (Giedd et al. 1999). A consensus on sexual dimorphism of CC during adolescence could not be formed based on these studies (Giedd et al. 1999, Lenroot et al. 2007, Raunch and Jinkins 1994). Most adult studies, however, observed a female versus male advantage (Johnson et al. 1994, Bermudez and Zatorre 2000) in the relative volume of the total corpus callosum. This suggested that as individuals mature from childhood towards adulthood, the sexual dimorphism in their corpus callosum also increases. By combining children and adolescents together in one sample, it is difficult to elucidate the callosal sex differences that are specific to adolescence (Giedd et al. 1999, Lenroot et al. 2007, Raunch and Jinkins 1994). The SYS and IMAGEN studies examine sex differences in the volume of the corpus callosum in a large number of participants assessed only during adolescence, thus ensuring that their results reliably predict sexual dimorphism during this specific stage of life. Moreover both the studies are conducted using statistical models that control for inter-segmental dependence, not co-varied by most previous studies. Also, FreeSurfer is used to measure the multi-slice mid-sagittal volume of the corpus callosum in contrast to the single-slice mid-sagittal area done by previous studies (Giedd et al. 1999, Lenroot et al. 2007, Raunch and Jinkins 1994). This ensures that a 3D estimate of callosal size is normalized with the 3D volume of the total brain rather than a 2D estimate, such as area used by previous studies. Thus, in comparison with previous reports, these two studies provide more reliable

evidence of a female versus male advantage in the relative volume of the corpus callosum, during adolescence.

The sex difference observed in the relative volume of the total corpus callosum in the SYS study (F>M by 7.5%) is slightly greater than that observed in the IMAGEN study (F>M by 5%). This may be partly due to differences in the age of SYS and IMAGEN adolescents. The SYS adolescents (180 ±21 months) are on average eight months older than the IMAGEN adolescents (172 ±13 months); across duration of eight months the relative volume of the corpus callosum increases by 1%. Previous studies also report that sex differences in CC size are more robust in older as compared with younger individuals (refer to Section 1.6), thus the SYS adolescents being older have more sexually dimorphic CC as compared with the IMAGEN adolescents.

The female versus male advantage in the relative volume of the corpus callosum is mainly observed in the anterior, central and posterior segments of the corpus callosum, whereas the mid-anterior segment is seen to be greater in boys as compared with girls. No sex difference is observed in the relative volume of the mid-posterior segment. These findings are consistent across both SYS and IMAGEN sample, thus authenticating their significance. They also concur with previous accounts of sex differences in the relative area of callosal segments. The results of studies investigating sex differences in CC segments though mixed, in general do provide evidence for a female advantage. Giedd et al. (1999) observed a trend (p=0.07 to 0.10) for greater relative area of the genu, posterior midbody and isthmus in girls as compared with boys. Bermudez and Zatorre

(2000) and DeLacoste Utamsing et al. (1982) observed that the relative area of all segments of the corpus callosum were significantly greater in women as compared with men. Some studies, however, observed sex difference in the relative area of only one segment of the corpus callosum (Witelson et al. 1989 in the isthmus) or none at all (Allen et al. 2002, Constant and Ruther 1996). The SYS and IMAGEN studies by means of their large sample size, reliable FreeSurfer based volume measurement techniques, and well controlled statistical analysis provide reliable evidence to support a female versus male advantage in the relative volume of the total corpus callosum and its anterior, central and posterior segment, during adolescence. Additionally, these studies demonstrate for the first time a male versus female advantage in the relative volume of the mid-anterior segment of the corpus callosum. No callosal segment has previously been reported to have greater relative volume in boys as compared with girls, during adolescence. The main reason for this novel and significant finding can be attributed to the correction for inter-segmental dependence. The sex differences observed in the uncontrolled segmental volumes do not demonstrate any male versus female advantage. Thus, highlighting the importance of covarying for other segmental volumes when examining the association of sex with the relative volume of a given callosal segment.

Knowledge of the specific segments of the corpus callosum that are sexually dimorphic is important because different callosal segments connect different cortical areas based on a topographic representation of cortical areas across the length of the corpus callosum (Pandya and Seltzer 1971, De LaCoste et al. 1985,

Hofer e al. 2006, Zarei et al. 2006). Therefore, possible functional correlates of having a larger CC segment may vary depending on the specific cortical areas connected by that specific segment. The anterior, central and posterior segments are observed to be larger in girls as compared with boys. The anterior segment of the corpus callosum carries fibres between the prefrontal and premotor areas of the left and right hemisphere (Pandya and Seltzer 1971, De LaCoste et al. 1985, Hofer e al. 2006, Zarei et al. 2006). The rostral half of the central segment of the corpus callosum carries fibres between the left and right primary and supplementary motor areas, whereas the caudal half of the central segment is responsible for inter-hemispheric connection between the primary and secondary somatosensory areas (Pandya and Seltzer 1971, De LaCoste et al. 1985, Hofer e al. 2006, Zarei et al. 2006). The posterior segment of the corpus callosum is responsible for carrying fibres between the temporo-parietal association areas rostrally and the visual association areas caudally (Pandya and Seltzer 1971, De LaCoste et al. 1985, Hofer et al. 2006, Zarei et al. 2006). Callosal segment connecting the frontal areas, the sensori-motor areas and the visual association areas are, therefore, larger in women as compared with men. The microstructural factors responsible for this sex difference may be variations in the number and/or diameter of axons or degree of myelination of the callosal fibres in girls as compared with boys. At this point it can be conjectured that on account of sex differences observed in callosal segments, there may be sex differences in cortical functions subserved by the corpus callosum.

The relative volume of the total corpus callosum increases gradually with age, particularly in the posterior most segment of the CC, during adolescence. The SYS study demonstrates significant age-associated increases in the total volume of the corpus callosum that are mainly limited to its posterior segment. The IMAGEN study demonstrates a trend ($p=0.09$) for age-associated increase in the relative volume of the total corpus callosum, but the age-associated increase in the relative volume of the posterior CC segment in this study, like the SYS study, is significant. Based on these findings it is concluded that the posterior segment of the corpus callosum demonstrates small but significant age-associated increases, during adolescence. This is consistent with the report of Giedd et al. (1999) that examined the influence of age on the relative volume of CC segments via a longitudinal study design. They too demonstrated that growth was most robust and significant in the posterior most region of the corpus callosum, during adolescence ($n=139$, 5 to 18 years). It is important to note that controlling for the dependence of segments on each other, has a significant influence on the results of the association between age and callosal segments. Since results for the uncontrolled segmental volumes demonstrate a positive correlation of age with the relative volume of all segments, except the mid-anterior segment.

The posterior extreme of the corpus callosum carries a high concentration of large diameter fibres that connect visual association cortex of the left and right hemisphere (La Mantia and Rakic 1990, Aboitiz et al. 1992). Continued growth of the posterior segment of the corpus callosum during adolescence may, therefore, reflect pronounced development of inter-hemispheric connections

between regions important for various visuo-spatial abilities, during this period. This development may be brought about by microstructural changes such as increases in axonal calibre and/or myelination of the callosal axons.

Together, sex and age account for 11% of the variance in the relative volume of the total corpus callosum in the SYS study and 4% of the variance in the IMAGEN study. The reason for this difference in the variance may be because both age and sex are associated with the total CC in the SYS study but only sex demonstrates a significant association with the CC in the IMAGEN study.

4A.5 CONCLUSION

This SYS and IMAGEN study examines the association of age and sex with the volume of the corpus callosum during adolescence. These studies have various improvements over the numerous studies to have previously explored this topic. Their sample size is significantly larger and encompasses only adolescents not (pre-pubertal) children. They estimate the multi-slice mid-sagittal volume of the corpus callosum that is a 3D measure of callosal size and more appropriately correlated with the 3D volume of the brain; whereas previous studies normalized 2D CC area with 3D brain volume. Statistical methods used control for the dependence of segments on each other. In addition to these advantages, the results of both the SYS and IMAGEN study are consistent, thus validating them. Based on these results it may be concluded that:

- The relative (brain-corrected) volume of the total corpus callosum is larger in girls as compared with boys, during adolescence (Cohen's d=0.37-0.62). This female versus male advantage in relative volume mainly lies in the anterior (Cohen's d=0.15-0.18), central (Cohen's d=0.26-0.33) and posterior (Cohen's d=0.20-0.27) segment of the corpus callosum. The relative volume of the mid-anterior segment (Cohen's d=0.15-0.22) of the corpus callosum, however, is slightly larger in boys versus girls.
- The relative (brain-corrected) volume of the total corpus callosum continues to grow during adolescence (Cohen's d=0.40). This growth is most robust and significant in the posterior segment of the corpus callosum (Cohen's d=0.24).

5 ASSOCIATION OF THE CORPUS CALLOSUM WITH INTELLIGENCE (IQ) AND BIMANUAL MOTOR COORDINATION IN THE SAGUENAY YOUTH STUDY

5.1 INTRODUCTION

The corpus callosum is white-matter body composed of nerve fibres connecting different cortical areas. Over the past years considerable research has been done to identify the cortical functions supported by these underlying callosal connections contribute towards. There is evidence to suggest that the corpus callosum is associated with multiple cognitive abilities, including verbal comprehension, verbal fluency, processing speed, abstract reasoning and problem solving; and with multiple motor abilities relating to finger dexterity and bimanual coordination (Hines et al. 1992, Sauerwein et al. 1994, Kennedy et al. 2000, Luders et al. 2007, Paul et al. 2007, Hutchinson et al. 2009, Voineskos et al. 2010, Sullivan et al. 2006, Johansen-Berg et al. 2007, Meutzel et al. 2008, Moes et al. 2008, Mueller et al. 2009).

Classically researchers studied the function of the corpus callosum by examining the functional deficits that are present in individuals lacking the corpus callosum due to agenesis or transection. Chiarello et al. (1980) and Sauerwein et al. (1993) have published many case reports of cognitive deficits that arise in acallosal individuals (both agenesis and transection). They demonstrated that most acallosal individuals function at the lower end of the normal range of general

intelligence (IQ). A small proportion, however, could be found that falls in the high average IQ range. Moreover, majority of the cases (69%) did not have any significant difference in their verbal and performance IQ scores, suggesting that both VIQ and PIQ were equally compromised. Sauerwein et al. (1994) conducted a study to compare the IQ and motor skills of nine agenesis individuals (10 to 24 years old) with two age- and sex-matched, control groups; one group with healthy individuals and one with individuals having low IQ. They observed that agenesis individuals performed significantly poorly in all Wechsler's subscales, Purdue pegboard and simple finger tapping tasks as compared with healthy individuals. In comparison with individuals having low IQ, however, agenesis individuals performed significantly poorly only in the similarities subset of Wechsler Intelligence Scales (WAIS-R, Wechsler D. 1981 and WISC-R, Wechsler D. 1974) and Purdue pegboard. Thus, suggesting that verbal abstract reasoning skills (based on similarities test) and fine motor performance (based on Purdue pegboard) were the main cognitive and motor skills associated with the corpus callosum. Moes et al. (2008) collected information on 720 children (aged 5 to 15 years) with agenesis of the corpus callosum and 219 healthy siblings via caregiver-reported questionnaires. They demonstrated that children (aged 5 to 15 years) with agenesis of the corpus callosum performed significantly poorly in everyday tasks requiring bimanual coordination such as biking, brushing, bathing and dressing, as compared with their healthy siblings. This is one of the largest known studies examining motor skills in children with agenesis of the CC (720 agenesis children and 219 healthy siblings). One of the limitations of this study, however, was that bimanual coordination skills were assessed via caregiver

reports and not corroborated by direct testing. This method of measuring performance is subject to possible bias and omission of facts. Mueller et al. (2009) examined bimanual motor coordination of 13 agenesis individuals on the computerized version of etch-a-sketch task. They observed that agenesis individuals as compared with healthy individuals having intact CC are less accurate and slower. Additionally, bimanual coordination was also seen to be compromised in individuals with transection of the corpus callosum (Callie et al. 2005), in particular section of the anterior portion of the genu caused a deficit in motor coordination and middle portion caused a deficit in motor planning.

Currently, modern MR imaging techniques are used to investigate the functional correlates of the size (MRI estimated volume, thickness and length) and microstructural properties (DTI estimated FA and MD value) of the corpus callosum in healthy individuals. The results of MRI studies examining the correlation between IQ and CC size were inconsistent. Haier et al. (2004) used voxel based morphometry to identify the brain regions where grey-matter and white-matter volumes were correlated with IQ that is measured using Wechsler Adult Intelligence Scale (WAIS-R; Wechsler D. 1981), in a sample of 23 adults (range: 18 to 37 years, mean ±SD: 27 ±6 years). They observed no association between the white matter of the CC and IQ. Ganjavi et al. (2011) observed a negative correlation between IQ measured on the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler D. 1999) and relative area of the total CC in a large cohort (n=198) of healthy children and adolescents (6 to 18 years). Post-hoc analysis revealed that this correlation was mainly between total CC area and

performance IQ of male children (<12 years), whereas adolescents (≥12 years) demonstrated no correlation between CC and IQ. Hutchinson et al. (2009) observed a negative correlation between IQ (measured via WASI; Wechsler D. 1999) and relative area of the posterior half of the CC, in a sample of 71 adolescents and young adults (14 to 25 years; 19 ±3 yrs). Detailed analysis revealed that this correlation was mainly between relative area of the midbody and performance IQ. In contrast to Ganjavi et al. (2011) and Hutchinson et al. (2009), some researchers examined the relationship of IQ with measures of CC size other than relative area. Allin et al. (2007), for instance, examined the correlation of IQ (measured via WASI; Wechsler D. 1999) with absolute CC area in a small sample (n=34) of healthy adolescents (15 ±1 years) and demonstrated a negative correlation between the absolute area of the mid-posterior segment of the CC and verbal IQ. Luders et al. (2007) demonstrated a positive correlation between IQ (WAIS-R; Wechsler D. 1981) and thickness (width) of the anterior third and body of the CC in a sample of 62 healthy participants (aged 28 ±7 years).

Diffusion tensor imaging studies demonstrate a significant correlation between cognitive abilities and microstructural properties of the corpus callosum (Voineskos et al. 2010, Kennedy et al. 2000). Voineskos et al. (2010) investigated the correlation of the FA and MD value of the corpus callosum with the following domains of cognition: memory, executive functioning, motor speed, set shifting/flexibility and visuospatial construction in 64 healthy elderly volunteers (aged 49 ±17 yrs). They observed that the FA value in the posterior fifth of the CC

broadly predicted working memory and executive function only. Similarly, Kennedy et al. (2000) investigated the correlation of the FA and MD value of the corpus callosum with processing speed, working memory and executive functioning in 55 healthy elderly volunteers (aged 54 ±19 yrs). They observed a positive correlation between FA of the genu and both working memory and executive function and a negative correlation between MD of the genu and processing speed. Bimanual motor coordination has also been shown to be positively associated with the FA and MD value of the corpus callosum of both adolescents (n=92, 9-to-24 years; Muetzel et al. 2008) and adults (Sullivan et al. 2006, Johansen-Berg et al. 2007). The sub-regions of the corpus callosum that demonstrate this relation include the splenium (n=49, 44 ±15 years; Sullivan et al. 2006) and the midbody (n=10, 21 to 45 years; Johansen-Berg et al. 2007). Additionally, fMRI studies demonstrate that upon performing bimanual coordination tasks BOLD responses are activated within the body of the corpus callosum (Fabri et al. 2011).

The above-mentioned agenesis and MR studies give clear evidence of a positive (excitatory) role of the CC in bimanual coordination; since performance on bimanual coordination tasks that require interhemispheric processing is better in individuals with a CC versus those without it (Mueller et al. 2009, Moes et al. 2008) and improves with better callosal integrity, that is, callosal FA value (Muetzel et al. 2008, Johansen-berg et al. 2007). The role of the CC in cognitive functioning, however, is less clear. Agenesis studies demonstrate a positive role of the CC on IQ, but MR studies give discordant results for the relationship

between the size of the CC and IQ (negative correlation: Hutchinson et al. 2009; positive correlation: Luders et al. 2007; no correlation: Ganjavi et al. 2011). In order to better understand the role of the CC in cognitive functions we may consider the role of the CC in interhemispheric processing of lateralized language tasks that involve higher order processing. Clarke and Zaidel (1993) investigated the association between the mid-sagittal area of the CC and performance on a lexical decision task with associative priming in 60 healthy adults (30 males and 30 females, 21 to 43 years). In this task the participants were exposed to a priming word (concrete noun) in one visual field and then a target word (related noun or unrelated non-word) in the same or opposite visual field and were asked to respond only if the target word is an actual word. They observed that performance on the *inter*hemispheric conditions of this task were unrelated to the CC area but the laterality index derived from the *intra*hemispheric conditions of this task was negatively correlated with the CC area of males. Thus, greater callosal connectivity reduced the left hemispheric advantage in the lexical decision task by allowing interhemispheric sharing of information. Previous findings from split-brain patients suggest that the left versus right hemisphere is superior in lexical decision making (Zaidel et al. 1994). This 'behavioural laterality-callosal morphometry' finding suggests that the CC plays a supportive (excitatory) role in the interhemispheric processing of high-order language based decision-making tasks with semantic features. Similarly, Yazgan et al. (1995) demonstrated that behavioural laterality based on speech perception and motor tasks (dichotic word listening, line bisection and turning bias tests) correlated inversely with the mid-sagittal area of the CC. These findings suggest that the

corpus callosum assumes an excitatory role when participants perform these tasks. It is important to note that relative to the tasks investigated by Yazgan et al. (1995) those investigated by Zaidel et al. (1994) involve higher order cognitive processing and are therefore more relevant to this study, which examines cognitive abiltites (IQ). Kompus and westerhausen et al. (2011) examined the association between functional asymmetry of episodic memory and inter-hemispheric connectivity based on CC size, in 37 healthy adult participants. Episodic memory was assessed based on performance on an encoding and retrieval task (face-name paired associates' task; Persson et al. 2011). This encoding relative to retrieval task is lateralized to the left frontal cortex. The degree of encoding-retrieval asymmetry in the frontal cortex (ventrolateral prefrontal region) was associated with the anterior CC size. This relationship was due to increasing recruitment of the non-dominant right venterolateral prefrontal cortex in individuals with larger anterior CC. Thus inter-hemispheric connectivity provided by the CC allows for greater recruitment of prefrontal areas during tasks of episodic memory.

This study aims to examine the association of the relative volume of the corpus callosum with: 1) four subscales of intelligence (IQ) estimated via Wechsler intelligence scale (WISC-III), namely verbal comprehension (VCIQ), working memory (WMIQ), perceptual reasoning (PRIQ) and processing speed (PSIQ); and 2) bimanual coordination assessed via performance on the Thurston finger tapping task. For the above-mentioned cognitive and motor functions, those that demonstrate a correlation with the total CC are further investigated to identify

the specific segments of the corpus callosum that are responsible for these structure-function relations. An alternate means of exploring this structure-function relationship would be to set a specific *a priori* hypothesis on regional basis. This method was not used in view of inter-indivudual variation in callosal topography (Fabri et al. 2011). Moreover, different studies have reported different segments to be associated with cognitive abilities (genu: Kennedy et al. 2000; splenium: Voineskos et al. 2010) and bimanual coordination (midbody: Johansen-Berg et al. 2007; splenium: Muetzel et al. 2008). These structure-function relations are examined in 737 12-to-18 year old adolescents from the Saguenay Youth study only and not the IMAGEN study because bimanual motor coordination is not assessed in the IMAGEN study and only few of the WISC tests have been conducted on adolescents from the IMAGEN sample. It is important to note that the choice of behavioural tasks investigated in this study is opportunistic given the fact that we are working with a large, existing dataset.

Previous MR studies examining the correlation between the size of the corpus callosum and bimanual coordination are lacking. Nevertheless, based on the deficits observed in bimanual coordination in individuals lacking the CC (Mueller et al. 2009, Moes et al. 2008) and the positive correlation between the FA value of the CC and bimanual coordination (Muetzel et al. 2008, Johansen-berg et al. 2007), it is hypothesized that the volume of the CC like its FA would be positively correlated with bimanual coordination. Similarly, overall review of agenesis, MR and laterality studies provides greater evidence in support of a positive (excitatory) versus negative (inhibitory) role of the CC in cognition. It is,

therefore, hypothesized that the volume of the CC would be positively correlated with measures of IQ, particularly performance IQ, which was seen to be correlated with the CC more often that verbal IQ.

There are multiple advantages of this study over the few existing MR studies on 'CC volume-IQ' relation. In addition to the large sample size and improved statistical models (that control for inter-segmental dependence) described previously, this study carries out a more detailed investigation of the specific areas of intelligence associated with the corpus callosum, as compared with previous studies; In particular, previous studies only examine the association of the CC with verbal IQ and performance IQ but this study does so with the individual subsets of verbal IQ (verbal comprehension, working memory) and performance IQ (Processing speed, perceptual reasoning). Moreover, this relation is investigated separately in boys and girls. This is particularly important since sex differences have been demonstrated in cognitive abilities. In general males are seen to be superior to females in mathematical abiltites (Halpern 2000, Lynn et al. 1994, Ankney et al. 1995) and mental rotation tasks (Halpern 2000, Kaufman et al. 2007). Females, on the other hand, are seen to be superior to males in verbal abilities including reading comprehension (Lynn and Malik 2009), fluency and memory (Kimura 1992, Ankney et al. 1995). In case of IQ scores on the Wechsler tests of intelligence (WAIS and WISC) the most robust and significant sex difference is observed in processing speed IQ (Cohen's *d*=0.3; Longman et al. 2007), particularly the coding task (Cohen's *d*=0.55; Jensen et al. 1983 and Lynn et al. 1991). Sex differences in the remaining IQ scores are subtle

with males having slightly greater verbal comprehension, working memory and perceptual reasoning scores (Longman et al. 2007), particularly in the vocabulary, information, comprehension, arithmetic, picture completion and block design tasks (Cohen's *d*=0.1 to 0.3; Jensen et al. 1983 and Lynn et al. 1991).

This thesis examines sexual dimorphism of the corpus callosum, during adolescence. So far it demonstrates that the relative volume of the corpus callosum is sexually dimorphic, during adolescence, with a female over male advantage. This study further explores sexual dimorphism in the CC by examining sex differences in the functional correlates of the corpus callosum.

5.2 METHODOLOGY

This study is conducted using data from 737 adolescent participants of the Saguenay Youth Study. Methodological details of the study design and participants, their recruitment and testing procedures have been provided in an earlier chapter (Chapter 2, Section 2A) of this thesis. Here the methodological approaches relevant to this study are briefly discussed.

5.2.1 Participants

This dataset consists of a total of 737 adolescents (356 boys and 381 girls) of French-Canadian origin belonging to the Saguenay Lac Saint-Jean (SLSJ) region of Quebec, Canada, with known founder effect (Moreau et al. 2011). All participants are aged between 12 to 18 years.

5.2.2 Wechsler IQ Battery

General intelligence is measured using the Wechsler Intelligence Scale for Children III. This neuropsychological battery consisted of 12 tests, conducted over a 70 to 80 minute period. These tests evaluate general cognitive ability over the following four domains of intelligence:

Verbal Comprehension: Verbal comprehension tests measure cognitive abilities of verbal comprehension, verbal reasoning and problem solving based on general knowledge and vocabulary. The total score for performance in the verbal comprehension tests is called verbal comprehension index (VCI).

Working Memory: Working memory tests measure cognitive abilities reasoning about, and solving, mathematical problems based on calculation skills and memory. The total score for performance in the working memory tests is called working memory index (WMI).

Perceptual Reasoning: Perceptual reasoning tests measure cognitive abilities of reasoning about and solving, abstract non-verbal problems involving designs and patterns based on logical thinking. The total score for performance in the perceptual reasoning tests is called perceptual reasoning index (PRI).

Processing Speed: Processing speed tests measure speed of solving abstract non-verbal problems involving designs and patterns based on logical thinking. The total score for performance in the perceptual reasoning tests is called processing speed index (PSI).

For more details on the tests included in each of these four domains, and their comparison with other neuropsychological tests of intelligence refer to Section 2A.9.

5.2.3 Thurston Bimanual Tapping Task

The Thurston finger tapping task was introduced by Thurston et al. in 1944. This task is a more complex form of the simple altered finger tapping task (Petellier et al. 1993). It requires accurate and fast performance of bimanual movements in a spatially ordered sequence.

Thurston finger tapping task is conducted on an apparatus specially designed for this task; it consists of two brass plates mounted on a wooden board, with a stylus next to each plate. Each plate is divided into four equal pie-shaped sections labelled 1, 2, 3 and 4, respectively. The styli are wired such that each time both the styli touched corresponding sections of the two plates an electric circuit was closed, activating a mechanical counter. The participant is asked to hold a stylus in each hand and tap corresponding sections of the two brass plates in ascending order as quickly as possible. The total number of taps made in a 30 second interval is recorded. Prior to the actual trial, a test trial is run to familiarize the participant with the task. Two trials are conducted and their mean calculated to more reliably assess bimanual coordination skills of the participant. This is recorded as the mean taps for bimanual performance on the Thurston tapping task. For more details on the Thurston tapping task and other tasks used to measure bimanual motor coordination refer to Section 2A.10.

5.2.4 MRI Acquisition

For each participant T1 weighted MRI scans are obtained on a Philips 1.0 T superconducting magnet. Acquisition parameters of these structural volume scans are as follows: 3D RF spoiled gradient-echo scan, 140-160 high resolution T1-weighted images, with TR=25 ms, TE=5 ms, flip angle=30^0 and 1 mm isotropic resolution.

5.2.5 MRI Volumetric Analysis

FreeSurfer version 5.0.0 is used for the automated volumetric analysis, performed on native, high resolution T1-weighted images. The FreeSurfer analysis pipeline has been described in detail in Chapter 4, Section 4A.2.3.

5.2.6 Statistical Analysis

All statistical analysis is performed using SPSS 18.0. Linear regression analysis is employed to assess the association of the relative volume of the total corpus callosum with: 1) VCIQ, WMIQ, PRIQ and PSIQ scores of WISC-III battery; and 2) mean bimanual taps performed on the Thurston finger tapping task. Separate analyses are conducted for boys and girls. The rationale for this sex-specific analysis is the presence of sex differences in cognitive functions and bimanual coordination (Ankney et al. 1994, Lynn et al. 1994, Kimura et al. 1994, Camarata et al. 2006, Wolff et al. 1976). The linear regression model is run with the performance measures (*i.e.* performance on Thurston finger tapping task and WISC IQ scores) specified as the dependent variables. There are two blocks of

independent variables. The first block controls for the following potential confounding factors: 1) prenatal exposure to maternal cigarette smoking (PEMCS); 2) adolescent age in months; and 3) use of oral contraceptive pill (OCP), in girls only. Relative volume of the total corpus callosum is specified as the independent variable in the second block. Subsequently, the cognitive (IQ) and motor (bimanual coordination) functions that demonstrate correlations with the total corpus callosum are further investigated to identify the specific segments of the corpus callosum that are responsible for these structure-function relations. This is done via a linear regression model similar to the one described above except that the relative volumes of CC segments (anterior, mid-anterior, central, mid-posterior and posterior) instead of the total CC are specified as the independent variables in the second block. This model controls for the inter-relatedness of the CC segments. For each linear regression model the change in adjusted R^2 (specifying variance), standardized regression coefficient (β), t value for the regression coefficient and the significance of the t value (p) are calculated.

5.3 RESULTS

5.3.1 Descriptive Statistics

There are a total of 356 boys with mean age of 180 (SD: ±22) months and mean puberty stage of 3.38 (±0.87). The percentage of boys who have no prenatal exposure to maternal cigarette smoking is 55. There are a total of 381 girls with mean age of 181 (±23) months and mean puberty stage of 4.08 (±0.74). The

percentage of girls who have no prenatal exposure to maternal cigarette smoking is 50. Of the total 381 girls, 56 were using oral contraceptives and the remaining 325 were not. Descriptive statistics of the demographics, mean IQ scores for WISC and mean bimanual taps on the Thurston tapping task are shown in Table 5.1.1.

Table 5.1.1 SYS: Sample Demographics and Descriptive Statistics for IQ and Bimanual Coordination				
	Boys		**Girls**	
	n	**Mean (SD)**	**n**	**Mean (SD)**
Age (months)	*355*	*180 (22)*	*379*	*181 (23)*
Puberty Stage	*342*	*3.38 (0.87)*	*377*	*4.08 (0.74)*
Girls Using OCP (n)	*-*	*-*	*56*	*-*
Percentage with no PEMCS (%)		*55*		*50*
VCIQ	*322*	*104.5 (12)*	*350*	*103.9 (11)*
WMIQ	*324*	*100.4 (15)*	*349*	*98.5 (12)*
PRIQ	*322*	*106.3 (13)*	*350*	*105.8 (13)*
PSIQ	*324*	*105.5 (15)*	*349*	*112.5 (13)*
Mean Bimanual Taps	*303*	*30.6 (11)*	*314*	*34.2 (11)*

Table 5.1.1 *SYS: The sample size (n) along with the mean and SD (in brackets) for the following variables in both boys and girls: age (months), puberty, PEMCS, WISC IQ tests (verbal comprehension, VCIQ; working memory, WMIQ; perceptual reasoning, PRIQ; processing speed, PSIQ) and number of bimanual taps (in Thurston tapping task).*

Pearson's correlations between the variables employed in the linear regression analyses to estimate the association of relative volumes of total corpus callosum with performance on WISC and Thurston tapping task, in boys and girls are provided in Table 5.1.2 and 5.1.3, respectively. In addition, the correlations between different segments of the corpus callosum with WISC-III IQ scores and bimanual mean taps on the Thurston tapping task are also included.

Table 5.1.2 SYS: Correlation Matrix for Boys (Pearson's Correlation Coefficient)								
	Age	**PEMCS**	**CC Anterior**	**CC Mid Anterior**	**CC Central**	**CC Mid Posterior**	**CC Posterior**	**CC Total**
PEMCS	*0.044*							
CC Volume								
Total	*0.216***	*0.014*						
Anterior	*0.130**	*-0.009*						
MidAnterior	*0.176***	*0.045*	*0.470***					
Central	*0.131**	*0.063*	*0.309***	*0.691***				
MidPosterior	*0.190***	*-0.028*	*0.419***	*0.572***	*0.598***			
Posterior	*0.255***	*-0.024*	*0.550***	*0.385***	*0.257***	*0.460***		
VCIQ	*-0.025*	*0.040*	*-0.076*	*-0.055*	*-0.033*	*-0.069*	*-0.024*	*-0.053*
WMIQ	*-0.016*	*0.123**	*-0.062*	*0.017*	*-0.013*	*-0.036*	*-0.080*	*-0.051*
PRIQ	*-0.003*	*0.098**	*0.041*	*0.055*	*-0.002*	*0.056*	*0.098*	*0.066*
PSIQ	*-0.145**	*0.139**	*0.014*	*-0.070*	*-0.045*	*-0.012*	*-0.002*	*-0.009*
BM Taps	*0.258***	*0.100**	*-0.032*	*0.027*	*-0.071*	*-0.074*	*0.022*	*-0.025*

Table 5.1.2 *SYS: The Pearson's correlation coefficient (r) between the following variables* ***in boys****: age (months), PEMCS, WISC IQ tests (VCIQ, WMIQ, PRIQ, PSIQ), bimanual (BM) taps (in Thurston task) and relative CC volumes (%; total and segmental). These variables are used in the analysis for the association of CC volumes with IQ & bimanual coordination. * refers to p<0.05 and ** refers to p<0.001.*

Table 5.1.3 SYS: Correlation Matrix for Girls (Pearson's Correlation Coefficient)								
	Age	**PEMCS**	**CC Anterior**	**CC Mid Anterior**	**CC Central**	**CC Mid Posterior**	**CC Posterior**	**CC Total**
PEMCS	*0.021*							
CC Volume								
Total	*0.194***	*-0.091*						
Anterior	*0.181***	*-0.039*						
MidAnterior	*0.161***	*-0.050*	*0.513***					
Central	*0.036*	*-0.010*	*0.221***	*0.579***				
MidPosterior	*0.159***	*-0.095*	*0.445***	*0.566***	*0.553***			
Posterior	*0.185***	*-0.112*	*0.578***	*0.462***	*0.245***	*0.523***		
OCP Use	*0.416***	*-0.059*	*0.072*	*0.030*	*0.022*	*0.040*	*0.082*	*0.095*
VCIQ	*0.189***	*-0.069*	*-0.005*	*-0.069*	*-0.074*	*-0.011*	*0.037*	*0.003*
WMIQ	*0.129**	*-0.040*	*0.026*	*-0.017*	*-0.048*	*-0.017*	*0.041*	*0.019*
PRIQ	*0.091**	*-0.035*	*0.038*	*-0.050*	*-0.037*	*0.009*	*-0.011*	*0.019*
PSIQ	*-0.061*	*-0.051*	*0.121**	*0.074*	*0.106**	*0.088*	*0.058*	*0.100**
BM Taps	*0.377***	*-0.001*	*0.139**	*0.007*	*0.053*	*0.107**	*0.130**	*0.124**

Table 5.1.3 *SYS: The Pearson's correlation coefficient (r) between the following variables* ***in girls****: age (months), PEMCS, OCP use, WISC IQ tests (VCIQ, WMIQ, PRIQ, PSIQ), bimanual (BM) taps (in Thurston task) and relative CC volumes (%; total and segmental). These variables are used in the analysis for the association of CC volumes with IQ and bimanual coordination. * refers to p<0.05 and ** refers to p<0.001. Correlations of OCP use with WISQ IQ and BM taps could not be included in the table and are as follows: VCIQ: 0.174**, WMIQ: 0.129*, PRIQ: 0.087, PSIQ: 0.026 and BM Taps: 0.251**.*

5.3.2 WISC-III: Association with Relative volume of the Total Corpus Callosum

There is no correlation between the relative volume of the total corpus callosum and the verbal comprehension score, the working memory score, and the perceptual reasoning score, in either sex. For details refer to Table 5.2.1 and 5.2.2.

The processing speed score, however, demonstrates a significant positive correlation (β=0.141, p=0.047) with the relative volume of the total corpus callosum in girls but not in boys. The relative volume of the corpus callosum accounts for 1% of the variance in the processing speed score of girls. Refer to Table 5.2.1 and 5.2.2. Further investigation is performed to identify the main segment of the corpus callosum associated with processing speed IQ in girls; this is observed to be the anterior segment. Refer to Table 5.2.3.

Table 5.2.1 SYS: Results for Association of Relative Total CC Volume with WISC IQ subsets in Boys			
	Results via Linear Regression Analysis		**Effect Size**
VCIQ	*ΔR^2=0.002, df=1,336, ΔF=0.79*	*β=-0.050, p=0.374, t=-0.89*	*d=-0.097, r=0.048*
WMIQ	*ΔR^2=0.002, df=1,338, ΔF=0.80*	*β=-0.049, p=0.373, t=-0.89*	*d=-0.097, r=0.048*
PRIQ	*ΔR^2=0.004, df=1,336, ΔF=1.52*	*β=0.068, p=0.219, t=1.23*	*d=0.134, r=0.067*
PSIQ	*ΔR^2=0.001, df=1,338, ΔF=0.17*	*β=0.023, p=0.678, t=0.42*	*d=0.045, r=0.022*

Table 5.2.1 *SYS: The results for association of relative volume (%) of the total CC with WISC IQ subsets **in boys**; and the effect sizes (effect size r, Cohen's d) of the results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance contributed by total CC), standardized regression coefficient (β; correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

Table 5.2.2 SYS: Results for Association of Relative Total CC Volume with WISC IQ subsets in Girls			
	Results via Linear Regression Analysis		Effect Size
VCIQ	*ΔR^2=0.002, df=1,353, ΔF=0.654*	*β=-0.043, p=0.419, t=-0.809*	*d=-0.086, r=0.043*
WMIQ	*ΔR^2=0.000, df=1,353, ΔF=0.032*	*β=-0.010, p=0.859, t=-0.178*	*d=-0.019, r=0.009*
PRIQ	*ΔR^2=0.000, df=1,354, ΔF=0.003*	*β=-0.003, p=0.958, t=-0.052*	*d=-0.006, r=0.003*
PSIQ	***ΔR^2=0.012, df=1,353, ΔF=4.246***	***β=0.111, p=0.040, t=2.061***	***d=0.219, r=0.109***

Table 5.2.2 *SYS: The results for association of relative volume (%) of the total CC with WISC IQ subsets **in girls**; and the effect sizes (effect size r, Cohen's d) of the results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance contributed by total CC), standardized regression coefficient (β; correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

Table 5.2.3 SYS: Results for Association of Relative Volumes of CC Segments with Processing Speed IQ in Girls		
CC Volumes (%)	**Results via Linear Regression Analysis**	**Effect Size**
Anterior	***β=0.141, p=0.047, t=1.990, df=346***	***d=0.214, r=0.106***
MidAnterior	*β=-0.039, p=0.613, t=-0.507, df=346*	*d=-0.055, r=0.027*
Central	*β=0.096, p=0.177, t=1.352, df=346*	*d=0.145, r=0.072*
MidPosterior	*β=0.018, p=0.811, t=0.239, df=346*	*d=0.026, r=0.013*
Posterior	*β=-0.029, p=0.683, t=-0.409, df=346*	*d=-0.044, r=0.022*

Table 5.2.3 *SYS: The results for association of relative volume (%) of CC segments with processing speed (PSIQ) in girls; and the effect sizes (effect size r, Cohen's d) of the results. These results are obtained via linear regression and the standardized regression coefficient (β) for the correlation of each segment with PSIQ, t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

To ascertain if the sex difference observed in "CC-processing speed IQ" relationship is based on performance, the "CC-processing speed IQ" correlation is investigated separately in boys with higher scores on processing speed IQ. The entire sample of boys is divided into two halves based on the median scores. Unlike girls, no significant correlation is observed between the relative volume of the corpus callosum and processing speed IQ in the subsample of boys with high processing speed IQ. For details see Table 5.2.4, 5.2.5 and 5.2.6.

Table 5.2.4 SYS: Descriptive Statistics for High vs. Low PSIQ boys sample		
	Boys: High PSIQ	**Boys: Low PSIQ**
Sample Size	*167*	*174*
Mean (SD)	*94 (8.2)*	*116 (9.6)*

Table 5.2.4 SYS: *The sample size (n) along with the mean and SD (in brackets) for the processing speed of high performing boys having PSIQ scores above median and low performing boys having PSIQ scores below median.*

Table 5.2.5 SYS: Results for Association of Relative Total CC Volume with Processing Speed in High vs. Low PSIQ boys sample			
BOYS	**Results via Linear Regression Analysis**		**Effect Size**
High PSIQ	*ΔR^2=0.008, df=1,164, ΔF=1.291*	*β=-0.094, p=0.257, t=-1.136*	*d=0.177, r=-0.088*
Low PSIQ	*ΔR^2=0.000, df=1,171, ΔF=0.013*	*β=0.009, p=0.908, t=0.115*	*d=0.017, r=0.008*

Table 5.2.5 *SYS: The results for association of relative volume (%) of the total CC with processing speed in high performing boys having PSIQ scores above median and low performing boys having PSIQ scores below median; and the effect sizes (effect size r, Cohen's d) of the results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance contributed by total CC), standardized regression coefficient (β; correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

Table 5.2.6 SYS: Results for Association of Relative Volumes of CC Segments Processing Speed in High vs. Low PSIQ boys sample		
CC Volumes	**Results via Linear Regression Analysis**	**Effect Size**
Anterior	*β=-0.132, p=0.173, t=1.368*	*d=0.201, r=0.104*
MidAnterior	*β=-0.143, p=0.227, t=-1.213*	*d=0.185, r=-0.092*
Central	*β=0.055, p=0.634, t=0.476*	*d=0.072, r=0.036*
MidPosterior	*β=0.049, p=0.625, t=0.490*	*d=0.075, r=0.037*
Posterior	*β=-0.059, p=0.553, t=-0.594*	*d=0.084, r=-0.042*

Table 5.2.6 *SYS: The results for association of relative volume (%) of CC segments with processing speed in high performing boys having PSIQ scores above median and low performing boys having PSIQ scores below median; and the effect sizes (effect size r, Cohen's d) of the results. These results are obtained via linear regression and the standardized regression coefficient (β) for the correlation of each segment with PSIQ, t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

5.3.3 Bimanual Mean Taps: Association with Relative volume of the Total Corpus Callosum

There is no correlation between the mean bimanual taps recorded during the Thurston finger tapping task and the relative volume of the total corpus callosum in either boys or girls. For details refer to Table 5.2.7 and 5.2.8.

Table 5.3.1 SYS: Results for Association of Relative Total CC Volume with Bimanual Coordination in Boys			
	Results via Linear Regression Analysis		Effect Size
Bimanual Taps	*ΔR^2=0.007, df=1,313, ΔF=2.38*	*β=-0.086, p=0.124, t=-1.543*	*d=-0.174, r=0.087*

Table 5.2.4 *SYS: The results for association of relative volume (%) of the total CC with bimanual coordination **in boys**; and the effect sizes (effect size r, Cohen's d) of the results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance contributed by total CC), standardized regression coefficient (β; correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted. Bimanual coordination is assessed in terms of number of bimanual taps in the thurston task.*

Table 5.3.2 SYS: Results for Association of Relative Total CC Volume with Bimanual Coordination in Girls			
	Results via Linear Regression Analysis		Effect Size
Bimanual Taps	*ΔR^2=0.003, df=1,325, ΔF=1.17*	*β=0.057, p=0.280, t=1.082*	*d=0.120, r=0.060*

Table 5.2.5 *SYS: The results for association of relative volume (%) of the total CC with bimanual coordination **in girls**; and the effect sizes (effect size r, Cohen's d) of the results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance contributed by total CC), standardized regression coefficient (β; correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted. Bimanual coordination is assessed in terms of number of bimanual taps in the thurston task.*

5.4 DISCUSSION

This study demonstrates that processing speed IQ correlates, albeit weakly, with the relative volume of the corpus callosum in girls but not boys; this is

particularly the case for the anterior segment of the corpus callosum. There is no significant association between the corpus callosum and the remaining domains of performance IQ and verbal IQ in both sexes. These findings help to resolve some of the inconsistencies in existing literature. Studies examining the association of the corpus callosum with performance IQ and verbal IQ were few with inconsistent results. In general, performance IQ has been associated with the corpus callosum more frequently than verbal IQ (Sauerwein et al. 1994, Hutchinson et al. 2009). Agenesis studies suggest a positive association between the corpus callosum and performance IQ (Sauerwein et al. 1994), whereas MRI studies in healthy adolescents and young adults demonstrate either a negative association between the relative CC volume and PIQ (Hutchinson et al. 2009) or none at all (Ganjavi et al. 2011). Variations in the results of previous studies can be attributed to differences in their sample sizes, age range and methodological techniques. The results of this study as compared with previous studies are more reliable by virtue of its larger sample size and well controlled statistical models (controlling for inter-segmental dependence). It addition, it is the first study to highlight that the specific subset of performance IQ associated with the CC is processing speed and not perceptual reasoning.

The association between the corpus callosum and processing speed IQ is mainly limited to the anterior segment. The anterior segment of the CC is responsible for carrying fibres between the prefrontal and premotor areas of the left and right hemisphere (Pandya et al. 1971, De LaCoste et al. 1985, Hofer et al. 2006, Zarei et al. 2006). Functional MRI studies demonstrated that (modified)

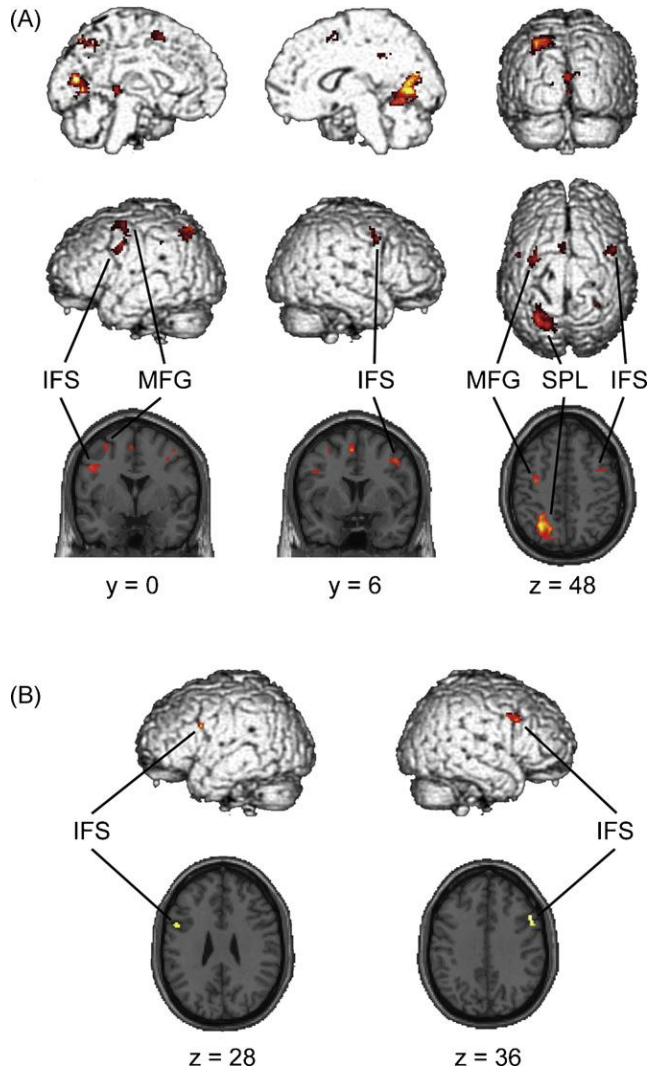

Figure 5A.1 *Brain areas involved in modified processing speed task. (A) BOLD responses relative to control task; and (B) BOLD responses positively correlated with processing speed task. IFS refers to Inferior Frontal Sulcus; MFG refers to Medial Frontal Gyrus; SPL refers to Superior Parietal Lobule. Schematic from Usui et al. 2009.*

processing speed tasks of the Wechsler scale were associated with greater BOLD responses in the bilateral inferior frontal sulci, left middle frontal gyrus and left posterior parietal cortex as compared with control tasks (see Figure 5A.1; Usui et al. 2009). Moreover, BOLD response in the inferior frontal sulcus was positively correlated with performance in the processing speed task (Usui et al. 2009). Thus, the inferior frontal sulcus of the prefrontal cortex appears to play a crucial role in processing speed IQ. Callosal fibres connecting the inferior frontal sulcus pass through the anterior segment of the CC (Pandya et al. 1971, De LaCoste et

al. 1985, Hofer et al. 2006). Thus, this study's finding is consistent with the known topographic distribution of callosal fibres connecting cortical areas engaged in tasks relevant for processes assessed by WISC-III in the processing speed domain. In contrast, previous studies observed that performance IQ was associated with the central segment of the CC (Hutchinson et al. 2009).

Processing speed of girls is seen to be significantly ($p<0.0001$) greater than that of boys (Cohen's d=0.50). For the remaining domains of IQ, namely verbal comprehension, working memory and perceptual reasoning, boys have a slightly higher score than girls but the difference does not reach significance. These results are similar to the sex differences reported for IQ by previous studies (Longman et al. 2007, Lynn et al. 1991, Jensen et al. 1983, Camarata et al. 2006). Longman et al. (2007) investigated sex differences in WAIS-III cognitive scores of the Amerian (n=2450) and Canadian (n=1104) standardized samples. In case of the Canadian sample they observed a significant sex difference in processing speed only, whereas in case of the American sample they observed a robust sex difference in processing speed with a female over male advantage (Cohen's d=0.30) and slight sex differences in verbal comprehension, working memory and perceptual reasoning with a male over female advantage. Lynn et al. (1991) and Jensen et al. (1983) examined sex differences in the WISC-R scores of the standardized Scottish (n=1400) and American samples (n=1868), respectively. They reported sex differences for individual tests of verbal comprehension, working memory, perceptual reasoning and processing speed. The most robust and significant sex difference was observed for the coding task of processing

speed IQ with a female over male advantage (Cohen's *d*=0.55). Relatively subtle male advantages (Cohen's *d*: 0.10 to 0.30) were observed in the individual tasks of verbal comprehension (vocabulary, information and comprehension), working memory (arithmetic) and perceptual reasoning (picture completion, block design and object assembly). Camarata et al. (2006) compared sex differences in cognitive abilities on the Woodcock Johnson tests of the standardized American sample (WJ III: n=1987, WJ-R: n=4253, WJ-77: n=4225). They observed that females outperform males in processing speed (Cohen's *d*=0.35) and males are superior to females in academic knowledge (Cohen's *d*=0.20). No sex differences were observed in verbal abilities, visuo-spatial thinking, long-term retrieval, short-term memory, auditory processing, fluid reasoning, reading, maths and writing.

Girls are, therefore, seen to be superior to boys in terms of both structure relating to anterior CC volume and function relating to processing speed. Accordingly, the structure-function relationship between the 'anterior CC volume-processing speed IQ' is also observed only in girls and not in boys, thus demonstrating that there is a sex difference not only in the structure of the CC but also its function with females having an upper hand in both cases. It is interesting to note that the sex difference observed in the callosal association with processing speed is not influenced by performance. This is evidenced by the fact that when boys with higher scores for processing speed (scores above median: n=174, Mean ±SD: 116 ±9.6, which are comparable to female scores) are

examined separately they, unlike girls, do not demonstrate any correlation between the CC volume and processing speed IQ (β=0.010, p=0.900).

No correlation is observed between relative volume of the total corpus callosum and bimanual coordination. No previous studies have examined this structure-function relationship using the CC volumes. Diffusion tensor imaging studies, however, demonstrated a positive association between the microstructural integrity of the corpus callosum (FA value) and bimanual motor coordination (Muetzel et al. 2008, Sullivan et al. 2006, Johansen-Berg et al.). Moreover, bimanual coordination was compromised in individuals with agenesis (Moes et al. 2008, Mueller et al. 2009) and transection (Callie et al. 2005) of the corpus callosum. Based on these studies, it was hypothesized that the volume of the corpus callosum will be positively associated with bimanual coordination. In contrast to this hypothesis, no such association is observed. It is possible that this is because bimanual coordination is determined by certain structural properties of CC white matter that influence its FA value but not its volume such as orientation of fibres (path geometry and crossing of fibre pathways) and axon density (Aboitiz et al. 1992, LaMantia et al. 1990, Beaulieu et al. 2002,). Fractional anisotropy (FA) reflects the directionality of diffusion of water molecules through CC white matter and is dependent on microstructural properties such as orientation of fibres (path geometry and crossing of fibre pathways) other than degree of myelination, axon size and density (Beaulieu et al. 2002). Gross anatomical measures of the corpus callosum, such as its mid-

sagittal area are not correlated with axon density (Aboitiz et al. 1992, LaMantia et al. 1990).

The average number of coordinated bimanual taps executed by the girls in the Thurston tapping task is significantly greater than that of boys. The results of previous studies examining sex differences in tapping tasks demonstrate that males usually perform better than females in simple tapping task (Dorfberger et al. 2009, Ruff et al. 1993), however, in the more complex forms on these tapping tasks, such as tapping to a steady rhythm, females performed better than males (Wolff et al. 1976). Females were also seen to perform better than males in the grooved pegboard task that involves fine motor skills (Ruff et al. 1993). Our findings are, therefore, consistent with those of previous studies since the Thurston task is a more complex test of bimanual coordination that involves both speed and accuracy. It is important to note, however, that some studies found no sex difference in speed and accuracy of motor performance (Nicholson and Kimura 1996, Mickeviciene et al. 2011, Moes et al. 2009).

In summary, this study demonstrates that the specific area of abstract or non-verbal intelligence associated with the relative volume of the corpus callosum is processing speed IQ. This relation is mainly with the anterior segment of the corpus callosum that connects the cortical areas activated during processing speed tasks (Usui et al. 2009, Pandya et al. 1971, Zarei et al. 2006). Moreover, this functional correlate of the corpus callosum, like its structure is sexually dimorphic (Chapter 4), with females having the upper hand in both cases.

In future it would be interesting to examine the relationship between the corpus callosum and IQ while controlling for the interdependance between the IQ subscales.

6 ASSOCIATION OF SEX HORMONES AND PUBERTY WITH RELATIVE VOLUME OF THE CORPUS CALLOSUM AND ITS SEGMENTS

6.1 INTRODUCTION

Sex differences have been observed in the relative volume of the corpus callosum (Section 1.6). These sex differences are subtle during the early years of life (Koshi et al. 1997, Hwang et al. 2004, Clarke et al. 1989) and gradually become more obvious during childhood and adolescence (Giedd et al. 1999 and Lenroot et al. 2007). They are most robust during late adolescence (Allen et al. 1991) and adulthood (Bermudez and Zatorre 2000, Johnson et al. 1994, Sullivan et al. 2001, Witelson et al. 1989). Adolescence is the period during which most of the physical differences between boys and girls begin to manifest (Grumbach et al. 2003, Guyton et al. 2000). These include the development of secondary sexual characteristics such as breasts, facial and pubic hair and voice changes. Sex hormones are known to play the most important role in the development of these sexually dimorphic physical attributes (Grumbach et al. 2003, Guyton et al. 2000). It is, therefore, possible that sex hormones are also responsible for the sexually dimorphic development of the corpus callosum during adolescence.

The level of sex hormones in both boys and girls remains low during early childhood. It is upon reaching puberty that the gonads (testes and ovaries) begin producing increasing quantities of sex hormones (Grumbach et al. 2003). Puberty

refers to the development of physical changes (in the form of secondary sexual characteristics) in response to the increased production of sex hormones that result in the capacity to reproduce. The physiological process that underlies the development of puberty is the activation of the Hypothalimic-Pitutary-Gonadal (HPG) axis (Guyton et al. 2000). Neuroendocrine cells of the preoptic hypothalamic nuclei begin production of gonadotropic releasing hormone (GnRH) in a pulsatile fashion. In both boys and girls, GnRH pulsing subsequently stimulates production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary gland (Guyton et al. 2000). In boys, LH stimulates the production of testosterone by the Leydig cells of the testes. In girls, both LH and FSH stimulate the production of estrogen and progesterone by the theca and granulose cells of the ovaries. The regular production of these sex hormones (testosterone, estrogen and progesterone) is maintained via a negative feedback mechanism whereby they act on the brain to inhibit production of GnRH (Guyton et al. 2000).

Testosterone, estrogen and progesterone are the main sex steroids produced in the body, although smaller amounts of other sex hormones are also present. They are lipid-soluble compounds and therefore cross the lipophilic blood-brain barrier to enter the brain. Inside the brain, these hormones penetrate the neuronal membrane and bind to intracellular steroid receptors (reviewed by Beyer 1999). Multiple areas of the brain express receptors for sex steroids (reviewed by Beyer 1999). By binding to these receptors sex steroids are able to exert their influence on the brain.

There is evidence from animal studies to suggest that sex steroids play a role in developmental processes responsible for sex-specific organization of brain structure and behaviour during the perinatal period (Bimonte et al. 2000a, Fitch et al. 1989b, Jacobson and Gorski 1981, Dohler et al. 1984, Thornton et al. 2009). Jacobson and Gorski (1981) showed that estrogen (produced via aromatization of androgens) is responsible for the sexual dimorphism of the brain displayed in the immediate postnatal period in the rat. Specifically, they found a sub-region of the hypothalamic pre-optic nucleus called the sexually dimorphic nucleus to be approximately five times larger in newborn male rats as compared with their female counterparts. Thornton et al. (2009) demonstrated that testosterone and dihydrotestosterone (directly without aromatization) organize neural mechanisms in rhesus monkeys, which in turn affect sexually dimorphic behaviour (such as non-sexual play behaviour). In addition to the sexual dimorphism in neural circuits associated with early perinatal sex hormone exposure, sex-steroid dependent organization of brain structure and behaviour is also observed during adolescence (Schluz et al. 2009, Sisk and Zehr 2005).

A number of animal studies provide evidence for the role of sex steroids as trophic factors influencing brain development and plasticity. Thus, sex steroids have been shown to stimulate dendritic branching, synapse formation, myelination, cell death and survival (Review papers by Beyer et al. 1999, Peper et al. 2011). Baulieu et al. (2000) demonstrated that progesterone increases the number of myelin-generating oligodendrocytes, the formation of myelin sheaths and the synthesis of myelin proteins in neonatal rat brains. Similarly, Marin-

Husstege et al. (2004) demonstrated that estrogen and progesterone influence oligodendrocytes' proliferation and maturation; specifically, estrogen increased sheet formation and progesterone enhanced cellular branching of neonatal rodent brains. Stocker et al. (1994) showed that testosterone enhanced the maturation of myelin in the brains of canaries at a later stage of development. Levy et al. (1996) demonstrated that estrogen increases neuronal sprouting and the number of synapses in neonatal rat brains; and testosterone causes early onset of myelination. Research in rat brains has demonstrated that sex steroids are responsible for the development of sex differences in the absolute area of the CC (M>F; Berrebi et al. 1988). Specifically, testosterone has a masculinising (incremental) effect on the CC of both male and female rats. Testosterone exerts this organizational effect on male rats prenatally and on female rats during the early postnatal period (Fitch et al. 1990 and 1991b). Ovarian hormones, unlike testosterone, were seen to exert a feminizing (decrimental) influence on the CC of female rats only (Bimonte et al. 2000a). The CC of female rats upon exposure to adult estrogen decreased in size when the female rats had prior neonatal exposure to estrogen but increased in size when they did not have any prior exposure to neonatal estrogen (Bimonte et al. 2000a). Thus, neonatal exposure to ovarian hormones primed the female rat CC to respond to the ovarian hormones produced later in life in a characteristic female manner. It is important to note that ovariectomy after day 78 was seen to have no effect on callosal size, suggesting that after the initial priming 'organizational' effect of neonatal estrogen the influence of subsequent ovarian hormone exposure on CC is established prior to adulthood (day 78; Mack et al. 1994). These studies examine

the effects of sex steroids on the absolute size (area and width) of the CC. The absolute CC size was not adjusted for total brain size because consistent correlations were not observed between the CC and the brain size (Fitch et al. 1991a). Human studies examining the relationship of sex steroids with white matter are fewer in comparison with animal studies. Human studies provide evidence that increasing level of testosterone during puberty was associated with increases in white-matter volume in boys during adolescence (Perrin et al. 2009). Perrin et al. (2008) investigated the association between the level of bioavailable testosterone and the relative volume of total white matter in the brains of 408 adolescents, aged 12 to 18 years. They demonstrated a positive correlation between the level of bioavailable testosterone and relative volume of total white matter in the brains of boys; this relationship was moderated by a genetic variation in the androgen-receptor gene. Thus, suggesting that testosterone may be one of the factors responsible for development of white matter in the brain, during adolescence. Peper et al. (2009) conducted an *in-vivo* magnetic resonance study on 104 prepubertal children, aged 9 years and showed that higher level of Luteinizing hormone (LH) was associated with higher relative volume of white matter, in the total sample and in boys and girls separately. Luteinizing hormone, as previously mentioned is responsible for stimulating increased production of sex hormones (Guyton et al. 2000).

The study described in this chapter is the first to examine the association of sex hormones with the relative volume of the total corpus callosum and its segments, during adolescence. This is done by examining the correlation (β,

standardized regression coefficient) of relative CC volumes with the following factors linked directly or indirectly with sex hormones, namely: 1) level of bioavailable testosterone; 2) “sex hormone exposure period” defined as the duration of exposure to female sex hormones from menarche up to the test day; and 3) puberty stage based on the Puberty Development Scale (PDS), in 681 12-to-18 year old adolescents (356 boys and 325 girls) from the SYS study and 1,925 13-to-15 year old adolescents (964 boys and 961 girls) from the IMAGEN study. The level of bioavailable testosterone is not available for the IMAGEN adolescents; hence the association between this variable and relative CC volume is not examined in the IMAGEN study sample. In addition, the girls in the IMAGEN study demonstrate limited variability in the stage of pubertal development; nearly 80% (754 from a total of 947 girls) of all girls in the IMAGEN sample have the same stage of pubertal development (stage 4), whereas in case of the boys the distribution is more uniform (across stages 3 and 4). Thus, the association of pubertal stage with the relative volume of the corpus callosum and its segments is examined in only boys from the IMAGEN sample. This study hypothesises that sex differences observed in the relative volume of the corpus callosum and its segments, during adolescence (see Chapter 4) are related to the increased production of sex hormones during this period. Therefore, this study hypothesizes that all three indices of sex hormones, namely: 1) the level of sex hormones; 2) duration of exposure to sex hormones; and 3) degree of sexual maturity, will demonstrate a positive association with the relative volume of the corpus callosum and its segments. Previous studies also demonstrate a positive association between sex hormones and the volume of white matter in the brain

(Perrin et al. 2008). Pubertal stage is closely related to the level of sex hormones (Shirtcliff et al. 2009) and hence predicted to exhibit a similar relation.

This study is the first study to examine the relationship of sex hormones with the relative volume of the corpus callosum. There are several strengths of this study, including: 1) two large study samples, which affords high statistical power to detect any possible association and also afford the ability to validate the results of the two studies against each other; 2) the SYS sample encompasses the pubertal years vital for sexual development in which the production of sex hormones accelerates and gains impetus, thus this sample is suitable to explore the association between sex hormones and the CC; 3) reliable statistical techniques accounting for inter-segmental dependence of the CC segments; and 4) FreeSurfer based volumetric analysis techniques that reliably measure CC volume and provide appropriate estimation of the relative volume of the corpus callosum (Chapter 3).

6.2 METHODOLOGY

This study is conducted by analyzing data from 681 adolescents (356 boys and 325 girls) of the Saguenay Youth Study and 1,925 adolescents (964 boys and 961 girls) of the IMAGEN study. Methodological details of the SYS and IMAGEN study design and participants, their recruitment and testing procedures have been provided in an earlier chapter (Chapter 2) of this thesis. Here the methodological approaches relevant to this study are briefly discussed. Section 6A, describes the methodological details for the SYS study and Section 6B for the IMAGEN study.

6A.2 METHODOLOGY: SYS STUDY

6A.2.1 Participants

This study is conducted on 681 adolescents of French-Canadian origin recruited from local high schools in Saguenay Lac Saint-Jean (SLSJ) region of Quebec, Canada. All participants are aged between 12 to 18 years. The total sample is comprised of 356 boys and 325 girls, all of whom do not use oral contraceptives. In the sample of 356 boys, the association of: 1) the level of bioavailable testosterone (cnmol/L); and 2) puberty stage, with the relative volume of the total corpus callosum and its segments is investigated; whereas in the sample of 325 non-OCP using girls, the association of: 1) sex hormone exposure period; and 2) puberty stage with the relative volume of the total corpus callosum and its segments is investigated.

6A.2.2 Serum Levels of Bioavailable Testosterone (cnmol/L)

Fasting blood samples of all participants are collected in the morning between 8:00 and 9:00 a.m. The blood level of testosterone (nanomoles per litre) and sex hormone binding globulin (nanomoles per litre) are analyzed using radioimmunoassay (Testosterone RIA DSL-4000; Diagnostic Systems Laboratory) at the Hospital Hôtel-Dieu in Montreal. Bioavailabale level of testosterone (nanomoles per litre) is measured using an equation proposed by Sodergard et al. (1982), which calculates the balance between total, bound and free (bioavialable) testosterone level in the body. (For more details refer to Section 2A.11).

6A.2.3 Puberty Development Scale

In addition to hormonal indices, there are other ways of determining sexual development. These include determining development of secondary sexual characteristics via clinical examination or interview. This study uses the Puberty Development Scale (PDS) questionnaire to determine sexual maturity. (For more details on PDS refer to Section 2A.6).

6A.2.4 Oral Contraceptive Pill Use

Oral contraceptive pill-use in girls is assessed via a medical questionnaire filled out by the mother during the initial home visit. In this questionnaire, mothers are asked to specify which medicines (if any) are taken by their children. Based on the answer to this question, use of oral contraceptive pills in girls is assessed. (For more details refer to Section 2A.7).

6A.2.5 MRI Acquisition

T1 weighted MRI scans of the participants are obtained on a Philips 1.0 T superconducting magnet. Acquisition parameters of these structural volume scans are as follows: 3D RF spoiled gradient-echo scan, 140-160 high resolution T1-weighted images, with TR=25 ms, TE=5 ms, flip angle=30^0 and 1 mm isotropic resolution.

6A.2.6 MRI Volumetric Analysis

FreeSurfer version 5.0.0 is used for the automated volumetric analysis, performed on native, high resolution T1-weighted images. Details of the volumetric analysis have been described previously in Chapter 4, Section 4A.2.3.

6A.2.7 Statistical Analysis

All statistical analysis is performed using SPSS 18.0. In the boys sample, two separate linear regression analysis are run to analyze the correlation (β, standardized regression coefficient) between: 1) level of bioavailable testosterone (cnmol/L) and relative volume (%) of the total corpus callosum and its segments; and 2) puberty stage and the relative volume (%) of the total corpus callosum and its segments. Each linear regression model is run with the CC volume measures (relative volume of the total corpus callosum and its five segments, namely, anterior, mid-anterior, central, mid-posterior and posterior segment) specified as the dependent variables. There are two blocks of independent variables. The first block consists of the following independent variables: 1) prenatal exposure to maternal cigarette smoking (PEMCS); and 2) adolescent age in months. This is to control for the potential confounding effect of maternal exposure to cigarette smoking and age on the volume of the corpus callosum. The level of bioavailable testosterone (cnmol/L) or puberty stage is specified as the independent variable in the second block. The callosal segmental volumes are seen to be well correlated. Therefore, in order to control for inter-segmental dependence when examining the association of the level of

bioavialable testosterone or puberty stage with a specific CC segment, all the remaining segments are covaried by specifying them as independent variables in a preliminary block. For each linear-regression model, the adjusted R^2, standardized regression coefficient (β), t value for the regression coefficient and the significance of the t value are calculated. The standardized regression coefficient (β) represents the standard deviation units by which the dependent variable changed for each standard unit change in the independent variable, thus explaining how well the dependent and independent variables are correlated with each other. Results of a two-tailed T-test for the significance of the regression coefficient (t value and p value) are also calculated to demonstrate if the correlation (β) observed is significant or not. Adjusted R^2 explained the percentage variance in the dependent variable contributed by the independent variables. Adjusted R^2 is calculated and displayed by SPSS in a stepwise sequence, based on the blocks of independent variables entered. Adjusted R^2 is displayed by SPSS for each block in the model summary, adding on the variance contributed by the independent variables of the previous blocks. By subtracting the adjusted R^2 of the first block of independent variables from the total adjusted R^2 of the model, the percentage variance in the dependent CC volume contributed specifically by the level of bioavialable testosterone (cnmol/L) or puberty stage is calculated.

In the sample of (non-OCP using) girls (n=325), the correlation (β, standardized regression coefficient) of the sex hormone exposure period (SHEP) and puberty stage with the relative (%) volumes of the total corpus callosum and its segments

is assessed via linear regression. Two separate linear regression analysis are run to analyze the association of each of the two factors, sex hormone exposure period and puberty stage, with callosal volumes. Each linear regression model is run with the CC volume measures (relative volume of the total corpus callosum and its five segments, namely, anterior, mid-anterior, central, mid-posterior and posterior segment) specified as the dependent variables. There are two blocks of independent variables. The first block consists of the following independent variables: 1) prenatal exposure to maternal cigarette smoking (PEMCS); and 2) adolescent age in months, thus controlling for the potential confounding effect of prenatal cigarette exposure and age on the volume of the corpus callosum. The sex hormone exposure period in months (SHEP) or puberty stage is specified as the independent variable in the second block. Additionally, when examining the association of sex hormone exposure period or puberty stage with a specific CC segment, all the remaining segments are specified as independent variables in a preliminary block; in order to control for the inter-relatedness of the segments with each other. For each linear regression model, the adjusted R^2, standardized regression coefficient (β), t value for the regression coefficient and the significance of the t value are calculated.

This study considered only the relative volume, that is, the brain-size corrected volumes of the total corpus callosum and its segments, not their absolute volumes.

6B.2: METHODOLOGY: IMAGEN STUDY

6B.2.1 Participants

This study is conducted on 1,925 adolescents recruited from eight centres across England, Ireland, France and Germany. All participants are aged between 13 to 15 years. The total sample is comprised of 964 boys and 961 girls, all of whom do not use oral contraceptives. In the sample of 964 boys, the association of puberty stage with the relative volume of the total corpus callosum and its segments is investigated. In the sample of 961 non-OCP using girls, the association of "sex hormone exposure period" with the relative volume of the total corpus callosum and its segments is investigated.

6B.2.2 Puberty Development Scale

Pubertal stage is measured using the same Puberty development scale (PDS) used in the Saguenay Youth study (Section 2A.6).

6B.2.3 Oral Contraceptive Pill Use

Oral contraceptive pill use in girls is assessed via a time line follow back (TLFB) questionnaire on drug use filled out by a researcher interviewing the child. In this questionnaire, the researcher asks the participants to specify which prescription medicines (if any) were taken by them in the last month. Based on the answer to this question, use of oral contraceptive pills in girls is assessed.

6B.2.4 MRI Acquisition

T1 weighted MRI scans of the participants are obtained on a Philips 3.0 T superconducting magnet, from a variety of manufacturers (Siemens, Philips, General Electric and Bruker). Acquisition parameters of these structural volume scans are as follows: 160-170 high resolution T1W images, with TR=2300 ms, TE=2.8 ms, flip angle=8-9^0 and 1.1 mm isotropic resolution.

6B.2.5 MRI Volumetric Analysis

FreeSurfer version 5.0.0 is used for the automated volumetric analysis, performed on native, high resolution T1-weighted images. The procedure of volumetric analysis is identical to that explained for the Saguenay Youth Study (Section 4A.2.3).

6B.2.6 Statistical Analysis

All statistical analysis is performed using SPSS software, PASW Statistics 18.0. The association (β, standardized regression coefficient) of sex hormone exposure period and puberty stage with the relative volumes (%) of the total corpus callosum and its segments is assessed in girls and boys sample, respectively, via a linear regression model, similar to that used in the Saguenay Youth Study (described above Section 6A.2.7). The only difference in the linear regression model used is that it did not control for prenatal exposure to maternal cigarette smoking (PEMCS) and that it corrects for the possible confounding effect of

acquisition site by incorporating seven dummy variables that represent the eight acquisition sites.

6A.3 RESULTS: SYS STUDY

6A.3.1 Descriptive Statistics

There are a total of 356 boys with mean age of 180 (SD: ±22) months and mean puberty stage of 3.38 (±0.87). The percentage of boys with no prenatal exposure to maternal cigarette smoking is 55. Bioavailable testosterone levels are available for 245 boys; their mean level is 8.68 (±5.56) cnmol/L. There are a total of 325 girls not using oral contraceptive pills, with mean age of 177 (±22) months, mean puberty stage of 4.01 (±0.70) and mean sex hormone exposure period of 25 (±24; range: 0 to 96) months. The percentage of girls with no prenatal exposure to maternal cigarette smoking is 48. For more details on these descriptive statistics refer to Table 6A.1.1.

Pearson's correlations between the variables employed in the linear regression analysis to estimate the association of CC volumes with: 1) level of bioavailable testosterone; 2) Puberty stage of boys, are provided in Table 6A.1.2. Pearson's correlations between the variables employed in the linear regression analysis to estimate the association of CC volumes with: 1) sex hormone exposure period (SHEP); and 2) puberty stage of total sample of girls not using oral contraceptives (n=325) are provided in Table 6A.1.3.

Table 6A.1.1 SYS: Sample Demographics and Descriptive Statistics for Sex Hormones				
	Boys		Girls	
	n	Mean (SD)	n	Mean (SD)
Age (months)	*355*	*180 (22)*	*322*	*177 (22)*
Puberty Stage	*337*	*3.38 (0.87)*	*303*	*4.01 (0.70)*
Percentage with no PEMCS (%)	*356*	*55*	*325*	*48*
Bioavailable Testosterone (cnmol/L)	*245*	*8.68 (5.56)*	*-*	*-*
Sex Hormone Exposure Period (months)	*-*	*-*	*319*	*25 (24)*

Table 6A.1.1 *SYS: The sample size (n) along with the mean and SD (in brackets) for the following variables in both boys and girls: age (months), puberty, PEMCS, male bioavailable testosterone (cnmol/l) and female sex hormone exposure period (SHEP) that is estimated since menarche (months).*

Table 6A.1.2 SYS: Correlation Matrix for Boys (Pearson's Correlation Coefficient)								
	Age	Testo-sterone	Puberty	PEMCS	CC Anterior	CC Mid Anterior	CC Central	CC Mid Posterior
Age								
Testosterone	*0.664***							
Puberty	*0.656***	*0.683***						
PEMCS	*0.058*	*0.042*	*0.079*					
CC Volumes								
Total	*0.199***	*0.116**	*0.154**	*0.004*				
Anterior	*0.113**	*0.057*	*0.063*	*-0.033*				
MidAnterior	*0.186**	*0.127**	*0.178**	*0.026*	*0.465***			
Central	*0.147**	*0.128**	*0.076*	*0.054*	*0.322***	*0.668***		
MidPosterior	*0.209***	*0.118**	*0.129**	*-0.029*	*0.429***	*0.578***	*0.599***	
Posterior	*0.218***	*0.122**	*0.224**	*-0.019*	*0.542***	*0.377***	*0.276***	*0.462***

Table 6A.1.2 *SYS: The Pearson's correlation coefficient (r) between the following variables in boys: age (months), testosterone (cnmol/l), puberty stage, PEMCS and relative CC volumes (%; total and segmental). These variables are employed in the statistical models used in boys to analyze the association of testosterone and puberty with relative CC volumes. * refers to p<0.05 and ** refers to p<0.001.*

Table 6A.1.3 SYS: Correlation Matrix for Girls (Pearson's Correlation Coefficient)								
	Age	**SHEP**	**Puberty**	**PEMCS**	**CC Anterior**	**CC Mid Anterior**	**CC Central**	**CC Mid Posterior**
Age								
SHEP	*0.736***							
Puberty	*0.679***	*0.705***						
PEMCS	*0.055*	*0.048*	*0.060*					
CC Volume								
Total	*0.161**	*0.146**	*0.182***	*-0.101*				
Anterior	*0.153**	*0.145**	*0.172**	*-0.029*				
MidAnterior	*0.156**	*0.134**	*0.149**	*-0.037*	*0.496***			
Central	*0.038*	*0.040*	*0.047*	*0.018*	*0.206***	*0.609***		
MidPosterior	*0.162**	*0.148**	*0.157**	*-0.101**	*0.445***	*0.573***	*0.533***	
Posterior	*0.162**	*0.107**	*0.163**	*-0.118**	*0.573***	*0.439***	*0.251***	*0.530***

Table 6A.1.3 *SYS: The Pearson's correlation coefficient (r) between the following variables in girls: age (months), sex hormone exposure period (SHEP) since menrache (months), puberty stage, PEMCS and relative CC volumes (%; total and segmental). These variables are employed in the statistical models used in girls to analyze the association of SHEP and puberty with relative CC volumes. * refers to p<0.05 and ** refers to p<0.001.*

6A.3.2 Relative Volume of the Total Corpus Callosum and its Segments: Association with Bioavialable Testosterone Level of Boys

There is no correlation between the level of bioavialable testosterone (cnmol/L) and the relative volume of the total corpus callosum, above and beyond chronological age. For details refer to Table 6A.2.

There is no correlation between the level of bioavialable testosterone (cnmol/L) and the relative volume of any callosal segment, above and beyond chronological age. For details refer to Table 6A.2.

Table 6A.2 SYS: Results for Association of Bioavailable Testosterone with Relative CC Volumes			
Relative CC Volume (%)	**Results via Linear Regression Analysis**		**Effect Size**
Total	*ΔR^2=0.001, df=234, ΔF=0.12*	*β=-0.030, p=0.726, t=-0.351*	*d=-0.046, r=0.023*
Anterior	*ΔR^2=0.000, df=226, ΔF=0.01*	*β=0.005, p=0.940, t=0.076*	*d=0.010, r=0.005*
MidAnterior	*ΔR^2=0.000, df=226, ΔF=0.03*	*β=0.011, p=0.859, t=0.178*	*d=0.024, r=0.012*
Central	*ΔR^2=0.001, df=226, ΔF=0.46*	*β=0.042, p=0.497, t=0.681*	*d=0.091, r=0.045*
MidPosterior	*ΔR^2=0.001, df=226, ΔF=0.65*	*β=-0.051, p=0.420, t=-0.808*	*d=-0.107, r=0.054*
Posterior	*ΔR^2=0.000, df=226, ΔF=0.06*	*β=-0.018, p=0.800, t=-0.253*	*d=-0.034, r=0.017*

Table 6A.2 *SYS: The results for association of male bioavailable testosterone (cnmol/l) with relative volume (%) of the total CC and its segments; and the effect sizes (effect size r, Cohen's d) of these results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance explained by testosterone), standardized regression coefficient (β; for correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

6A.3.3 Relative Volume of the Total Corpus Callosum and its Segments: Association with Sex Hormone Exposure Period of Girls

There is no correlation between sex hormone exposure period and the relative volume of the total corpus callosum or any of its segments, above and beyond chronological age. For details refer to Table 6A.3.

Table 6A.3 SYS: Results for Association of Female Sex Hormone Exposure Period with Relative CC Volumes			
Relative CC Volume (%)	**Results via Linear Regression Analysis**		**Effect Size**
Total	*ΔR^2=0.001, df=300, ΔF=0.27*	*β=0.046, p=0.605, t=0.517*	*d=0.060, r=0.030*
Anterior	*ΔR^2=0.002, df=292, ΔF=0.92*	*β=0.067, p=0.339, t=0.958*	*d=0.112, r=0.056*
MidAnterior	*ΔR^2=0.000, df=292, ΔF=0.00*	*β=-0.003, p=0.966, t=-0.042*	*d=-0.005, r=0.002*
Central	*ΔR^2=0.000, df=292, ΔF=0.00*	*β=-0.005, p=0.947, t=-0.066*	*d=-0.008, r=0.004*
MidPosterior	*ΔR^2=0.001, df=292, ΔF=0.58*	*β=0.050, p=0.447, t=0.761*	*d=0.089, r=0.044*
Posterior	*ΔR^2=0.003, df=292, ΔF=1.56*	*β=-0.087, p=0.212, t=-1.250*	*d=-0.146, r=0.073*

Table 6A.3 *SYS: The results for association of female sex hormone exposure period (SHEP) estimated since menarche (months) with relative volume (%) of the total CC and its segments; and the effect sizes (effect size r, Cohen's d) of these results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance explained by SHEP), standardized regression coefficient (β; for correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

6A.3.4 Relative Volume of the Total Corpus Callosum and its Segments: Association with PDS Puberty Stage

There is no correlation observed in both boys and girls between puberty stage and the relative volume of the total corpus callosum, above and beyond chronological age. For details see Table 6A.4.1 and 6A.4.2 for boys and girls, respectively.

In boys, significant but opposing correlations are observed between puberty stage and the relative volumes of only the anterior and mid-anterior segment of the corpus callosum, above and beyond chronological age. There is a significant negative correlation between puberty stage and relative volume of the anterior segment of the corpus callosum (β=-0.118, p=0.047), above and beyond chronological age. There is a significant positive correlation between puberty stage and relative volume of the mid-anterior segment of the corpus callosum (β=0.137, p=0.006), above and beyond chronological age. Puberty stage accounts for 1% of the variance in the relative volume each of the two (anterior and mid-anterior) segments of the corpus callosum of boys.

In case of girls, there is no correlation between the puberty stage and relative volume of any segment of the corpus callosum, above and beyond chronological age. For details see Table 6A.4.1 and 6A.4.2 for boys and girls, respectively.

Table 6A.4.1 SYS: Results for Association of Puberty Stage with Relative CC Volumes in Boys			
Relative CC Volume (%)	**Results via Linear Regression Analysis**		**Effect Size**
Total	*ΔR^2=0.000, df=320, ΔF=0.09*	*ß=0.023, p=0.756, t=0.311*	*d=0.035, r=0.017*
Anterior	***ΔR^2=0.008, df=312, ΔF=3.98***	***ß=-0.118, p=0.047, t=-1.996***	***d=-0.226, r=0.112***
MidAnterior	***ΔR^2=0.010, df=312, ΔF=7.60***	***ß=0.137, p=0.006, t=2.757***	***d=0.312, r=0.154***
Central	*ΔR^2=0.00, df=312, ΔF=2.55*	*ß=-0.081, p=0.111, t=-1.598*	*d=-1.181, r=0.090*
MidPosterior	*ΔR^2=0.001, df=312, ΔF=0.51*	*ß=-0.039, p=0.476, t=-0.714*	*d=-0.081, r=0.040*
Posterior	*ΔR^2=0.007, df=312, ΔF=3.67*	*ß=0.113, p=0.056, t=1.915*	*d=0.217, r=0.108*

Table 6A.4.1 *SYS: The results for association of puberty stage with relative volume (%) of the total CC and its segments **in boys**; and the effect sizes (effect size r, Cohen's d) of these results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance explained by puberty), standardized regression coefficient (ß; for correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

Table 6A.4.2 SYS: Results for Association of Puberty Stage with Relative CC Volumes in Girls			
Relative CC Volume (%)	**Results via Linear Regression Analysis**		**Effect Size**
Total	*ΔR^2=0.009, df=281, ΔF=2.60*	*ß=0.128, p=0.108, t=1.611*	*d=0.192, r=0.096*
Anterior	*ΔR^2=0.002, df=275, ΔF=0.89*	*ß=0.060, p=0.345, t=0.946*	*d=0.114, r=0.057*
MidAnterior	*ΔR^2=0.000, df=275, ΔF=0.00*	*ß=0.003, p=0.959, t=0.052*	*d=0.006, r=0.003*
Central	*ΔR^2=0.000, df=275, ΔF=0.18*	*ß=-0.026, p=0.668, t=-0.429*	*d=-0.052, r=0.023*
MidPosterior	*ΔR^2=0.001, df=275, ΔF=0.30*	*ß=0.032, p=0.586, t=0.545*	*d=0.066, r=0.033*
Posterior	*ΔR^2=0.000, df=275, ΔF=0.03*	*ß=0.010, p=0.874, t=0.159*	*d=0.019, r=0.009*

Table 6A.4.2 *SYS: The results for association of puberty stage with relative volume (%) of the total CC and its segments **in girls**; and the effect sizes (effect size r, Cohen's d) of these results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance explained by puberty), standardized regression coefficient (ß; for correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

6B.3 RESULTS: IMAGEN STUDY

6B.3.1 Descriptive Statistics

There are a total of 964 boys with mean age of 172 (SD: ±15) months and mean puberty stage of 3.28 (±0.68). There are a total of 961 girls, all of whom are not

using oral contraceptive pills, with mean age of 172 (±12) months. There is limited variability in the pubertal stage of the girls; nearly 80% of the girls (754 from 947) have the same stage of pubertal development (stage 4). The mean sex hormone exposure period of the girls is 18 (±12; range: 0 to 60) months. For more details on these descriptive statistics refer to Table 6B.1.1.

Pearson's correlations between the variables employed in the linear regression analysis to estimate the association of relative CC volumes with: 1) puberty stage of boys; and 2) sex hormone exposure period (SHEP) of girls are provided in Table 6B.1.2 and Table 6B.1.3, respectively.

Table 6B.1.1 IMAGEN: Sample Demographics and Descriptive Statistics for Sex Hormones

	Boys		Girls	
	n	**Mean (SD)**	**n**	**Mean (SD)**
Age (months)	*964*	*172 (15)*	*961*	*172 (12)*
Puberty Stage	*955*	*3.28 (0.68)*	*951*	*3.98 (0.45)*
Sex Hormone Exposure Period (months)	-	-	*947*	*18 (12)*

Table 6B.1.1 *IMAGEN: The sample size (n) along with the mean and SD (in brackets) for the following variables in both boys and girls: age (months), puberty and female sex hormone exposure period (SHEP) that is estimated since menarche (months).*

Table 6B.1.2 IMAGEN: Correlation Matrix for Boys (Pearson's Correlation)

	Age	**Puberty**	**CC Anterior**	**CC Mid Anterior**	**CC Central**	**CC Mid Posterior**
Puberty	*0.079**					
CC Volume						
Total	*-0.041*	*0.045*				
Anterior	*-0.058**	*-0.002*				
MidAnterior	*-0.050*	*0.042*	*0.390***			
Central	*0.011*	*0.055*	*0.245***	*0.656***		
MidPosterior	*0.004*	*0.026*	*0.427***	*0.565***	*0.507***	
CC-Posterior	*-0.064**	*0.038*	*0.522***	*0.447***	*0.265***	*0.550***

*Table 6B.1.2 IMAGEN: The Pearson's correlation coefficient (r) between the following variables **in boys**: age (months), puberty stage and relative CC volumes (%; total and segmental). These variables are employed in the statistical models used in boys to*

*analyze the association of puberty with relative CC volumes. * refers to p<0.05 and ** refers to p<0.001.*

Table 6B.1.3 IMAGEN: Correlation Matrix for Girls (Pearson's Correlation)						
	Age	**SHEP**	**CC Anterior**	**CC Mid Anterior**	**CC Central**	**CC Mid Posterior**
Age						
SHEP	*0.226***					
CC Volume						
Total	*0.046*	*0.020*				
Anterior	*0.012*	*-0.036*				
MidAnterior	*0.006*	*-0.017*	*0.524***			
Central	*-0.017*	*-0.020*	*0.375***	*0.659***		
MidPosterior	*0.043*	*-0.040*	*0.463***	*0.500***	*0.528***	
Posterior	*0.083**	*-0.043*	*0.523***	*0.451***	*0.320***	*0.547***

***Table 6B.1.3** IMAGEN: The Pearson's correlation coefficient (r) between the following variables **in girls**: age (months), sex hormone exposure period SHEP since menarche (months) and relative CC volumes (%; total and segmental). These variables are employed in the statistical models used to analyze the association of female shep with relative CC volumes. * refers to p<0.05 and ** refers to p<0.001.*

6B.3.2 Relative Volume of the Total Corpus Callosum and its Segments: Association with Sex Hormone Exposure Period of Girls

There is no correlation between sex hormone exposure period and the relative volume of the total corpus callosum, above and beyond chronological age. For details refer to Table 6B.2.

There is no correlation between sex hormone exposure period and the relative volume of any callosal segment, above and beyond chronological age. For details refer to Table 6B.2.

Table 6B.2 IMAGEN: Results for Association of Female Sex Hormone Exposure Period with Relative CC Volumes			
Relative CC Volume (%)	**Results via Linear Regression Analysis**		**Effect Size**
Total	*ΔR^2=0.000, df=899, ΔF=0.27*	*β=-0.017, p=0.603, t=-0.520*	*d=-0.035, r=0.017*
Anterior	*ΔR^2=0.000, df=883, ΔF=0.64*	*β=-0.022, p=0.423, t=-0.802*	*d=-0.054, r=0.027*
MidAnterior	*ΔR^2=0.000, df=883, ΔF=0.07*	*β=0.006, p=0.797, t=0.257*	*d=0.017, r=0.009*
Central	*ΔR^2=0.000, df=883, ΔF=0.30*	*β=0.013, p=0.586, t=0.546*	*d=0.036, r=0.018*
MidPosterior	*ΔR^2=0.002, df=883, ΔF=3.33*	*β=-0.047, p=0.086, t=-1.826*	*d=-0.123, r=0.061*
Posterior	*ΔR^2=0.002, df=883, ΔF=0.38*	*β=0.041, p=0.123, t=1.544*	*d=0.104, r=0.052*

Table 6B.2 *IMAGEN: The results for association of female sex hormone exposure period (months) with relative volume (%) of the total CC and its segments; and the effect sizes (effect size r, Cohen's d) of these results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance explained by SHEP), standardized regression coefficient (β; for correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

6B.3.3 Relative Volume of the Total Corpus Callosum and its Segments: Association with Puberty Stage of Boys

As mentioned previously, the correlation between puberty stage and CC volume is examined in boys only because of the limited variability in puberty stage of girls. No correlation is observed in boys between puberty stage and the relative volume of the total corpus callosum, above and beyond chronological age. For details refer to Table 6B.3.

No correlation is observed in boys between puberty stage and the relative volume of any segment of the corpus callosum, above and beyond chronological age. For details refer to Table 6B.3.

Table 6B.3 IMAGEN: Results for Association of Puberty Stage with Relative CC Volumes in Boys			
Relative CC Volume (%)	**Results via Linear Regression Analysis**		**Effect Size**
Total	*ΔR^2=0.002, df=878, ΔF=1.51*	*ß=0.040, p=0.219, t=1.229*	*d=0.083, r=0.041*
Anterior	*ΔR^2=0.001, df=863, ΔF=0.85*	*ß=-0.026, p=0.357, t=-0.921*	*d=-0.063, r=0.031*
MidAnterior	*ΔR^2=0.000, df=863, ΔF=0.05*	*ß=0.005, p=0.829, t=0.216*	*d=0.015, r=0.007*
Central	*ΔR^2=0.001, df=863, ΔF=1.03*	*ß=0.025, p=0.312, t=1.012*	*d=0.069, r=0.034*
MidPosterior	*ΔR^2=0.000, df=863, ΔF=0.17*	*ß=-0.010, p=0.682, t=-0.410*	*d=-0.028, r=0.014*
Posterior	*ΔR^2=0.001, df=863, ΔF=1.43*	*ß=0.031, p=0.232, t=1.197*	*d=0.081, r=0.041*

Table 6B.3 *IMAGEN: The results for association of puberty with relative volume (%) of the total CC and its segments in boys; and the effect sizes (effect size r, Cohen's d) of these results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance explained by puberty), standardized regression coefficient (ß; for correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

6.4 DISCUSSION

This study demonstrates that a higher stage of pubertal development (above and beyond chronological age) is associated with a smaller anterior and larger mid-anterior segment of the corpus callosum, in adolescent boys. This is consistent with the sex differences observed in the relative volume of the anterior (F>M) and mid anterior (M>F) segment of the corpus callosum, during adolescence (Chapter 4). Therefore, it may be speculated that sex differences in the volume of the corpus callosum are partly the result of sex hormones that bring about pubertal development (Guyton et al. 2000). Moreover, based on the absence of this "puberty stage-CC volume" relationship in girls, it may be conjectured that the relationship of male sex hormones with CC volume is possibly stronger as compared with that of female sex hormones. This is consistent with existing literature, which demonstrates an association between male sex hormones (testosterone) and white-matter volume (Perrin et al. 2008) but does not

comprise of any report of a similar relationship between female sex hormones and white-matter volume.

No correlation is observed between the level of bioavialable testosterone and the relative volume of the total corpus callosum or any of its segments, above and beyond chronological age. This is in contrast to the hypothesis of this study, which suggests that testosterone is one of the factors responsible for sexual differentiation observed in CC volume during adolescence (see Chapter 4) and, therefore, will demonstrate a correlation with the relative volume of the corpus callosum. Few researchers have explored the relation between testosterone and total or regional white-matter volume. Perrin et al. (2008) were one of the few research groups to have worked in this direction. They demonstrated a positive correlation between the level of bioavailable testosterone and the relative volume of total white matter (r^2=0.16, p<0.0001) in the brains of 204 male adolescents, aged 12 to 18 years, from the same SYS sample. This relationship was moderated by a genetic variation in the androgen-receptor gene. Moreover, they also observed a significant relationship between the level of bioavailable testosterone and age (r^2=0.42, p<0.0001); after correcting for age the relationship between testosterone and white-matter volume was no longer significant (p=0.082). Thus, correcting for age, which is well correlated with the level of bioavailable testosterone (r^2=0.44, p<0.0001) may be responsible for the absence of a significant association between the level of testosterone and CC volumes. Furthermore, the corpus callosum forms only a small fraction of the total white-matter volume in the brain. Hence the changes in the volume of the

corpus callosum are likely to be much smaller in magnitude as compared with the total white-matter changes. It is possible that the statistical power afforded by the study sample is large enough to identify the greater white-matter volume changes but not the smaller CC volume changes associated with testosterone level. Moreover, rat studies demonstrate that testosterone exerts its influence on the corpus callosum prenatally and not during adolescence (Fitch et al. 1990 and 1991b).

In girls, unlike in boys, the relation between the levels of female sex hormones and the relative volume of the corpus callosum is not examined. This is due to the challenges associated with measuring true peak levels of female sex hormones (estrogen and progesterone) by a single measurement taken at different phases of the menstrual cycle. The sex hormone levels vary considerably across the monthly menstrual cycle of each girl. Furthermore, the menstrual cycle of different girls also varies in terms of duration. As a result, it is challenging to measure peak estrogen and progesterone values in girls. The estrogen and progesterone levels are likely to be biased by the menstrual cycle phase in which they were measured. In view of the limited reliability of measured estrogen and progesterone level, this study examines instead the correlation of duration of exposure to female sex hormones, as indexed by menarche, with the relative volume of the total corpus callosum and its segments. Menarche refers to the start of the menstrual cycles in girls and marks the onset of production of biologically relevant levels of sex hormones by the ovaries.

In both the SYS and IMAGEN sample, no correlation is observed between the duration of exposure to female sex hormones and the relative volume of the total corpus callosum or any of its segments, above and beyond chronological age. This is in contrast to this study's hypothesis. There are sex differences in the volume of the corpus callosum of adolescents, with girls having greater relative volume of the corpus callosum as compared with boys (see Chapter 4). This study hypothesizes that the increased production of female sex hormones during adolescence is one of the factors responsible for this sexual dimorphism in the CC volume. Hence, a positive correlation is expected to be observed between duration of exposure to female sex hormones and CC volume. Multiple factors may be responsible for the absence of this relation. As previously mentioned, the first and possibly the most important factor is correcting for chronological age, which is done to ensure that the known association of age on CC volume (Chapter 4) does not confound the results. Age is well correlated with duration of exposure to female sex hormones, particularly more so in the SYS sample (r^2=0.60, p<0.0001) as compared with the IMAGEN sample (r^2=0.05, p<0.0001). The girls of the IMAGEN sample, however, have a shorter (18 ±12 months) and somewhat less variable (0 to 60 months) duration of exposure to female sex hormones as compared with the (slightly older) girls of the SYS sample (25 ±24 months; 0 to 96 months). Most girls achieve menarche at the age of 12 to 13 years and the age of most IMAGEN girls at the time of testing is close to 14 years, whereas in case of SYS girls their age at the time of testing is more evenly distributed from 12 to 18 years. Secondly, the changes in CC volume associated with sex hormone exposure period may be too subtle to be picked up by the

statistical power these studies afford. It is important to note that although these studies do not demonstrate any significant relationship between duration of exposure to female sex hormones and CC volumes they still are an important addition to existing literature because no previous study has investigated the association of the level or duration of exposure to female sex hormones with the total or regional white-matter volume of any brain region.

Puberty staging is a measure of the overall sexual maturation of the body by gonadal and adrenal sex hormones. Sex steroids are responsible for physical maturation, in the form of development of secondary sexual characteristics during puberty (Guyton et al. 2000, Grumbach et al. 2003). In 1962, Tanner introduced a method of describing the stage of pubertal development from 1 (no sexual development) to 5 (adult sexual development) based on physical examination of these secondary sexual characteristics, which include development of external genitalia, pubic hair and breasts (Dorn et al. 2006, Shirtcliff et al. 2009). Since sex hormones are responsible for guiding the development of puberty, sex hormone levels and Tanner stages are closely associated (Grumbach et al. 2003, Granger et al. 2003, Maskarinec et al. 2005). Tanner stage, however, encompasses the external/physical aspect of sexual development only. The physical development of secondary sexual characteristics in boys is induced by multiple gonadal and adrenal hormones, such as testosterone, dihydrotestosterone, dehydroepiandrosterone (DHEA) and androstenedione (Guyton et al. 2000). Testosterone mainly guides development of external genitalia and adrenal androgens (DHEA and androstenedione) cause

development of pubic hair and skin changes (Shirtciff et al. 2007, Guyton et al. 2003, Morrison et al. 1997). Bioavailable testosterone level and Tanner stage, therefore, measure two separate but correlated dimensions of sexual development. Testosterone is the primary sex hormone that guides sexual development at both an internal and external level, whereas Tanner stage defines external sexual development mediated by multiple hormones. Hence, in addition to bioavialable testosterone, the correlation of pubertal stage with the relative volume of the total corpus callosum and its segments is also examined. Puberty stage of girls (PDS based), like that of boys, is a measure of the physical sexual development induced by gonadal and adrenal hormones, mainly, estrogen, testosterone and DHEA (Guyton et al. 2000, Grumbach et al. 2003, Shirtcliff et al. 2009). Testosterone and DHEA are responsible for the development of pubic hair, whereas, estrogen induces breast development (Guyton et al. 2000, Grumbach et al. 2003, Shirtcliff et al. 2009). This study estimates pubertal stage of the adolescents by means of a self-report questionnaire (PDS) that includes questions on growth of pubic hair, facial hair, voice changes, breast development, and menarche. Puberty development scale (PDS) based puberty stages are well correlated with physician-assessed Tanner stage (r=0.65 to 0.75), which is regarded as the gold standard for measuring puberty stage (Brooks-Gunn et al. 1987, Shirtcliff et al. 2009). Moreover, PDS has been reported to capture basal sex hormone levels in parallel to physical exam (Shirtcliff et al. 2009).

In girls, there is no correlation between puberty stage and the relative volume of the total corpus callosum or any of its segments, above and beyond chronological age. In case of boys, however, puberty stage demonstrates opposing correlations with relative volume of the anterior (negative correlation) and mid-anterior (positive correlation) segments of the corpus callosum, above and beyond chronological age. It may be speculated that the absence of a relationship between puberty stage and CC volumes in girls, in contrast to that in boys, is because the influence of female sex hormones driving sexual maturation on the relative volume of the CC segments is not as strong as that of male sex hormones. It is important to note that these opposing correlations between puberty stage and the relative volumes of the anterior and mid-anterior CC segments are observed only in the boys of the SYS study and not the IMAGEN study. This may be because the boys of the SYS study demonstrate greater variance across the pubertal stages (5^{th} to 95^{th} percentile: 1.5 to 5; Tanner Stage=n boys: 2=48, 3=118, 4=143, 5=22) as compared with boys of the IMAGEN study (5^{th} to 95^{th} percentile: 2 to 4.5; Tanner Stage=n boys: 2=80, 3=500, 4=353, 5=10). Pubertal development occurs during adolescence; since the SYS study as compared with the IMAGEN study includes boys across a wider period of adolescence, these SYS boys encompass better the stages of pubertal development. Thus, studying the brains of the SYS versus IMAGEN participants is more likely to reveal the effects of puberty on the CC. The opposing correlations observed in this study between puberty stage and the relative volumes of the anterior (negative) and mid-anterior (positive) CC segments are consistent with the sex differences observed in the relative volume of the anterior (F>M) and

mid-anterior (M>F) segment in Chapter 4. Based on these findings it appears that the relative volume of the mid-anterior segment of the corpus callosum increases with sexual maturation (higher pubertal stage) more significantly in boys as compared with girls, thus resulting in a larger mid-anterior segment in boys versus girls, during adolescence. The relative volume of the anterior segment of the corpus callosum, on the other hand, decreases with sexual maturation (higher pubertal stage) more significantly in boys as compared with girls, thus resulting in a smaller anterior segment in boys versus girls, during adolescence. Sexual maturation of boys (reflected by higher pubertal stage) is induced by multiple gonadal and adrenal hormones, such as testosterone, dihydrotestosterone, dehydroepiandrosterone (DHEA) and androstenedione (Guyton et al. 2000) and relates well with the levels of these hormones (Shirtcliff et al. 2009); therefore, these findings further strengthen the hypothesis that sex differences observed in the corpus callosum during adolescence are attributed at least partly to the increased production of sex hormones during this period. It can be conjectured at this point that the increasing production of sex hormones, which drive the sexual maturation of males during adolescence, are responsible for influencing the growth of the corpus callosum. In addition, it is also speculated that the combined signals provided by multiple sex hormones of gonadal and adrenal origin have a stronger influence on the corpus callosum as compared with testosterone signal alone; since gonadal and adrenal hormone dependent pubertal stage is associated with callosal volumes but testosterone level alone is not. The specific effect of different male sex hormones on the volume of specific callosal segments, however, remains unproven. Further

research in this direction using longitudinal studies is required to establish such a cause-effect relationship.

There is a topographic representation of cortical areas connected by callosal fibres along its antero-posterior length (Pandya et al. 1971, De LaCoste et al. 1985, Hofer et al. 2006, Zarei et al. 2006). Knowledge of this topographic representation enables us to speculate on possible functional correlates of sex hormones. The anterior segment of the corpus callosum, which is negatively associated with puberty stage in boys, is responsible for carrying fibres between the prefrontal areas of the left and right frontal lobe, whereas the mid-anterior segment of the corpus callosum, which is positively associated with puberty stage in boys, is responsible for carrying fibres between the premotor areas of the left and right frontal lobe (Pandya et al. 1971, De LaCoste et al. 1985, Hofer et al. 2006, Zarei et al. 2006). It is possible that the opposing changes observed in the volume of the anterior and mid-anterior segments with sexual maturation reflect differential development of inter-hemispheric connections between the cortical areas connected by these two segments, respectively.

In summary, this study demonstrates a possible role of gonadal and adrenal male sex hormones, which drive pubertal maturation, in sexual differentiation of the relative volume of the corpus callosum, particularly its anterior and mid-anterior region, during adolescence. This is the first time a possible role of sex hormones in sexual dimorphism of CC volume has been demonstrated in humans.

7 VOLUME AND STRUCTURAL PROPERTIES OF THE CORPUS CALLOSUM AS A FUNCTION OF MENSTRUAL CYCLE AND ORAL CONTRACEPTIVES

7.1 INTRODUCTION

Sexual dimorphism in the structure and function of the corpus callosum is an extensively researched topic (Section 1.6). Reviewing the literature revealed that early adolescence was the period when reports of a sex difference in the relative volume of the corpus callosum first began to appear (see Section 1.6). These reports became more frequent towards late adolescence and, by adulthood, sex differences in the relative volume of the corpus callosum were most robust (see Section 1.6). Moreover, this thesis confirms the presence of sex differences in the relative volume of the CC during adolescence (see Chapter 4). It is possible that sex hormones, which induce development of the differentiating secondary sexual characteristics of boys and girls during adolescence, are also one of the factors responsible for development of sex differences in the corpus callosum. This chapter examines the role of sex steroid-hormones in the macro-structural (volume), micro-structural (magnetization transfer ratio as an index of myelin content) and functional (bimanual coordination) properties of the corpus callosum. This is done by studying these variables in four phases of the menstrual cycle of both freely cycling women and women using oral contraceptive pills (OCP).

The main sex hormones, namely testosterone, estrogen and progesterone, are lipid-soluble steroids that are synthesized and secreted primarily by the gonads. These steroids cross the blood-brain barrier and enter the brain where they bind to intracellular steroid receptors and exert their influence on the brain (reviewed by Beyer 1999). Sex steroids are known to act as trophic factors that influence various aspects of brain development, particularly myelination of white matter (Baulieu et al. 2000, Marin-Husstege et al. 2004, reviewed by Beyer et al. 1999 and Peper et al. 2011,). This provides a biological basis underpinning the hypothesis that sex steroids may be one of the factors responsible for sexual differentiation of the relative volume of the corpus callosum.

There is dearth of literature investigating the effects of menstrual cycle-phase on brain structure and function. Most of the research on reproductive cyclicity has been done on experimental animals. In one such study, Lagnub and Watson (1994) investigated the effect of estrous cycle-phase on the morphology of the anteroventral paraventricular nucleus of the rat hypothalamus. They found a 39% increase in axosomatic synapses during the estrus (ovulatory) versus proestrus (early follicular) phase and a 22% decrease in axosomatic synapses in the metestrus (early luteal) versus estrus phase. But this finding does not indicate necessarily general effects of cycle-phase on the brain because the anteroventral paraventricular nucleus of the hypothalamus is responsible for stimulating the secretion and feedback regulation of the sex steroids. It is, therefore, the classic target area for estrogen and has the highest number of estrogen and progesterone receptors in the brain. Nonetheless, sex steroids have

been found to affect other areas of the brain as well. Carillo and Collado (2007) observed that cycle phase affects morphology of the anteroventral medial amygdala in the rat. They showed an increase in the number of Nissl stained neurons and NADPH diaphorese positive neurons in this region during the estrus (follicular) phase as compared with the diestrus (ovulatory) phase. This clearly demonstrates that the neuronal architecture within the brain, even in regions other than those directly involved in regulating the menstrual cycle, were influenced by the cyclic variation in sex steroids over a short period (4-5 days for rats).

Human studies reporting the effects of menstrual cycle-phase and oral contraceptives on brain volume and morphology are few. In one such report, Protopopescu et al. (2008) studied the structural changes in the hippocampus and basal ganglia across the menstrual cycle. They observed that the absolute volume of grey matter in the right anterior hippocampus to be greater in late follicular phase (day 10 to day 12) as compared with the luteal phase. On the other hand, the absolute volume of grey matter in the right dorsal basal ganglia was seen to be greater in luteal phase as compared with the late follicular phase. Similarly Pletzer et al. (2010) observed that women had significantly greater grey-matter volume in the right fusiform/parahippocampal gyrus during the early follicular phase as compared with the mid-luteal cycle phase. They also observed significantly greater regional volumes of grey matter in the prefrontal cortex, pre- and postcentral gyri, parahippocampal and fusiform gyri and temporal regions, in women using oral contraceptives as compared with those not using

oral contraceptives. Regarding the association of oral contraceptives with the brain, they observed that the grey-matter volume of the prefrontal cortices, pre- and postcentral gyri, parahippocampal and fusiform gyri and temporal regions was significantly greater in women using oral contraceptives as compared with those not using oral contraceptives. Some studies examined the association of hormone replacement therapy (HRT) with global and regional brain volumes. Hormone replacement therapy is a treatment in the form of synthetic estrogen and progesterone, typically given to post-menopausal women to supplement their diminished circulating levels of natural sex steroids. Hence it artificially increases levels of sex steroids of post-menopausal women. Ha et al. (2007) compared the volume of white matter, grey matter and ventricular CSF of 10 postmenopausal women having over 20 years exposure to synthetic estrogen (23 ±9 years of HRT) with 10 age-matched postmenopausal women having no estrogen exposure and 10 healthy young women. They observed that postmenopausal women using HRT compared with non-HRT users had significantly greater white-matter and smaller CSF volume and similar grey-matter volume. Postmenopausal women using HRT compared with young women had significantly greater CSF and smaller grey-matter volume and similar white-matter volume. Similarly, Erikson et al. (2005) also investigated the association of hormone replacement therapy with the volume of grey matter and white matter in the brain in three groups of elderly postmenopausal women; 16 current HRT users, 14 past users of HRT (not uses HRT for the last year) and 13 non users in their study. They generated standardized grey- and white-matter maps from high resolution MR images and employed voxel-based morphometry

to assess the impact of hormone replacement therapy on the volume of grey matter and white matter in a voxel-wise fashion. They observed that HRT users (irrespective of group) as compared with non-HRT users had greater volume of white matter in the medial temporal lobe regions and greater volume of grey matter in the prefrontal, parietal and temporal brain regions (see Figure 7.1).

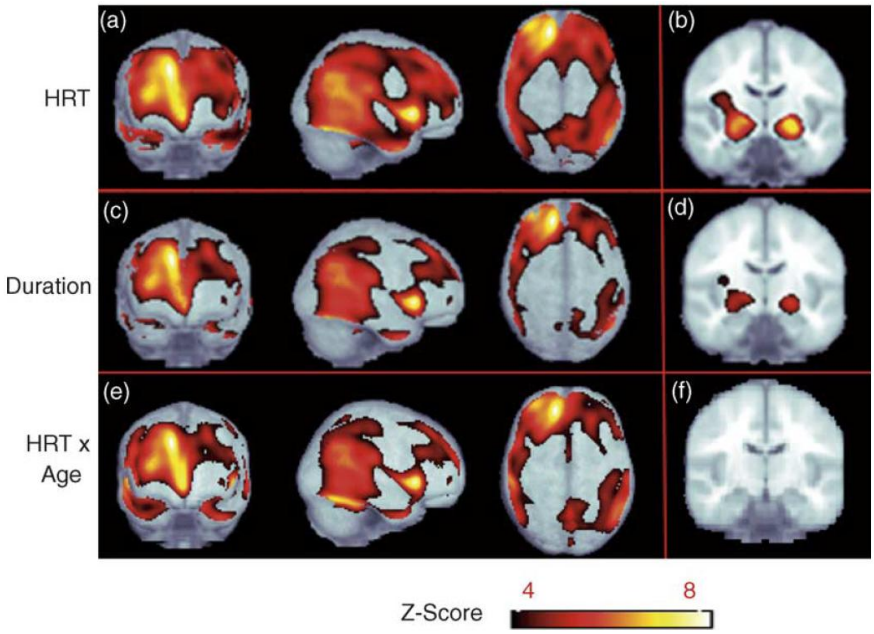

Figure 7.1: MR maps showing (a) the effect of HRT on grey-matter and (b) white-matter volume; (c) the effect of duration of HRT use on grey-matter and (d) white-matter volume; (e) the interactive effect of HRT and age on grey-matter and (f) white-matter volume. From Erikson et al. 2005.

Fluctuating levels of sex steroids across the menstrual cycle are known to influence both cortical functions and the cerebral organization of these cortical functions, such as lateralization. Hampson et al. (1990) examined the performance of women (n=45, 19 to 39 years) in multiple cognitive domains across the menstrual and mid-luteal phases of the menstrual cycle. They demonstrated that women perform significantly better in tasks of spatial ability (space relations test, hidden figures test and portable rod & frame test) and deductive reasoning (inference test) during the menstrual versus the mid-luteal

phase, whereas the reverse pattern was observed for articulation (speeded counting, colour reading & naming and syllable repetition). Manual speed (Purdue pegboard, simple finger tapping and manual sequence box) and perceptual speed (number comparisons, identical pictures and subtraction & multiplication) were not significantly different between the two phases. Similarly, Hausmann et al. (2000) demonstrated that women (n=12, 23 to 38 years) perform significantly better in 3D mental rotation task during the menstrual phases as compared with the mid-luteal phase. Maki et al. (2001) observed that women (n=16, 18 to 28 years) perform better in tests of implicit memory, verbal fluency and fine motor performance (grooved pegboard) during the mid luteal versus the menstrual phases, whereas the opposite pattern was observed for performance on mental rotation tasks. Based on these studies it seems that cognitive abilities in which males excel over females, such as mental rotation (Kaufman et al. 2007, Halpern et al. 2000, Lynn et al. 1994) are performed better in estrogen and progesterone poor versus rich phases of the menstrual cycle; whereas cognitive abilities in which females excel over males, such as fluency and memory (Ankney et al. 1995, Kimura 1992) are performed better in estrogen and progesterone rich (mid-luteal) versus poor (menstrual) phases of the menstrual cycle. Functional asymmetry in behavioural tasks such as dichotic listening tasks and tachistoscopic tasks also varies across the menstrual cycle (Cowell et al. 2011, Sanders et al. 1998). Language lateralization based on the asymmetry scores for speech perception is greater during the mid-luteal phases as compared with the menstrual phase (Cowell et al. 2011, Sanders et al. 1998). This change in asymmetry results from a decrease in left ear scores and an

increase in right ear scores as the women go from the menstrual to mid-luteal phase. Similarly, laterality scores for tachistoscopic tests suggest greater laterality during the mid-luteal phase as compared with the menstrual phase (Bibawi et al. 1994, Mead and Hampson 1996).

This study is aimed at examining the influence of female sex steroids on the structure and function of the corpus callosum. This possibility is explored by studying the association between the phase of the menstrual cycle and the use of oral contraceptives on one hand with the structure (volume and MTR) and function (bimanual coordination) of the corpus callosum on the other hand. Specifically, this study investigates the effect of menstrual cycle phase and oral contraceptive use on the following structural and functional properties of the corpus callosum: 1) relative volume of the total corpus callosum and its five segments (anterior, mid-anterior, central, mid-posterior and posterior); 2) MTR value of the total corpus callosum and its five segments; and 3) performance on an altered finger-tapping task. At the time of designing this study the lack of a correlation between the CC and bimanual coordination (in Chapter 5) was not known. This is done in the “Cycle” study by examining a sample that is comprised of two groups of young women, those who are freely cycling and those who are using the oral contraceptive pill. All women are tested in four different phases of their menstrual cycle. For each time point, the relative volume and MTR value of the total corpus callosum and its segments is estimated, and bimanual coordination in the altered finger-tapping task is assessed. Measuring MTR values along with the CC volumes enables determination of whether (expected)

variations in the volumes might be related to the variations in the content of myelin. The myelinated axonal membranes of white matter have been shown to be the prime determinants of MTR (Schmierer et al. 2004). Thus, volumetric changes associated with likewise changes in MTR value can be attributed to an increase in the degree of myelination of callosal axons.

In addition to the “Cycle” study, this chapter also examines the association of the use of oral contraceptives with the relative volume of the total corpus callosum and its segments in two subsamples from the SYS and IMAGEN adolescent studies, respectively; each of these two subsamples consists of 50 OCP using girls and 50 age-matched non-OCP using girls. It is hypothesized that individuals with different sex hormonal milieus related to differences in the phase of their menstrual cycle or in the use of oral contraceptives will exhibit differences in the structure (relative volume and MTR) and function (bimanual coordination) of the corpus callosum. Based on the positive association of sex steroids, both male (Perrin et al. 2008) and female (Ha et al. 2007, Erikson et al. 2005) with the structure of the corpus callosum, a positive effect of high sex steroid states versus low sex steroid states is expected to be observed on the structure (volume and MTR) of the corpus callosum with possible consequences on its function (bimanual coordination). High sex steroid states include the estrogen rich ovulatory phase and progesterone rich luteal phase as opposed to the menstrual and follicular phase. Oral contraceptives alter the normal sex steroidal environment of the body by suppressing the natural production of testosterone, estrogen and progesterone by the gonads, but at the same time they supply an

exogenous source of synthetic estrogen and progesterone to the body. The levels of these synthetic female sex steroids fluctuate considerably across the day with greater peaks and troughs and lower mean daily levels as compared with freely cycling women. Based on the suppressed production of testosterone and reduced mean daily levels of estrogen and progesterone in OCP using women, they are considered to constitute a low sex steroid state as compared with freely cycling women.

7A.2 METHODOLOGY: CYCLE STUDY

7A.2.1 Participants

All participants are women between 18 to 30 years recruited from the University of Nottingham. Posters advertising the study are posted on campus and interested women are recruited after an initial interview assessing the following eligibility criteria. Inclusion criteria are as follows: 1) Age between 18 to 30 years; 2) Regular menstrual cycle of 28 to 35 day length, in the case of free-cycling women; and 3) Regular use of monophasic pill, in the case of pill-using women. Exclusion criteria are as follows: (1) MRI contraindications; (2) Brain anomalies noted on MRI scans. A total of 26 subjects are included in the study: 13 freely cycling women and 13 OCP using women.

For each participant, four scanning sessions are organized during four different phases of their menstrual cycle (with the first day of menstruation regarded as Day 1). These phases are as follows: 1) menstrual phase (Day 5 +/- 2); 2) follicular

phase (Day 11 +/- 2); 3) mid-cycle phase (Day 15 +/-2); and 4) premenstrual phase (Day 28 +/- 2). The cycle length of the free-cycling women was variable, unlike that of the pill-using women. At each visit the participants had a morning blood sample taken, followed by an MRI examination and then performed an alternate finger tapping test.

All women completed four visits except for one who left the study prematurely and is not scanned in the luteal phase. An equal number of participants started the protocol in each cycle-phase so that the visit number does not confound the effect of cycle-phase on the results.

This study is approved by the Ethics Board of the Medical School of the University of Nottingham.

7A.2.2 Serum 17 β-Estradiol and Progesterone

Blood samples are taken in the morning between 9:00 and 10:30 a.m. For each participant 5 ml of blood is collected. Following collection the blood is centrifuged to separate the serum. The separated serum is alliquoted into 2 ml polypropylene tubes and stored at -70 degrees until required for assays.

The serum level of 17 β-estradiol (pmol/L) is analyzed at the Queens Medical Centre in Nottingham using the ADVIA Centaur Estradiol assay (Siemens). This is an immunoassay that uses chemiluminescent technology to measure the level of estradiol in participant serum. It is a competitive immunoassay based on antigen-linked technology and chemilumenescence detection. It uses monoclonal

antibody derived by coupling estradiol immunogen at the specificity enhancing sixth position.

The serum level of progesterone (nmol/L) is analyzed at the Queens Medical Centre in Nottingham using the ADVIA Centaur Progesterone assay (Siemens). This is also an immunoassay that uses chemiluminescent technology to measure the level of progesterone in participant serum.

7A.2.3 Alternating Finger Tapping Task

This task is developed in the Paus laboratory at the Montreal Neurological Institute by Dr. Valeria Della Maggiore. It is designed to measure bimanual coordination based on the degree of accuracy with which an individual can oppose his fingers to a rhythm of increasing frequency (Johansen-Berg et al. 2007).

Each participant listens to 10 rhythms, each comprised of a 20-second beep sequence starting at a frequency of 1 Hz with a stepwise increase of 0.25 Hz to reach a maximum frequency of 3.25 Hz. The participants are expected to coordinate their finger movements to the beeps in such a fashion that they produce a right index finger-thumb opposition movement to each beep and a left index finger-thumb opposition movement in between two beeps (i.e. out of phase movement of the left and right index/thumb opposition). Special sensors attached along the metacarpo-pharangeal joint of the index finger record these movements as a series of graphical curves. The movements are recorded for a

12-second period for each frequency and subsequently analyzed to determine the degree of coordination between the angle time-series for both hands. This is calculated as a correlation value for each of the 10 frequencies, at each visit. The 'Best Correlation' is selected as the correlation value that is closest to +/-1. The 'average correlation' is the mean of the correlations across the 10 frequencies. Best and Average correlation are the two main measures of bimanual performance on this task.

During the index finger-thumb opposition movement the wire of the sensors curves along the C-shaped contour formed beside the junction of the index finger and the thumb. In some cases, however, the wire inverted in an opposing direction to this C-shaped curve. This resulted in abnormally low correlation values being recorded. Correlation values for bimanual coordination of three participants are excluded because they experienced this problem during multiple (>1) visits. Of the remaining 23 participants, 4 participants are missing correlation values for a single time point (participant number × time points: 4×1 missing of 23×4) and 2 participants have correlation values beyond 3 standard deviation for a single time point (2×1 beyond ±3 SD of 23×4).

7A.2.4 MR Image acquisition

All scanning is performed on a 1.5 T scanner (Philips Achieva Scanner, Philips Medical System, Best, The Netherlands). The images acquired for each woman across four time points and their acquisition parameters are as follows: (1) 160 slice high resolution T1W image (3D magnetization prepared rapid acquired

gradient echo, that is MPRAGE, sequence) with TR=9.9 ms, TE=4.6 ms and 1 mm isotropic resolution; 2) 70 slice Magnetization Transfer (MT) image acquired using 3D Fast Field Echo (FFE) magnetization transfer sequence with TR=25 ms, TE=4.6 ms, flip-angle=10^0, 2 mm isotropic resolution with and without an MT saturation pulse (composite RF pulse with 90 degrees y-180 degrees x-90 degrees y sequence applied on-resonance).

7A.2.5 MRI Volumetric Analysis

FreeSurfer version 5.0.0 is used for the automated volumetric analysis, performed on native, high resolution T1-weighted images. The FreeSurfer analysis pipeline employed has been described in detail in Chapter 4, Section 4A.2.3. Briefly, the T1W images are intensity-corrected and normalized and subsequently brain extraction performed. Following this pre-processing, the native images undergo non-linear registration to the MNI305 template (Evans et al. 2003). The CC is segmented on this standardized T1W image by utilizing a probabilistic atlas. The segmented images are converted back to their native form by applying the inverse of the transformation matrix previously used and absolute volume of the total corpus callosum and its segments is then calculated. The total corpus callosum is also parcellated into five segments (posterior, mid-posterior, central, mid-anterior and anterior) and their volumes calculated. The relative volumes of the total corpus callosum and its five segments are then calculated as a percentage of the total brain volume. The CC segmentations of all scans are visually inspected by a trained expert (INK) as part of a quality check, which resulted in no exclusions. Moreover, all volumes calculated are within 3

standard deviations of the mean. One participant is missing a value for the CC volume of a single time point (participant number × time points: 1×1 of 26×4). For more details on the volumetric analysis refer to Chapter 4, Section 4A.2.3.

7A.2.6 MTR Analysis

Magnetization transfer (MT) images are acquired to provide an indirect index of myelination (Schmierer et al. 2004). Pre-processing of the MTR images involves brain extraction, which is performed using the BET2 command (http://www.fmrib.ox.ac.uk/analysis/research/bet/). Computational analysis of the MT images is subsequently performed using minc software developed by MNI BIC (http://www.bic.mni.mcgill.ca/ServicesSoftware/HomePage). The ratio between MT on and MT off images is calculated to generate MTR maps (Pike, 1996). These MTR maps are registered to the native T1W images using a 6-parameter transformation; the FreeSurfer corpus callosum masks are used to "mask" the MTR maps to calculate the mean MTR value for the white matter in the corpus callosum in each segment. These 6-parameter registrations are visually inspected by a trained expert (INK) and all scans that are poorly registered are excluded (n=4). Additionally, outliers whose mean MTR value is above or below three standard deviations (SD) of the mean are also excluded (n=2). For statistical analysis this study includes MTR data of women whose MTR values for up to a single visit are unavailable (due to poor quality or MTR values beyond ±3 SD) but not that of women whose MTR values are unavailable across multiple visits (>1). Overall statistical analysis is performed on MTR values of 24 participants. Of these 24 participants: 1) three participants are missing the MTR

values for a single time point (3×1 missing of 24×4); 2) one participant has an MTR value beyond 3 standard deviation for a single time point (1×1 beyond ±3 SD of 24×4); and 3) three participants have unreliable MTR values for a single time point due to poor quality of the scans (3×1 poor quality of 24×4).

7A.2.7 Statistical Analysis

All statistical analysis is performed using the SPSS software, PASW Statistics 18.0. The association of OCP use and phase of the menstrual cycle with all dependent variables (sex steroid levels, relative CC volumes and task performance) are analyzed using repeated-measures ANOVA. Each analysis is conducted using pill status as the between-subject factor and menstrual cycle-phase as the within-subject factor. Post-hoc analysis is conducted using pairwise analysis to compare different cycle phases in the two groups, OCP using group and freely cycling group, separately. Independent T tests are used to compare freely cycling group with OCP using across specific phases of the menstrual cycle.

Missing values, removal of outliers (±3 Standard Deviation) and quality check resulted in loss of 1% of the data values. When conducting repeated measure ANOVA, SPSS includes only those participants with values for all time points; participants with missing values for any one of the four time points, that is cycle phases, are automatically excluded. Hence, in order to maintain sample size imputation is performed. In the case of sex steroid levels and relative volumes of the corpus callosum, mean substitution is performed in view of the limited amount of data missing for these variables at random (0.01%). This is done by

calculating each variable mean across the four phases of the two groups, OCP using group and freely cycling group, separately and subsequently replacing the missing values by the mean value for that particular phase and group. It is important to note that the results for effect of cycle phase and pill use on these variables remains the same both before and after imputations. This chapter reports the results for these variables that are obtained post imputation.

Mean substitution causes loss in variance and the extent of this loss depends on the amount of missing data. Thus, in case of the MTR values of the CC and correlation scores for bimanual coordination multiple imputations using linear regression are performed because relatively more values are unavailable as compared with volumetric and hormonal data, on account of three factors: missing values (0.04% for both), poor quality check (0.04% for MTR only) and outliers beyond ±3 SD (0.02% for both). Specifications of the multiple imputation method employed are as follows: 1) Imputation method: the default automatic method selected Fully Conditional Specification (FCS); 2) number of imputations: five, since three to five imputed datasets are said to be sufficient for good inferences; and 3) model for scale variables: linear regression. The predictor variables entered into the multiple imputation model include all the analysis variables with missing data, that is, MTR value for the total corpus callosum and all its segments along with cycle phase and pill status. Repeated measure ANOVA analysis is performed on five separate datasets created via multiple imputations and the average parameter estimates for these five analyses are reported. It is important to note that the results for effect of cycle phase and pill use on these

variables that are obtained via both mean substitution and multiple imputation method is similar. This chapter demonstrates the results for these variables that are obtained post multiple imputation method. Additionally, it is also important to note that the pattern of missingness for the bimanual coordination scores and MTR data is random (MAR: missing at random). This is assessed by replacing the missing values for each variable with “0” and the available ones with “1”, and then varying the pattern of missingness for each variable as a function of variables employed in the analysis (mentioned above), along with other major variables such as age, length of cycle and pill duration. No significant correlations are observed between the pattern of missingness and all major variables.

Before considering the results, a brief discussion on the confounding factors that are controlled for is provided. When examining the association of the menstrual cycle phase and OCP use with the structure and function of the corpus callosum, age is controlled by matching the age of the free cycling women with the pill using women. There is no significant difference between the mean age of the free cycling and the pill using women. The second possible confounder corrected for is total brain volume. Furthermore, the volumes of the callosal segments correlate with each other and so do the MTR values of the CC segments. Unlike in the analyses reported in the previous chapters, here this inter-segmental dependence is not corrected for due to the limitations provided by this study’s sample size. Using the segmental volumes and MTR value as covariates while examining the association of OCP use and cycle phase on a specific segment will provide too stringent a test given the small sample size of this study. Moreover,

the within-subject design of the study reduces the potential limitations of not correcting for inter-segmental dependence. This is because each participant is being compared with herself across different phases of the menstrual cycle, thus resulting in limited variation, if any, in inter-segmental co-relatedness across the phases.

7B.2 METHODOLOGY: SYS STUDY

7B.2.1 Participants

This study is conducted on a subsample of 106 girls from the SYS study sample, which is comprised of 12-to-18 year old adolescents of French-Canadian origin recruited from local high schools in Saguenay Lac Saint-Jean (SLSJ) region of Quebec, Canada. This subsample is comprised of 53 OCP using girls and 53 age-matched girls not using oral contraceptive pills. For the matching, first all the girls not using oral contraceptives are identified who have the same age (in months) as each OCP using participant. Of all the non-OCP using girls age-matched for each OCP using girl, one is selected at random. In this subsample, the association of the use of oral contraceptive pill with the relative volume of the total corpus callosum and its segments is investigated. (For more details on the SYS participants refer to Chapter 2, Section 2A).

7B.2.2 Oral Contraceptive Pill Use

Oral contraceptive pill-use in girls is assessed via a medical questionnaire filled out by the mother during the initial home visit. In this questionnaire, mothers are

asked to specify which medicines (if any) are taken by their children. Based on the answer to this question, use of oral contraceptive pills in girls is assessed. (For more details refer to Section 2A.7).

7B.2.3 MRI Acquisition

For each participant T1 weighted MRI scans are obtained on a Philips 1.0 T superconducting magnet. Acquisition parameters of these structural volume scans are as follows: 3D RF spoiled gradient-echo scan, 140-160 high resolution T1 weighted images, with TR=25 ms, TE=5 ms, flip angle=30^0 and 1 mm isotropic resolution.

7B.2.4 MRI Volumetric Analysis

FreeSurfer version 5.0.0 is used for the automated volumetric analysis, performed on native, high resolution T1-weighted images. Details of the volumetric analysis have been described previously in Chapter 4, Section 4A.2.3.

7B.2.5 Statistical Analysis

All statistical analysis is performed using the SPSS software, PASW Statistics 18.0. The association (β, standardized regression coefficient) of oral contraceptive pill use with the relative (%) volumes of the total corpus callosum and its segments is assessed via linear regression. The linear regression model is run with the CC volume measures (relative volume of the total corpus callosum and its five segments, namely, anterior, mid-anterior, central, mid-posterior and posterior

segment) specified as the dependent variables. There are two blocks of independent variables. The first block consists of the following independent variables: 1) prenatal exposure to maternal cigarette smoking and 2) puberty stage. There is no need to correct for age, since the freely cycling girls are age-matched to the OCP using girls in this subsample. The use of oral contraceptive pill is specified as the independent variable in the second block. Just as in the cycle study, this study does not correct for inter-segmental dependence as well due to the limitations posed by sample size and in order to maintain uniformity in analytical method for better comparison of results. For each linear regression model, the adjusted R^2, standardized regression coefficient (β), t value for the regression coefficient and the significance of the t value are calculated.

7C.2: METHODOLOGY: IMAGEN STUDY

7C.2.1 Participants

The IMAGEN subsample consists of a total of 104, 13 to 15 year old girls recruited from eight centres across England, Ireland, France and Germany. The association of oral contraceptive pill use with the relative volume of the total corpus callosum and its segments is investigated in this subsample, comprising of 52 OCP using girls and 52 centre-matched and age-matched non-OCP using girls. For the matching, first all the girls not using oral contraceptives are identified who have the same age (in months) as each OCP using participant and belong to the same centre. Of all the non-OCP using girls centre- and age-matched for each

OCP using girl, one is selected at random. (For more details on the IMAGEN participants refer to Chapter 2, Section 2B).

7C.2.2 Oral Contraceptive Pill Use

Oral contraceptive pill use in girls is assessed via a time line follow back (TLFB) questionnaire on drug use filled out by a researcher interviewing the child. In this questionnaire, the researcher asks the participants to specify which prescription medicines (if any) were taken by them in the last month. Based on the answer to this question, use of oral contraceptive pills in girls is assessed.

7C.2.3 MRI Acquisition

T1 weighted MRI scans of the participants are obtained on a Philips 3.0 T superconducting magnet, from a variety of manufacturers (Siemens, Philips, General Electric and Bruker). Acquisition parameters of these structural volume scans are as follows: 160-170 high resolution T1W images, with TR=2300 ms, TE=2.8 ms, flip angle=8-9^0 and 1.1 mm isotropic resolution.

7C.2.4 MRI Volumetric Analysis

FreeSurfer version 5.0.0 is used for the automated volumetric analysis, performed on native, high resolution T1-weighted images. The procedure of volumetric analysis is identical to that explained for the Saguenay Youth Study (Section 4A.2.3).

7C.2.5 Statistical Analysis

All statistical analysis is performed using the SPSS software, PASW Statistics 18.0. The association (β, standardized regression coefficient) of OCP use with the relative volumes (%) of the total corpus callosum and its segments is assessed via a linear regression model, similar to that used in the SYS subsample of OCP using versus non-OCP using girls. The only difference in the linear regression model used is that it does not control for prenatal exposure to maternal cigarette smoking (PEMCS).

7A.3 RESULTS: CYCLE STUDY

7A.3.1 Demographics

The mean age of women in the freely cycling (n=13) and OCP using (n=13) groups are 21 (SD: ±3) years and 22 (±3) years, respectively. There is no significant difference in their mean age. Duration for which the OCP using women consumed oral contraceptives varies from 3 to 36 months (mean: 29 ±29 months). Three main kinds of oral contraceptives are used, microgynon, ovranette and femodene. Descriptive statistics of these demographic details are provided in Table 7A.1.1. Mean values of the: 1) plasma levels of sex steroids (Table 7A.1.2); 2) relative volumes of the total corpus callosum and its segments (Table 7A.1.3 and 7A.1.4); 4) MTR values of the total CC and its segments (Table 7A.1.3 and 7A.1.5); and 5) average and best correlation scores for performance on the AFT task (Table 7A.1.6), of the freely cycling and OCP using women across

different menstrual cycle phases are described in Table 7A.1.2 to 7A.1.6. Pearson's correlation between the volume and MTR value of the corpus callosum of the freely cycling and OCP using women across different phases of the menstrual cycle are described in Table 7A.1.7.

Table 7A.1.1 Cycle Study: Sample Demographics and Descriptive Statistics for Menstrual Cycle and OCP use				
	Sample Size	**Age (yrs) Mean (SD)**	**Cycle Length (days) Mean (SD)**	**Pill Duration (months) Mean (SD)**
FC	*13*	*21.2 (3)*	*29 (2)*	*0 (0)*
OCP	*13*	*22 (3)*	*28 (0)*	*29 (29)*

Table 7A.1.1 *Cycle Study: The sample size (n) along with the mean and SD (in brackets) for the following variables: age (years), length of menstrual cycle (days) and duration of oral contraceptive pill (OCP) use (months) in both freely cycling (FC) and oral contraceptive pill using (OCP) women.*

Table 7A.1.2 Cycle Study: Descriptive Statistics for Sex Steroid Levels		
	Progesterone (nmol/L) Mean (SD)	**Estrogen (pmol/L) Mean (SD)**
FC	**9.33 (10.96)**	**400 (354)**
Menstrual	*3.78 (1.39)*	*148 (62)*
Follicular	*3.85 (1.11)*	*388 (325)*
Ovulatory	*9.93 (11.51)*	*553 (449)*
Luteal	*19.77 (13.79)*	*511 (339)*
OCP	**2.63 (0.88)**	**112 (67)**
Menstrual	*2.83 (0.98)*	*137 (114)*
Follicular	*2.68 (0.74)*	*117 (46)*
Ovulatory	*2.52 (0.94)*	*94 (41)*
Luteal	*2.50 (0.92)*	*99 (32)*

Table 7A.1.2 *Cycle Study: The mean and SD (in brackets) for the level of estrogen (pmol/l) and progesterone (nmol/l) in both freely cycling (FC) and oral contraceptive pill (OCP) using women across the four phases of the menstrual cycle (menstrual, follicular, ovulatory and luteal phase).*

Table 7A.1.3 Cycle Study: Descriptive Statistics for Relative Volume and MTR value of the Total Corpus Callosum		
	Relative Volume (%) Mean (SD)	**MTR Value Mean (SD)**
FC	**0.289 (0.02)**	**0.643 (0.01)**
Menstrual	*0.289 (0.02)*	*0.642 (0.01)*
Follicular	*0.289 (0.02)*	*0.646 (0.01)*
Ovulatory	*0.287 (0.02)*	*0.644 (0.01)*
Luteal	*0.292 (0.02)*	*0.640 (0.01)*
OCP	**0.273 (0.03)**	**0.641 (0.01)**
Menstrual	*0.272 (0.03)*	*0.642 (0.01)*
Follicular	*0.272 (0.03)*	*0.640 (0.01)*
Ovulatory	*0.276 (0.03)*	*0.644 (0.01)*
Luteal	*0.274 (0.03)*	*0.638 (0.01)*

Table 7A.1.3 *Cycle Study: The mean and SD (in brackets) for the relative volume (%) and MTR value of the total corpus callosum in both freely cycling (FC) and oral contraceptive pill (OCP) using women across the four phases of the menstrual cycle (menstrual, follicular, ovulatory and luteal phase).*

Table 7A.1.4 Cycle Study: Descriptive Statistics for Relative Volumes of the CC Segments (%)					
	Anterior Mean (SD)	**MidAnterior Mean (SD)**	**Central Mean (SD)**	**MidPosterior Mean (SD)**	**Posterior Mean (SD)**
FC	**0.0775 (0.01)**	**0.0438 (0.01)**	**0.0451 (0.01)**	**0.0423 (0.01)**	**0.0805 (0.01)**
Menstrual	*0.0764 (0.01)*	*0.0443 (0.01)*	*0.0445 (0.01)*	*0.0424 (0.01)*	*0.0813 (0.01)*
Follicular	*0.0779 (0.01)*	*0.0432 (0.01)*	*0.0450 (0.01)*	*0.0427 (0.01)*	*0.0800 (0.01)*
Ovulatory	*0.0762 (0.01)*	*0.0441 (0.01)*	*0.0449 (0.01)*	*0.0421 (0.01)*	*0.0801 (0.01)*
Luteal	*0.0795 (0.01)*	*0.0438 (0.01)*	*0.0460 (0.01)*	*0.0421 (0.01)*	*0.0806 (0.01)*
OCP	**0.0747 (0.01)**	**0.0397 (0.01)**	**0.0408 (0.01)**	**0.0389 (0.01)**	**0.0792 (0.01)**
Menstrual	*0.0743 (0.01)*	*0.0396 (0.01)*	*0.0399 (0.01)*	*0.0394 (0.01)*	*0.0790 (0.01)*
Follicular	*0.0749 (0.01)*	*0.0389 (0.01)*	*0.0406 (0.01)*	*0.0380 (0.01)*	*0.0793 (0.01)*
Ovulatory	*0.0747 (0.01)*	*0.0403 (0.01)*	*0.0423 (0.01)*	*0.0393 (0.01)*	*0.0799 (0.01)*
Luteal	*0.0750 (0.01)*	*0.0401 (0.01)*	*0.0404 (0.01)*	*0.0391 (0.01)*	*0.0789 (0.01)*

Table 7A.1.4 *Cycle Study: The mean and SD (in brackets) for the relative volume (%) of the CC segments in both freely cycling (FC) and oral contraceptive pill (OCP) using women across four phases of the menstrual cycle (menstrual, follicular, ovulatory and luteal phase).*

Table 7A.1.5 Cycle Study: Descriptive Statistics for MTR Values of the CC Segments					
	Anterior Mean (SD)	**MidAnterior** Mean (SD)	**Central** Mean (SD)	**MidPosterior** Mean (SD)	**Posterior** Mean (SD)
FC	**0.653 (0.01)**	**0.643 (0.01)**	**0.635 (0.01)**	**0.627 (0.01)**	**0.647 (0.01)**
Menstrual	*0.655 (0.01)*	*0.642 (0.01)*	*0.632 (0.01)*	*0.626 (0.01)*	*0.645 (0.01)*
Follicular	*0.655 (0.01)*	*0.647 (0.01)*	*0.639 (0.01)*	*0.634 (0.01)*	*0.649 (0.01)*
Ovulatory	*0.651 (0.01)*	*0.644 (0.01)*	*0.639 (0.01)*	*0.632 (0.01)*	*0.647 (0.01)*
Luteal	*0.651 (0.01)*	*0.639 (0.01)*	*0.629 (0.01)*	*0.621 (0.02)*	*0.647 (0.02)*
OCP	**0.652 (0.01)**	**0.639 (0.01)**	**0.629 (0.01)**	**0.625 (0.01)**	**0.647 (0.01)**
Menstrual	*0.652 (0.01)*	*0.641 (0.01)*	*0.628 (0.01)*	*0.626 (0.01)*	*0.648 (0.01)*
Follicular	*0.651 (0.01)*	*0.638 (0.01)*	*0.639 (0.01)*	*0.622 (0.01)*	*0.645 (0.01)*
Ovulatory	*0.656 (0.01)*	*0.643 (0.01)*	*0.634 (0.01)*	*0.629 (0.01)*	*0.648 (0.01)*
Luteal	*0.648 (0.01)*	*0.635 (0.02)*	*0.626 (0.02)*	*0.618 (0.01)*	*0.645 (0.01)*

Table 7A.1.5 *Cycle Study: The mean and SD (in brackets) for the MTR value of the segments of the corus callosum in both freely cycling (FC) and oral contraceptive pill (OCP) using women across four phases of the menstrual cycle (menstrual, follicular, ovulatory and luteal phase).*

Table 7A.1.6 Cycle Study: Descriptive Statistics for Bimanual Performance on Altered Finger Tapping Task		
	Average Correlation Mean (SD)	**Best Correlation** Mean (SD)
FC	**0.623 (0.107)**	**0.853 (0.076)**
Menstrual	*0.606 (0.132)*	*0.866 (0.086)*
Follicular	*0.604 (0.142)*	*0.832 (0.089)*
Ovulatory	*0.645 (0.091)*	*0.853 (0.075)*
Luteal	*0.634 (0.116)*	*0.861 (0.053)*
OCP	**0.665 (0.117)**	**0.885 (0.057)**
Menstrual	*0.687 (0.104)*	*0.905 (0.046)*
Follicular	*0.689 (0.141)*	*0.890 (0.063)*
Ovulatory	*0.645 (0.112)*	*0.870 (0.066)*
Luteal	*0.641 (0.151)*	*0.875 (0.056)*

Table 7A.1.6 *Cycle Study: The mean and SD (in brackets) for the best correlation and mean correlation score in both freely cycling (FC) and oral contraceptive pill (OCP) using women across four phasesof the menstrual cycle (menstrual, follicular, ovulatory and luteal phase). These correlation scores demonstrate the degree of coordination in bimanual performance on the alternating finger tapping (AFT) task.*

Table 7A.1.7 Cycle Study: Correlations between the Volume and MTR value of the Total CC and its segments						
	Total	**Anterior**	**MidAnterior**	**Central**	**MidPosterior**	**Posterior**
FC	**0.195**	**-0.278**	**-0.150**	**0.073**	**0.178**	**-0.216**
Menstrual	*0.422*	*-0.378*	*0.027*	*0.301*	*0.137*	*-0.006*
Follicular	*0.581**	*-0.266*	*0.360*	*-0.173*	*0.357*	*-0.170*
Ovulatory	*0.340*	*-0.366*	*-0.34*	*-0.115*	*0.067*	*-0.152*
Luteal	*-0.050*	*-0.116*	*-0.234*	*-0.159*	*0.140*	*-0.426*
OCP	**-0.027**	**-0.066**	**-0.163**	**0.119**	**0.233**	**-0.305***
Menstrual	*-0.079*	*-0.122*	*-0.273*	*-0.045*	*0.171*	*-0.074*
Follicular	*0.142*	*0.104*	*-0.071*	*0.189*	*0.292*	*-0.457*
Ovulatory	*-0.123*	*0.055*	*-0.704**	*-0.162*	*-0.117*	*-0.273*
Luteal	*-0.013*	*-0.207*	*0.077*	*0.286*	*0.599**	*-0.459*

Table 7A.1.7 *Cycle Study: The correlation (Pearson's Correlation Coefficient) between the relative volume (%) and MTR value of the Total CC and its segments in both freely cycling (FC) and oral contraceptive pill (OCP) using women across four phases of the menstrual cycle (menstrual, follicular, ovulatory and luteal phase). * refers to p<0.05.*

7A.3.2 17 β-Estradiol and Progesterone levels: Association with Oral Contraceptive Pill Status and Menstrual Cycle Phase

There is an interactive effect of pill status and cycle phase on progesterone level ($F_{3,72}$ =11.97, p=0.001). Post-hoc analysis reveals that the progesterone level of freely cycling women is greater than that of pill using women in the follicular (p=0.004, t=3.152), mid-cycle (p=0.039, t=2.315) and luteal phase (p=0.001, t=4.506) but not in the menstrual phase. Additionally, in freely cycling women progesterone level is significantly greater in the luteal phase as compared with all other phases (menstrual: p=0.001, t=-4.266; follicular: p=0.001, t=-4.340; and ovulatory: p=0.012, t=-2.961). In the case of the pill using women, on the other hand, there are no significant differences in the progesterone levels across the cycle. Refer to Table 7A.2.1 and Figure 7A.1.1.

There is also an interactive effect of pill status and cycle phase on 17 β-estradiol level ($F_{3,72}$ =5.27, p=0.002). Post-hoc analysis reveals that the 17 β-estradiol level of freely cycling women is greater than that of pill using women in the follicular (p=0.011, t=2.976), mid-cycle (p=0.003, t=3.681) and luteal phase (p=0.001, t=4.356) but not in the menstrual phase. Additionally, in freely cycling women 17 β-estradiol level is significantly lower in the menstrual phase as compared with all other phases (follicular: p=0.025, t=-2.549; ovulatory: p=0.009, t=-3.142; and luteal: p=0.001, t=-4.184). In the case of the pill using women, on the other hand, there are no significant differences in the estrogen levels across the cycle. Refer to Table 7A.2.1 and Figure 7A.1.2.

It is important to note that some studies (Cowell et al. 2011) assess postovulatory status based on progesterone levels above 10 nmol/L in the mid-luteal phase (day 18 to 25). In the Cycle study, however, progesterone levels are assessed in the late-luteal phase (day 28 ±2) and are, therefore, not likely to be as high as that in the mid-luteal phase (day 18 to 25). Four of the freely cycling women (n=13) do not demonstrate luteal levels above 10nmol/L, but given the time of the cycle this does not exclude ovulation in the given participants.

Table 7A.2.1 Cycle Study: Results for Association of Cycle Phase and OCP use with Sex Steroids

Sex Steroids	Pill Status			Cycle Phase			Interactive Effect		
	df	F	p	df	F	p	df	F	p
Progesterone	***1,24***	***16.93***	***0.001***	***3,72***	***11.19***	***0.001***	***3,72***	***11.97***	***0.001***
Estrogen	***1,24***	***30.262***	***0.001***	***3,72***	***3.421***	***0.022***	***3,72***	***5.27***	***0.002***

Table 7A.2.1 *Cycle Study: The results for association of cycle phase and OCP use with the levels of female sex hormones (estrogen, pmol/l; progesterone, nmol/l). These results are obtained using repeated measure ANOVA with OCP use as between and cycle phase as within subject variables. The result statistics quoted include: F ratio for between and within group variance (F), level of significance (p) and degree of freedom (df).*

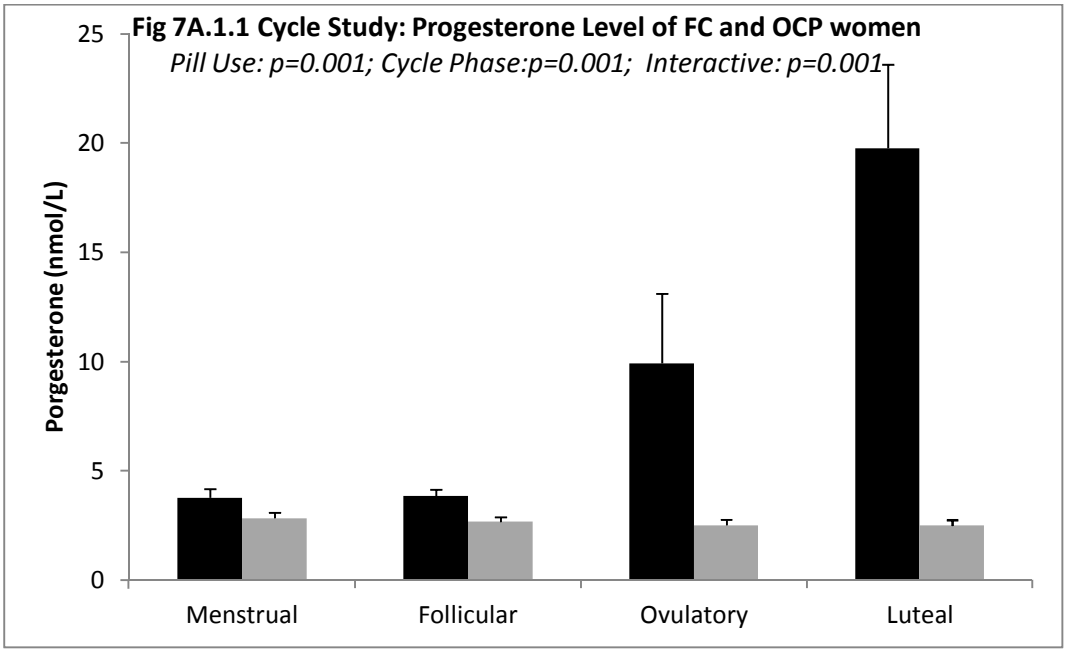

Figure 7A.1.1 *Cycle Study: Bar chart for the level of progesterone (nmol/l) in freely cycling (FC) women and oral contraceptive pill (OCP) using women. Freely cycling women are shown in black and OCP using women in grey. Error bars represent standard error of mean.*

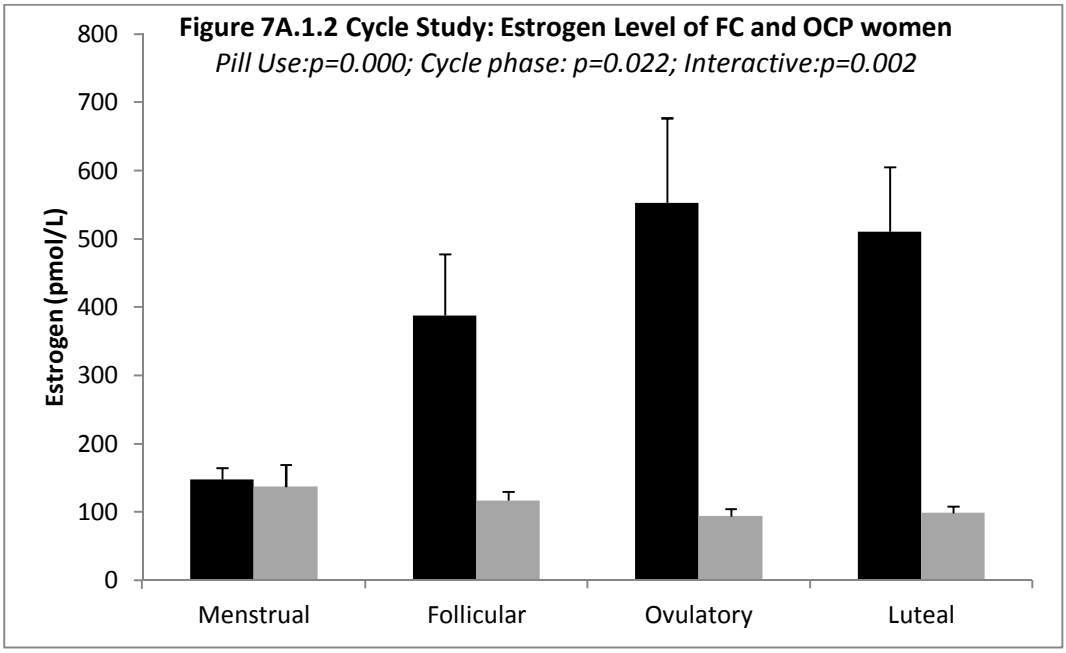

Figure 7A.1.2 *Cycle Study: Bar chart for the level of estrogen (pmol/l) in freely cycling (FC) women and oral contraceptive pill (OCP) using women. FC women are shown in black and OCP using women in grey. Error bars represent standard error of mean.*

7A.3.3 Relative Volume of the Total Corpus Callosum: Association with Oral Contraceptive Pill Status and Menstrual Cycle Phase

There is an interactive effect of pill status and cycle phase on the relative volume of the total corpus callosum ($F_{3,72}$=4.190, p=0.015). Post-hoc analysis reveals that, in the freely cycling women, the relative volume of the total corpus callosum in the ovulatory phase is significantly lower than in the follicular phase preceding it (p=0.023, t=2.595), and the luteal phase following it (p=0.020, t=-2.691); thus, the relative volume of the total corpus callosum of freely cycling women decreases from the follicular towards the ovulatory phase after which it begins to increase during the luteal phase. On the other hand, in women using oral contraceptives the relative volume of the total corpus callosum is significantly greater in the ovulatory phase as compared with the follicular phase preceding it (p=0.003, t=-3.656); thus, the relative volume of the total CC of women using contraceptives begins to increase earlier than that of freely cycling women, from the follicular towards the ovulatory phase. Refer to Table 7A.2 and Figure 7A.2.1.

Table 7A.2 Cycle Study: Results for Association of Cycle Phase and OCP use with Relative CC Volumes

Relative CC Volumes (%)	Pill Status			Cycle Phase			Interactive Effect		
	df	F	p	df	F	p	df	F	p
Total	*1,24*	*2.289*	*0.143*	*3,72*	*2.163*	*0.116*	***3,72***	***4.19***	***0.015***
Anterior	*1,24*	*0.595*	*0.448*	***3,72***	***3.122***	***0.031***	*3,72*	*1.604*	*0.196*
MidAnterior	*1,24*	*2.744*	*0.111*	*3,72*	*1.425*	*0.243*	*3,72*	*0.355*	*0.785*
Central	*1,24*	*1.556*	*0.224*	***3,72***	***2.698***	***0.050***	***3,72***	***2.924***	***0.040***
MidPosterior	*1,24*	*2.200*	*0.151*	*3,72*	*0.421*	*0.670*	*3,72*	*1.502*	*0.232*
Posterior	*1,24*	*0.145*	*0.707*	*3,72*	*0.578*	*0.632*	*3.72*	*2.573*	*0.061*

Table 7A.2 *Cycle Study: The results for association of cycle phase and OCP use with relative volume (%) of the total CC and its segments. These results are obtained using repeated measure ANOVA with OCP use as between and cycle phase as within subject variables. The result statistics quoted include: F ratio for between and within group variance (F), level of significance (p) and degree of freedom (df).*

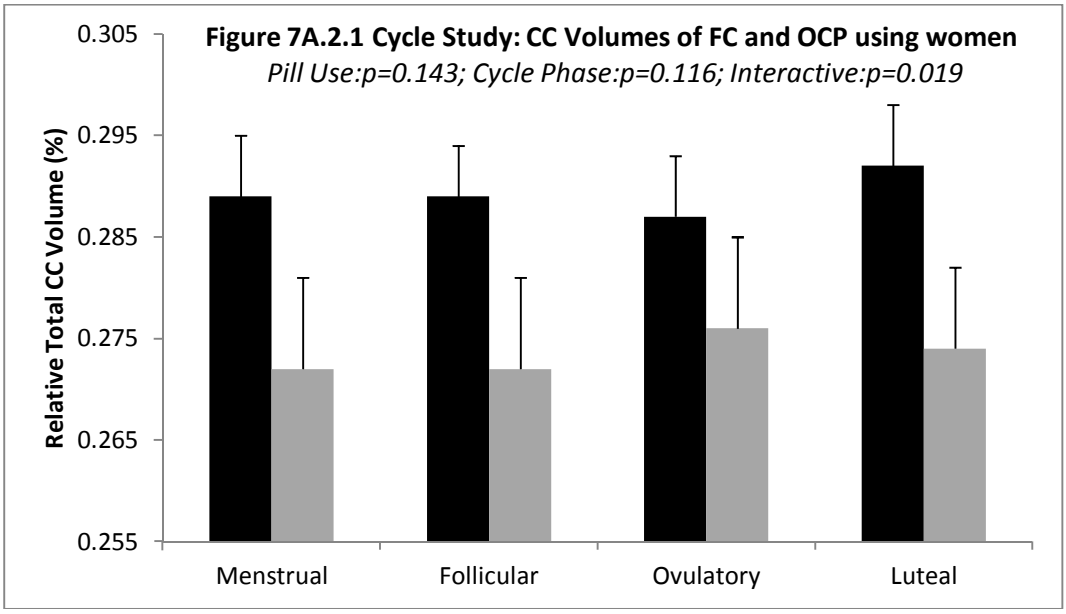

Figure 7A.2.1 *Cycle Study: Bar chart of the relative volume (%) of the total CC in freely cycling and OCP using women. Freely cycling women are shown in black and OCP using women in grey. Error bars represent standard error of mean.*

7A.3.4 Relative Volume of the Corpus Callosum Segments: Association with Oral Contraceptive Pill Status and Menstrual Cycle Phase

There is an interactive effect of pill status and cycle phase on the relative volume of the central segment of the corpus callosum ($F_{3,72}$ =2.924, p=0.040). Post-hoc analysis reveals that, in freely cycling women, the relative volume of the central CC segment in the luteal phase is significantly greater than the menstrual (p=0.002, t=-3.953) and follicular phase (p=0.040, t=-2.280) preceding it; thus, there is a significant and gradual increase in the relative volume of the central CC segment from menstruation onwards. In OCP women, the relative volume of the central CC segment is significantly greater in the ovulatory phase as compared with the earlier menstrual phase (p=0.010, t=-3.052); thus, there is a significant increase in the relative volume of the central CC segment of OCP using women

during the first half of the cycle, that is from menstrual to ovulatory phase. Refer to Table 7A.2 and Figures 7A.2.2 & 7A.2.3.

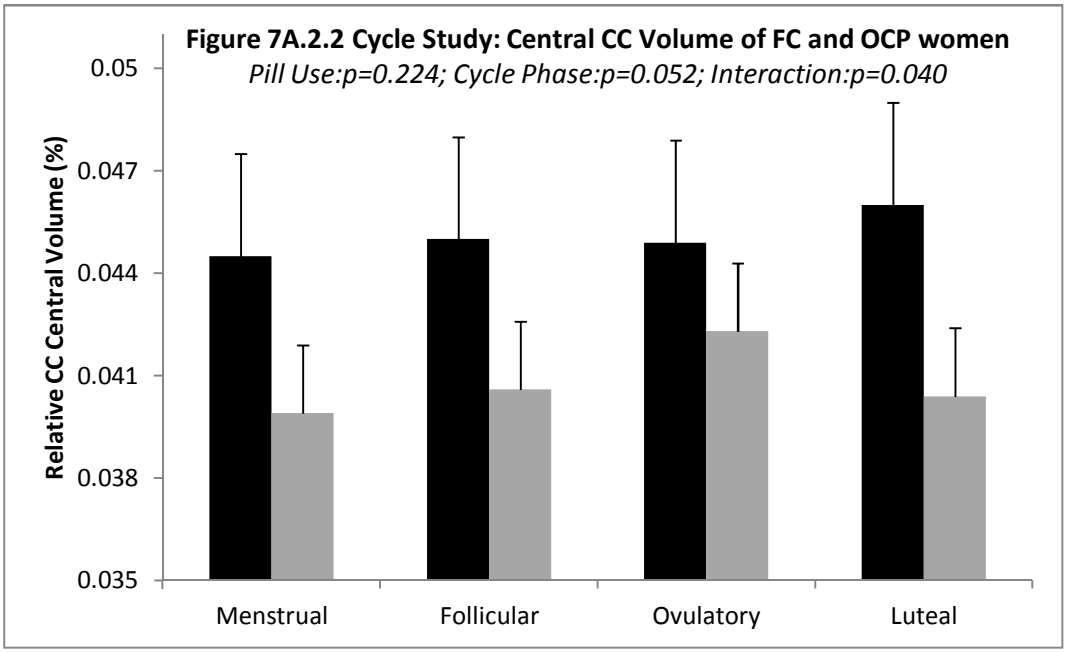

Figure 7A.2.2 *Cycle Study: Bar chart of the relative volume (%) of the central CC segment in freely cycling (FC) and OCP using women. Freely cycling women are shown in black and OCP using women in grey. Error bars represent standard error of mean.*

There is a main effect of cycle phase on the relative volume of the anterior CC segment ($F_{3,72}$ =3.122, p=0.031). Post hoc analysis reveals that the relative volume of the anterior CC segment is significantly greater in the luteal phase versus the menstrual (p=0.034, t=-2.238) and the ovulatory phase (p=0.047, t=-2.085).

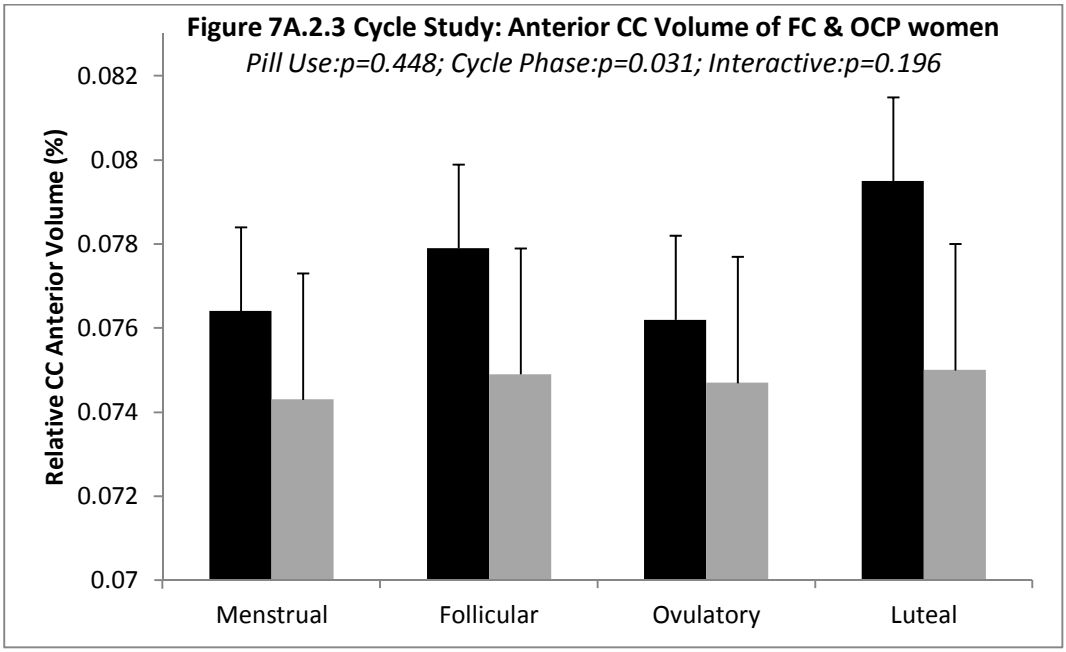

Figure 7A.2.3 *Cycle Study: Bar chart of the relative volume (%) of the anterior CC segment of freely cycling (FC) and oral contraceptive pill (OCP) using women. Freely cycling women are shown in black and OCP using women in grey. Error bars represent standard error of mean.*

7A.3.5 MTR Value of the Total Corpus Callosum: Association with Oral Contraceptive Pill Status and Menstrual Cycle Phase

There is a main effect of cycle phase on the MTR value of the total corpus callosum ($F_{3,66}$ =3.959, p=0.045). The MTR value of the total corpus callosum is significantly lower in the luteal phase as compared with all three phases, the menstrual phase (p=0.05, t=1.878) the follicular (p=0.028, t=2.441) and ovulatory phase (p=0.041, t=2.365). There is no main effect of pill use or interactive (pill × cycle phase) effect on the MTR value of the total corpus callosum. Refer to Table 7A.3 and Figure 7A.3.1.

Table 7A.3 Cycle Study: Results for Association of Cycle Phase and OCP use with MTR Values of the Total CC and its Segments (Repeated Measure ANOVA)									
CC MTR Values	**Pill Status**			**Cycle Phase**			**Interactive Effect**		
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Total	*1,22*	*0.291*	*0.653*	***3,66***	***3.959***	***0.045***	*3,66*	*0.935*	*0.403*
Anterior	*1,22*	*0.180*	*0.728*	*3,66*	*2.328*	*0.111*	*3,66*	*0.594*	*0.619*
MidAnterior	*1,22*	*0.439*	*0.666*	*3,66*	*2.573*	*0.100*	*3,66*	*0.490*	*0.661*
Central	*1,22*	*3.714*	*0.086*	*3,66*	*2.287*	*0.138*	*3,66*	*0.534*	*0.631*
MidPosterior	*1,22*	*0.953*	*0.341*	***3,66***	***4.733***	***0.018***	*3,66*	*0.860*	*0.443*
Posterior	*1,22*	*0.168*	*0.715*	*3,66*	*1.050*	*0.404*	*3,66*	*0.934*	*0.450*

Table 7A.3 *Cycle Study: The results for association of cycle phase and OCP use with the MTR values of the total CC and its segments. These results are obtained using repeated measure ANOVA with OCP use as between and cycle phase as within subject variables. The result statistics quoted include: F ratio for between and within group variance (F), level of significance (p) and degree of freedom (df).*

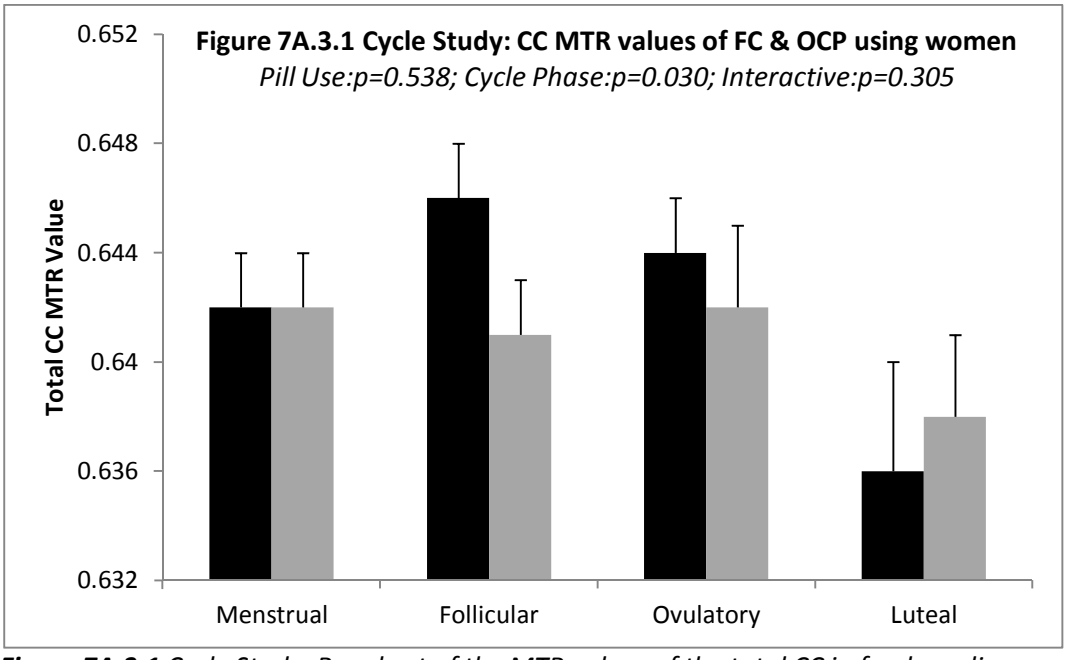

Figure 7A.3.1 *Cycle Study: Bar chart of the MTR values of the total CC in freely cycling (FC) and oral contraceptive pill (OCP) using women. Freely cycling women are shown in black and OCP using women in grey. Error bars represent standard error of mean.*

7A.3.6 MTR Value of the Corpus Callosum Segments: Association with Oral Contraceptive Pill Status and Menstrual Cycle Phase

There is a main effect of cycle phase on the MTR value of the mid-posterior segment of the corpus callosum only ($F_{3,66}$ =4.733, p=0.018). The MTR value of the mid-posterior segment of the corpus callosum is significantly lower in the luteal phase as compared with the menstrual phase (p=0.043, t= 2.241), follicular phase (p=0.011, t=2.983) and ovulatory phase (p=0.015, t=2.697) of the cycle. Refer to Table 7A.3 and Figure 7A.3.3.

There is no main effect of OCP use or interactive (pill × cycle phase) effect on the MTR value of any segment of the corpus callosum. Refer to Table 7A.3 and Figure 7A.3.2.

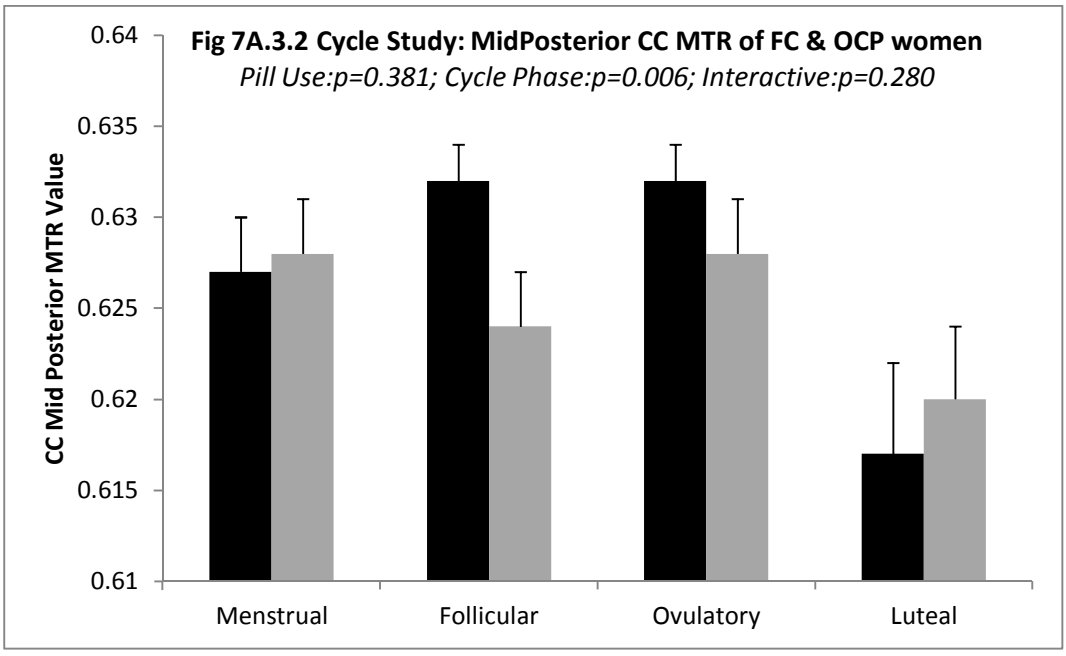

Figure 7A.3.2 *Cycle Study: Bar chart of the MTR value of the mid-posterior CC segment of freely cycling (FC) and oral contraceptive pill (OCP) using women. Freely cycling women are shown in black and OCP using women in grey. Error bars represent standard error of mean.*

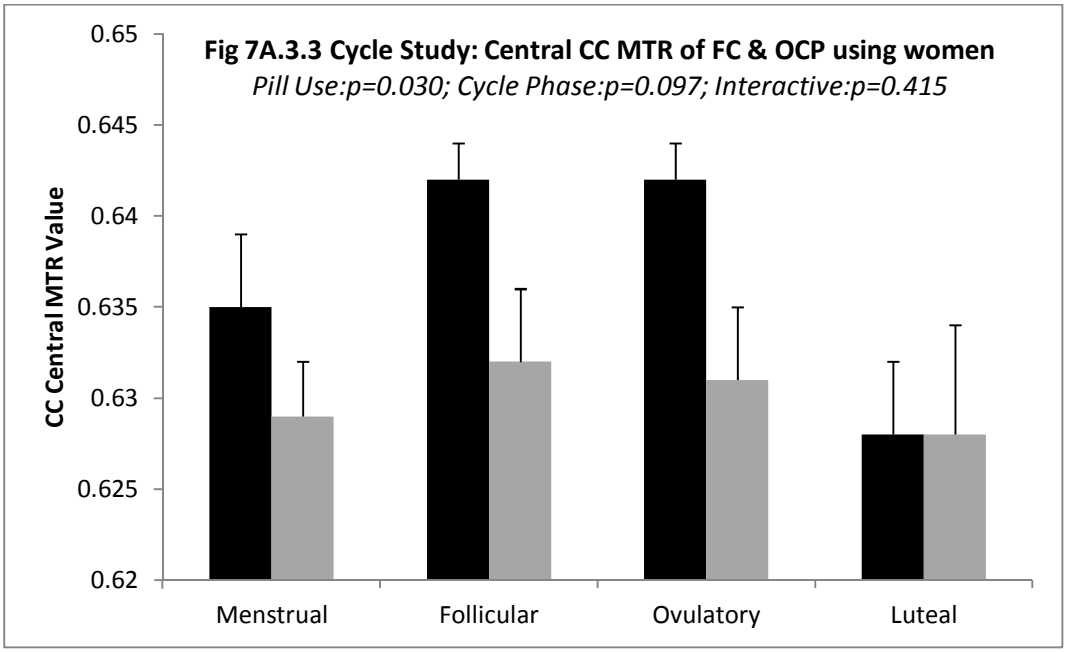

Figure 7A.3.3 *Cycle Study: Bar chart of the MTR value of the central CC segment of freely cycling (FC) and oral contraceptive pill (OCP) using women. Freely cycling women are shown in black and OCP using women in grey. Error bars represent standard error of mean.*

7A.3.7 Performance on Bimanual Coordination Task: Association with Oral Contraceptive Pill Status and Menstrual Cycle Phase

There is no main or interactive effect of oral contraceptive pill use and menstrual cycle phase on both the standard correlation and the best correlation measures of bimanual coordination in the alternating finger tapping task. See Table 7A.4.

Table 7A.4 Cycle Study: Results for Association of Cycle Phase and OCP use with Performance on the Altered Finger Tapping Task

AFT Task Performance	Pill Status			Cycle Phase			Interactive Effect		
	df	F	p	df	F	p	df	F	p
Average Correlation	*1,21*	*0.929*	*0.347*	*3,63*	*0.200*	*0.893*	*3,63*	*1.751*	*0.170*
Best Correlation	*1,21*	*0.869*	*0.391*	*3,63*	*1.554*	*0.249*	*3,63*	*1.991*	*0.237*

Table 7A.4 *Cycle Study: The results for association of cycle phase and OCP use with best and average correlation score for bimanual performance on the altered finger tapping (AFT) task. These results are obtained using repeated measure ANOVA with OCP use as between and cycle phase as within subject variables. The result statistics quoted include: F ratio for between and within group variance (F), level of significance (p) and degree of freedom (df).*

7B.3 RESULTS: SYS STUDY

7B.3.1 Descriptive Statistics

The SYS study sample consists of 53 OCP using girls with mean age of 203 (SD: ±15) months and mean puberty stage of 4.70 (±0.46). The percentage of OCP using girls with no prenatal exposure to maternal cigarette smoking is 57. The OCP using girls are age-matched with 53 non-OCP using girls (mean age of 203 ±15 months) having mean puberty stage of 4.45 (±0.57). The percentage of non-OCP using girls with no prenatal exposure to maternal cigarette smoking is 54. For more details on descriptive statistics refer to Table 7B.1.1.

Table 7B.1.1 SYS: Demographics and Descriptive Statistics for Sex Hormones of the OCP/Non-OCP Sample

	Non-OCP using Girls		OCP Using Girls	
	n	Mean (SD)	n	Mean (SD)
Age (months)	*53*	*203 (15)*	*53*	*203 (15)*
Puberty Stage	*53*	*4.45 (0.57)*	*53*	*4.70 (0.46)*
Percentage with no PEMCS (%)	*53*	*54*	*53*	*57*
Bioavailable Testosterone (cnmol/L)	*36*	*0.411 (0.28)*	*44*	*0.321 (0.24)*
Testosterone (nmol/L)	*36*	*1.867 (0.82)*	*44*	*1.509 (0.76)*
Estradiol (pmol/L)	*35*	*218 (247)*	*47*	*89 (139)*
SHBG (nmol/L)	*36*	*87.47 (36)*	*45*	*88.86 (30)*

Table 7B.1.1 *SYS: The sample size (n) along with the mean and SD (in brackets) for the following variables in both OCP using and age-matched non-OCP using girls: age (months), puberty, PEMCS, bioavailable testosterone (cnmol/l), testosterone (nmol/l), estradiol (pmol/l) and sex hormone binding globulin (SHBG; nmol/l).*

Pearson's correlations between the variables employed in the linear regression analysis to estimate the association of CC volumes with OCP use in the subsample of OCP using and age-matched non-OCP using girls (n=100) are provided in Table 7B.1.2.

Table 7B.1.2 SYS: Correlation Matrix for OCP/Non-OCP sample (Pearson's Correlation Coefficient)								
	Age	**OCP use**	**Puberty**	**PEMCS**	**CC Anterior**	**CC Mid Anterior**	**CC Central**	**CC Mid Posterior**
Age								
OCP use	*-0.013*							
Puberty	*0.615***	*0.249**						
PEMCS	*0.003*	*-0.057*	*-0.018*					
CC Volume								
Total	*0.171**	*-0.165**	*0.154*	*-0.109*				
Anterior	*0.155*	*-0.220**	*0.066*	*-0.049*				
MidAnterior	*0.187**	*-0.205**	*0.161**	*-0.090*	*0.480***			
Central	*0.131*	*-0.064*	*0.200**	*-0.114*	*0.264***	*0.564***		
MidPosterior	*0.111*	*-0.067*	*0.134*	*-0.079*	*0.502***	*0.560***	*0.647***	
Posterior	*0.116*	*-0.135*	*0.042*	*-0.053*	*0.631***	*0.442***	*0.225**	*0.532***

Table 7B.1.2 *SYS: The Pearson's correlation coefficient (r) between the following variables in the OCP/non-OCP sample: age (months), puberty, PEMCS, OCP use and relative CC volumes (%; total and segmental). These variables are employed in the statistical model used to analyse the association of OCP use with relative volume (%) of the total CC and its segments. * refers to p<0.05 and ** refers to p<0.001.*

7B.3.2 Relative Volume of the Total Corpus Callosum and its Segments: Association with OCP use

There is a significant negative correlation between the use of oral contraceptive pills and the relative volume of the total corpus callosum (β=-0.233, p=0.025); the relative volume of the total corpus callosum of OCP using girls is significantly lower than that of age-matched non-OCP using girls. The use of oral contraceptives accounts for 5% of the variance in the relative volume of the total corpus callosum. For details refer to Table 7B.2.1 and 7B.2.2 and Figure 7B.1 below.

There is a significant negative correlation between the use of oral contraceptive pills and the relative volume of the anterior (β=-0.261, p=0.010) and mid-anterior (β=-0.265, p=0.008) segment of the corpus callosum. The central, mid-posterior

and posterior segments of the corpus callosum are not associated with the use of oral contraceptives. For details refer to Table 7B.2.1 and 7B.2.2 and Figure 7B.2 below.

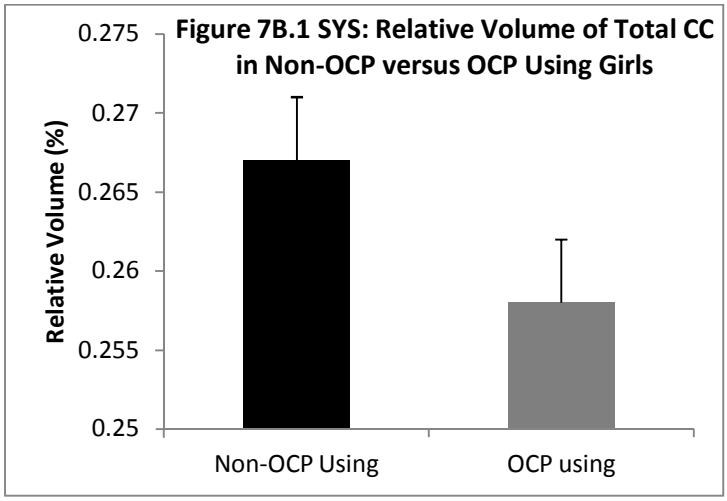

Figure 7B.1 *SYS: Bar chart of the relative volume (%) of the total corpus callosum in non-OCP versus OCP using girls. Non-OCP using girls are shown in black and OCP using girls in grey. Error bars represent standard error of mean.*

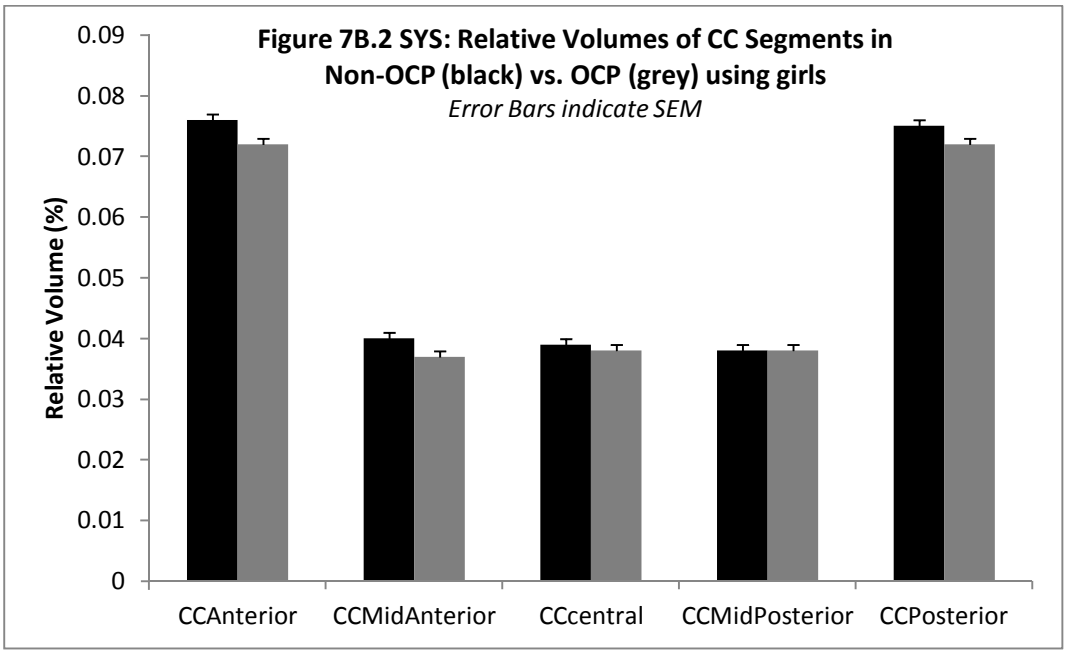

Figure 7B.2 *SYS: Bar chart of the relative volumes (%) of CC segments in non-OCP versus OCP using girls. Non-OCP using girls are shown in black and OCP using girls in grey. Error bars represent standard error of mean.*

Table 7B.2.1 SYS: Descriptive Statistics for Relative CC Volumes in Non-OCP versus OCP using Girls				
Relative CC Volume (%)	**Non-OCP using**		**OCP Using**	
	n	**Mean (SD)**	**n**	**Mean (SD)**
Total	***53***	***0.267 (0.029)***	***53***	***0.258 (0.027)***
Anterior	***53***	***0.076 (0.010)***	***53***	***0.072 (0.007)***
MidAnterior	***53***	***0.040 (0.006)***	***52***	***0.037 (0.006)***
Central	*53*	*0.039 (0.006)*	*52*	*0.038 (0.007)*
MidPosterior	*53*	*0.038 (0.006)*	*53*	*0.038 (0.006)*
Posterior	*53*	*0.075 (0.009)*	*53*	*0.072 (0.010)*

Table 7B.2.1 *SYS: The sample size (n) along with the mean and SD (in brackets) for the relative volume (%) of the total CC and its segments (anterior, mid-anterior, central, mid-posterior and posterior) in both OCP using and age-matched non-OCP using girls.*

Table 7B.2.2 SYS: Results for Association of OCP use with Relative CC Volumes			
Relative CC Volume (%)	**Results via Linear Regression Analysis**		**Effect Size**
Total	***ΔR^2=0.047, df=103, ΔF=5.18***	***β=-0.233, p=0.025, t=-2.276***	***d=-0.449, r=0.219***
Anterior	***ΔR^2=0.063, df=103, ΔF=6.95***	***β=-0.261, p=0.010, t=-2.636***	***d=-0.519, r=0.251***
MidAnterior	***ΔR^2=0.066, df=103, ΔF=7.38***	***β=-0.265, p=0.008, t=-2.717***	***d=-0.535, r=0.259***
Central	*ΔR^2=0.015, df=103, ΔF=1.60*	*β=-0.126, p=0.209, t=-1.265*	*d=-0.249, r=0.124*
MidPosterior	*ΔR^2=0.008, df=103, ΔF=0.80*	*β=-0.090, p=0.373, t=-0.895*	*d=-0.176, r=0.088*
Posterior	*ΔR^2=0.025, df=103, ΔF=2.66*	*β=-0.165, p=0.106, t=-1.632*	*d=-0.321, r=0.159*

Table 7B.2.2 *SYS: The results for association of OCP use with relative volumes (%) of the total CC and its segments; and the effect sizes (effect size r, Cohen's d) of these results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance explained by OCP use), standardized regression coefficient (β; for correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

7C.3 RESULTS: IMAGEN STUDY

7C.3.1 Descriptive Statistics

The IMAGEN subsample is comprised of 52 OCP using girls and 52 centre- and age-matched non-OCP using girls. The mean ages of both the OCP using and non-OCP using girls are 176 (SD: ±5) months and their mean puberty stage is 4.1 (±0.4). For more details refer to Table 7C.1.1.

Table 7C.1.1 IMAGEN: Demographics of the OCP/Non-OCP Sample				
	Non-OCP Using Girls		OCP Using Girls	
	n	Mean (SD)	n	Mean (SD)
Age (months)	*52*	*176 (5)*	*52*	*176 (5)*
Puberty Stage	*52*	*4.05 (0.37)*	*52*	*4.15 (0.36)*

Table 7C.1.1 *IMAGEN: The sample size (n) along with the mean and SD (in brackets) for the following variables in both OCP using and age-matched non-OCP using girls: age (months) and puberty.*

Pearson's correlation between the variables employed in the linear regression analysis to estimate the association of CC volumes with OCP use is provided in correlation matrix of Table 7C.1.2.

Table 7C.1.2 IMAGEN: Correlation Matrix for OCP/Non-OCP sample (Pearson's Correlation Coefficient)							
	Age	OCP Use	Puberty	CC Anterior	CC Mid Anterior	CC Central	CC Mid Posterior
Age							
OCP Use	*0.000*						
Puberty	*0.166**	*0.132*					
CC Volume							
Total	*-0.029*	*-0.124*	*0.041*				
Anterior	*-0.049*	*-0.142*	*0.060*				
MidAnterior	*-0.060*	*-0.267**	*-0.061*	*0.704***			
Central	*-0.112*	*-0.216**	*-0.129*	*0.398***	*0.732***		
MidPosterior	*0.092*	*-0.087*	*-0.046*	*0.538***	*0.609***	*0.627***	
Posterior	*0.156*	*-0.080*	*0.057*	*0.461***	*0.485***	*0.318***	*0.592***

Table 7C.1.2 *IMAGEN: The Pearson's correlation coefficient (r) between the following variables in the OCP/non-OCP sample: age (months), puberty, OCP use and relative CC volumes (%; total and segmental). These variables are employed in the statistical model used to analyze the association of OCP use with relative volume (%) of the total CC and its segments. * refers to p<0.05 and ** refers to p<0.001.*

7C.3.2 Relative Volume of the Total Corpus Callosum and its segments: Association with OCP use in Girls

There is no correlation between the use of oral contraceptive pills and the relative volume of the total Corpus Callosum. For details refer to Table 7C.2.1 and 7C.2.2 and Figure 7C.1.

There is a significant negative correlation between the use of oral contraceptive pills and the relative volume of the mid-anterior segment (β=-0.230, p=0.023) and central segment (β=-0.190, p=0.055) of the corpus callosum. The mid-posterior and posterior segments of the CC are not associated with the use of oral contraceptives. For details refer to Table 7C.2.1 and 7C.2.2 and Figure 7C.1.

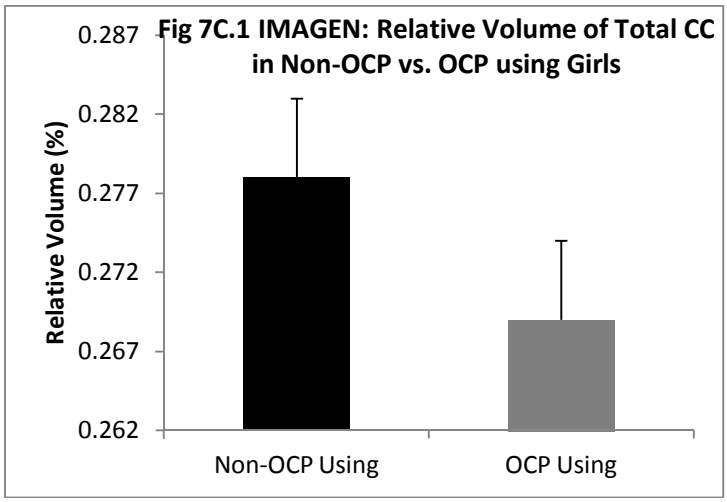

Figure 7C.1 *IMAGEN: Bar chart of the relative volume (%) of the total corpus callosum in non-OCP versus OCP using girls. Non-OCP using girls are shown in black and OCP using girls in grey. Error bars represent standard error of mean.*

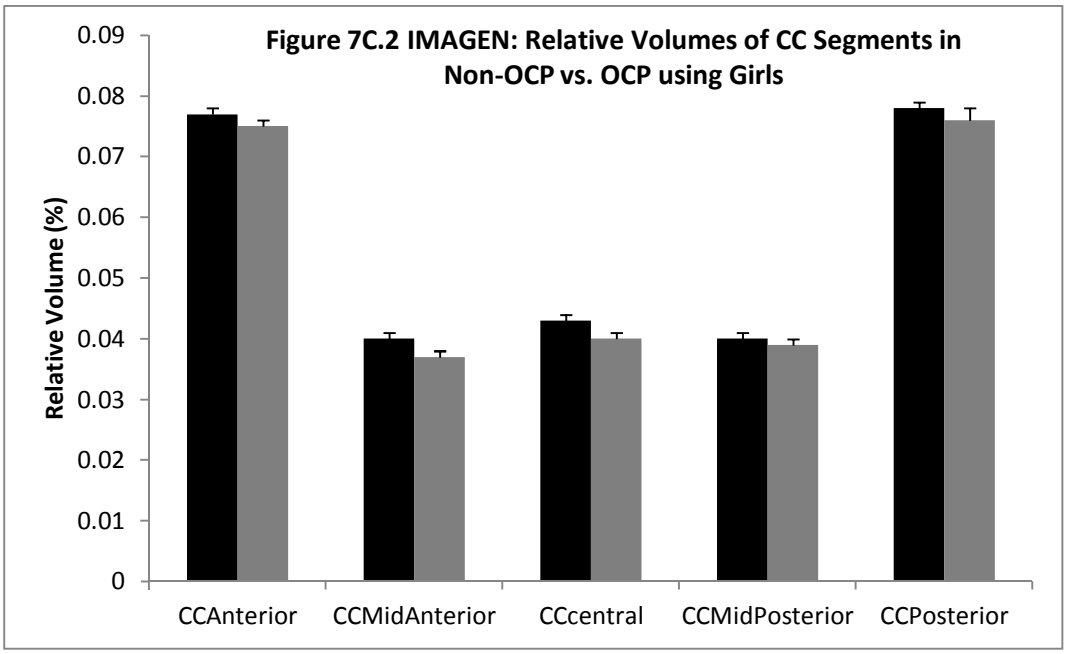

Figure 7C.2 *IMAGEN: Bar chart of the relative volumes (%) of the CC segments in non-OCP versus OCP using girls. Non-OCP using girls are shown in black and OCP using girls in grey. Error bars represent standard error of mean.*

Table 7C.2.1 IMAGEN: Descriptive Statistics for Relative CC Volumes in Non-OCP versus OCP using Girls				
Relative CC Volume (%)	**Non-OCP Using**		**OCP Using**	
	n	**Mean (SD)**	**n**	**Mean (SD)**
Total	*52*	*0.278 (0.034)*	*52*	*0.269 (0.038)*
Anterior	*52*	*0.077 (0.010)*	*52*	*0.075 (0.011)*
MidAnterior	***52***	***0.040 (0.006)***	***50***	***0.037 (0.006)***
Central	***52***	***0.043 (0.010)***	***52***	***0.040 (0.007)***
MidPosterior	*52*	*0.040 (0.008)*	*51*	*0.039 (0.007)*
Posterior	*52*	*0.078 (0.010)*	*52*	*0.076 (0.012)*

Table 7C.2.1 *IMAGEN: The sample size (n) along with the mean and SD (in brackets) for the relative volume (%) of the total CC and its segments (anterior, mid-anterior, central, mid-posterior and posterior) in both OCP using and age-matched non-OCP using girls.*

Table 7C.2.2 IMAGEN: Results for Association of OCP use with Relative CC Volumes			
Relative CC Volume (%)	**Results via Linear Regression Analysis**		**Effect Size**
Total	*ΔR^2=0.017, df=102, ΔF=1.76*	*β=-0.132, p=0.187, t=-1.328*	*d=-0.263, r=0.130*
Anterior	*ΔR^2=0.012, df=102, ΔF=1.29*	*β=-0.113, p=0.258, t=-1.136*	*d=-0.224, r=0.111*
MidAnterior	***ΔR^2=0.052, df=102, ΔF=5.64***	***β=-0.230, p=0.023, t=-2.316***	***d=-0.459, r=0.224***
Central	***ΔR^2=0.035, df=102, ΔF=3.71***	***β=-0.190, p=0.050, t=-1.926***	***d=-0.381, r=0.187***
MidPosterior	*ΔR^2=0.006, df=102, ΔF=0.61*	*β=-0.078, p=0.438, t=-0.778*	*d=-0.154, r=0.077*
Posterior	*ΔR^2=0.006, df=102, ΔF=0.60*	*β=-0.077, p=0.442, t=-0.772*	*d=-0.153, r=0.076*

Table 7C.2.2 *IMAGEN: The results for association of OCP use with relative volume (%) of the total CC and its segments; and the effect sizes (effect size r, Cohen's d) of these results. These results are obtained using linear regression models and for each model the adjusted R^2 (ΔR^2; change in variance explained by OCP use), standardized regression coefficient (β; for correlation), t value (t) for the regression coefficient and the significance of the t value (p) is quoted.*

7.4 DISCUSSION

This study is the first to investigate and, in turn, demonstrate effects of sex steroids on the volume and MTR value of the corpus callosum in females. The key findings of this study are that freely cycling women have: 1) greater CC volume and MTR values than those of women using oral contraceptives; and 2) greater CC volume but lower MTR values in the luteal phase as compared with the

ovulatory phase of the menstrual cycle. A summary of the significant findings of this study are provided in Table 7.4.1.

Before discussing the results, it is important to understand how and why female sex steroids vary across the cycle phases of both the freely cycling and OCP using women. Knowledge of hormonal variation across the menstrual cycle and its biological basis will enable us to put the results of this study into perspective.

Table 7.4.1 Chapter 7: Summary of Significant Findings			
	Main Effect Of OCP Use	**Main Effect Of Cycle Phase**	**Interactive Effect Of OCP use and Cycle Phase**
Volume	*Decreases*	*Increases in Luteal vs. Ovulatory Phase*	***FC women**:* *1) Increases in Luteal vs. Ovulatory Phase; and 2) Decreases in Ovulatory vs. Follicular Phase*
			***OCP women**:* *Increases in Ovulatory vs. Follicular Phase.*
MTR	*Decreases*	*Decreases in Luteal vs. Ovulatory Phase*	***FC Women**:* *Decreases in Luteal vs. Ovulatory Phase*
CC Sections	*Total CC, Anterior, Mid-Anterior and Central CC Segments*	*Total CC, Anterior & Mid-Posterior CC Segments*	*Total CC; Central and Anterior CC Segment*

***Table 7.4.1** Summary of significant findings of the effects of OCP use and cycle phase on the relative volumes (%) and MTR values of the corpus callosum and the specific segments that experience these effects.*

In healthy freely cycling women, during the menstrual phase of the cycle the levels of estrogen (150 pmol/L) and progesterone (0.64 nmol/L) are low (Stricker et al. 2006). Following the menstrual phase, production of estrogen increases during the follicular phase (450 pmol/L), at first gradually and then with a sudden and robust peak in the ovulatory phase (peak level: 900 pmol/L), which coincides with the release of the egg (Stricker et al. 2006). Following this sudden peak, estrogen levels gradually decline across the luteal phase (380 pmol/L; Stricker et

al. 2006). Estrogen levels of the freely cycling women in this study are seen to vary in a similar fashion across the cycle; the levels are significantly lower in the menstrual phase as compared with all other phases. In case of progesterone, following the menstrual phase its production remains low during the follicular phase (0.64 nmol/L); it begins to rise following the ovulatory phase (13.67 nmol/L) and peaks in the mid-luteal phase (36.25 nmol/L) after which is gradually declines (Stricker et al. 2006). Progesterone levels of the freely cycling women in this study are seen to vary in a similar fashion; the levels are significantly higher in the luteal phase as compared with all other phases. Figure 7.4.1 demonstrates the variation in the level of estrogen and progesterone across the cycle phases of freely cycling women.

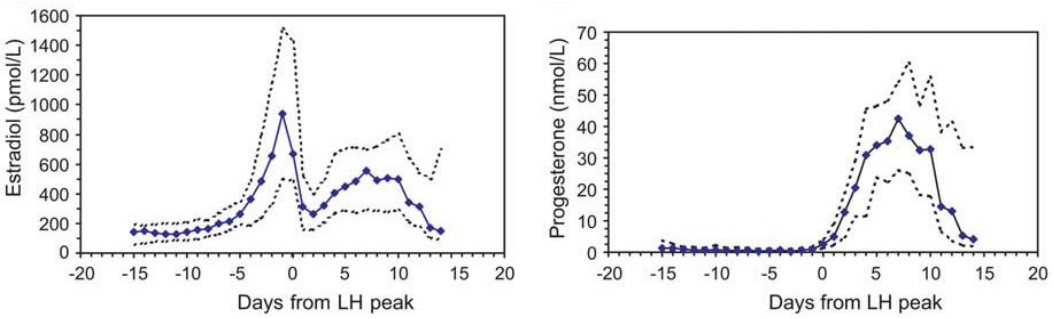

Figure 7.4.1: Variation in the level of estradiol (pmol/L) and progesterone (nmol/L) across the menstrual cycle in freely cycling women (Day -15 refers to first day of menstrual cycle). Solid lines represent median values and dotted lines represent 5th and 95th percentiles. From Stricker et al. 2006.

Combined oral contraceptives, such as those used by the women in this study, are comprised of synthetic estrogen and progesterone; Ovranette, Microgynon and Femodene all have the same amount of synthetic estrogen (30 µg Ethinyl Estradiol) and slightly different amounts of synthetic progesterone (150 µg of Levonogestrel in Ovranette and Microgynon and 75 µg of Gestogene in Femodene). These synthetic steroids act on the hypothalamic-pituitary axis and

inhibit secretion of FSH and LH. As a result the natural production of endogenous sex steroids by the ovaries is suppressed. In these women, the exogenous contraceptive steroids are the main source of sex steroids. Synthetic estradiol mainly differs from natural estradiol in that it contains the ethinyl group. Nearly 80 to 90 percent of Ethinyl Estradiol in the pill is absorbed from the gut (Edelman et al. 2010). Following absorption, Ethinyl Estradiol is rapidly conjugated in part with plasma compounds such as glucuronides and sulphates and undergoes renal excretion (Edelman et al. 2010). Van Den Heuvel et al. (2005) examined the pharmacokinetic profile of Ethinyl Estradiol (EE) from a combined oral contraceptive pill (Marvelon) containing 30 µg of Ethinyl Estradiol in women of similar age to those included in this study. Daily dosing of this oral contraceptive resulted in characteristic peaks and troughs in the serum concentration of Ethinyl Estradiol during the day. Figure 7.4.2 demonstrates the daily variation recorded in the serum level of EE across the course of one menstrual cycle.

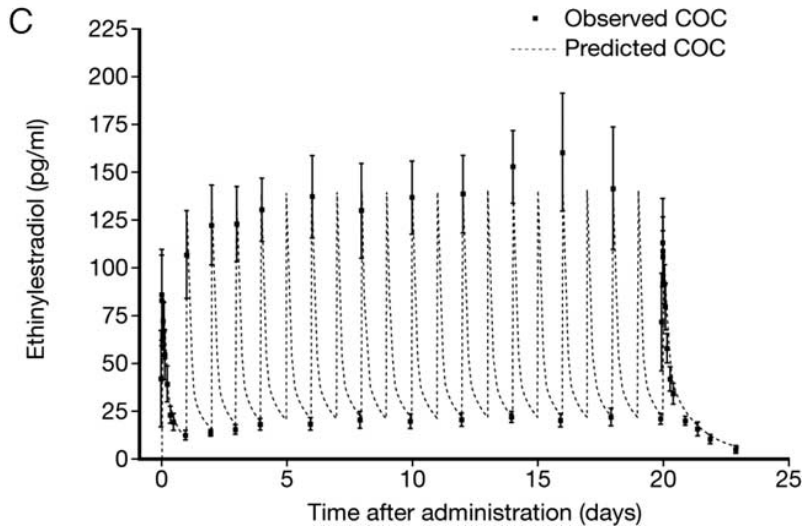

Figure 7.4.2: Variation in the level of ethinyl estradiol (pg/ml) across the menstrual cycle in women using oral contraceptives. Schematic taken from Van Den Heuvel et al. 2005.

The average daily concentration of EE in the serum of women consuming 30 µg of Ethinyl Estradiol daily was found to be 159 ±20 pmol/L, whereas the mean daily peak level was 616 ±110 pmol/L; this was the case for each of the 21 days during which the pill contains the same amount of EE. Similarly, Kuhnz et al. (1992) reported that daily consumption of a combined oral contraceptive (Microgynon) containing 30 µg of Ethinyl Estradiol results in average serum EE concentrations of 113 pmol/L (calculated from the area-under-the-curve values over 24 hour steady state), whereas the daily peak concentration was approximately 401 ±157 pmol/L (based on C_{max} values for two separate treatment cycles of oral contraceptives: 387 to 416 pmol/L). In this study, the level of synthetic estrogen and progesterone were not assayed, therefore, the results of previous studies are used to make assumptions about the possible level of synthetic sex steroids in OCP using women. Overall, the main differences in the female sex hormonal milieu of women using oral contraceptives and freely cycling women include: 1) women using oral contraceptives have considerable variations in their estradiol levels during the course of each day, however, the average daily level of estradiol is more constant across the 21 days of contraceptive use (139 to 180 pmol/L; Van Den Heuvel et al. 2005). Freely cycling women, on the other hand, have considerable variations in the daily level of sex steroids across the corresponding 21 days (330 to 700 pmol/L; Stricker et al. 2006); 2) the mean daily level of estradiol in OCP using women (113 pmol/L; Kuhnz et al. 1992) across the 21 days that oral contraceptives are consumed is lower than that of freely cycling women (440 pmol/L; Stricker et al. 2006) across the corresponding 21 days; 3) the women using oral contraceptives demonstrate

a daily peak in the level of estradiol (401 pmol/L, Kuhnz et al. 1992; 616 pmol/L, Van Den Heuvel et al. 2005), in case of freely cycling women, however, the levels are seen to peak only during the ovulatory phase (700 pmol/L; Stricker et al. 2006). In addition to the differences in the level of female sex steroids, oral contraceptives, by inhibiting the hypothalamic-pituitary-ovarian axis, reduce the ovarian production of testosterone; unlike estrogen and progesterone, testosterone is not contained in the contraceptive pill, thus freely cycling women have significantly higher level of testosterone as compared with women using oral contraceptives (Graham et al. 2006). In this study, the level of testosterone is also significantly lower in female adolescents using oral contraceptives as compared with freely cycling adolescents (SYS: $p=0.046$). Oral contraceptives also increase the hepatic production of sex hormone binding globulin (SHBG). Sex hormone binding globulin is a glycoprotein that binds to testosterone and estradiol in the plasma, thus resulting in lower levels of free testosterone and estradiol to bind with their respective receptors and induce their effects on the body (Hammond et al. 1984, Guyton et al. 2000).

Estradiol exerts its influence on the brain by binding with intracellular nuclear receptor proteins, namely, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ; Kuiper et al. 1998). Studies examining the receptor binding affinity (RBA) of synthetic versus natural estradiol had variable results; receptor binding affinity of synthetic estradiol has been reported to vary from less than half to more than twice that of natural estradiol (Kuiper et al. 1997, Salmaan et al. 1991, Blair et al. 2000). Classically estrogen is known to regulate reproductive function

via feedback influence on the hypothalamic-pituitary axis (Guyton et al. 2000). Recent evidence, however, suggests that estrogen may modulate physiological parameters other than reproduction, such as learning and memory (Xu et al. 1998). These effects are likely to be mediated by ERβ, since ERα is sparse in the learning and memory regions of the brain (Kuiper et al. 1998, Shughrue et al. 1997). Estrogen receptor β is also the predominant receptor in the oligodendroglial cells of white matter and activation of the receptor is shown to increase thickness of the myelin sheath (Zhang et al. 2004). Synthetic ethinyl estradiol is a selective agonist of ERα, thus suggesting that the influence of natural estradiol on white matter is likely to be greater than that of synthetic estradiol (Barkhem et al. 1998). Progesterone, like estradiol, acts on the brain primarily via nuclear steroid receptors PRB and PRA, which are distributed widely across the brain in all neural cell types including oligodendroglial cells (reviewed in Brinton et al. 2008). Progesterone increases expression of myelin basic protein and causes an increase in thickness of myelin sheath, diameter of myelinated fibre and axon diameter (Ghoumari et al. 2003, Sameni et al. 2008).

The use of oral contraceptive pills is associated with lower relative volume and MTR value of the corpus callosum. Although the relative volume of the total corpus callosum and its segments is lower in OCP using females versus non-OCP using females in all three studies, this difference reaches significance for the relative volumes of the total CC (r=0.22), anterior (r=0.25) and mid-anterior CC (r=0.26) segments in the SYS study, and only for the mid-anterior (r=0.22) and central CC (r=0.19) segments in the IMAGEN study. The MTR values of the total

corpus callosum and its segments are examined only in the cycle study; these MTR values are seen to be lower in OCP using women as compared with freely cycling women, with the difference showing a trend for the central segment of the CC only (r=0.28). Based on these finding it can be conjectured that the OCP associated decrease in relative volume of the corpus callosum may be driven, at least in part, by a lower degree of myelination. The absence of a significant relation between OCP use and CC volume in the Cycle study, unlike the SYS and IMAGEN study, is most likely due to its limited sample size. The SYS and IMAGEN study each has a sample of 52 OCP versus 52 non-OCP using girls, which is considerably greater than the 13 OCP using versus 13 freely cycling women included in the Cycle study. Moreover, the SYS and IMAGEN studies are carried out in adolescents whereas the Cycle study is carried out in young adults; the effect of contraceptives on CC volume might be greater during adolescence when the corpus callosum is growing than during adulthood when there is little development in the size of the corpus callosum (Johnson et al. 1994, Lee et al. 2009). The SYS, IMAGEN and Cycle studies successfully demonstrate that the use of oral contraceptives is associated with lower relative volume of the total corpus callosum, particularly its anterior, mid-anterior and central segments; and that OCP use exhibits a similar relationship with MTR value of only the central CC segment (OCP using < Non-OCP using). Based on these findings it may be conjectured that oral contraceptives exert this negative influence on the relative volume of the corpus callosum by lowering the level of sex steroid-hormones both male and female; and that this action is possibly underlined by reduction in the degree of myelination as indexed by the MTR value. Oral contraceptives

lower the production of testosterone by inhibiting the HPO axis and also reduce the level of free testosterone available to activate receptors by increasing the level of SHBG. Consistent with this proven effect of contraceptives on physiology, the level of testosterone in this study is observed to be lower in OCP using females as compared with non-OCP using females (SYS: p=0.046); thus, suggesting that testosterone is positively associated with the relative volume and MTR value of the CC. This hypothesis is consistent with the findings of previous studies that have demonstrated a positive role of testosterone on the relative volume of white matter (Perrin et al. 2008) and its degree of myelination (Stocker et al. 1994). Additionally, oral contraceptives also reduce the average daily level of female sex steroids (estradiol and progesterone) to which the body is exposed (Kuhnz et al. 1992, Van Den Heuvel et al. 2005). Similarly, in this study the level of estradiol (SYS: p=0.008; Cycle: p=0.001) and progesterone (Cycle: p=0.001) is significantly lower in OCP using females versus non-OCP using females; although it is important to keep in mind that the assays were not sensitive to synthetic estradiol and progesterone in OCP using females. Thus, there is some evidence to suggest that higher levels of female sex steroids may also contribute towards the larger CC and higher MTR value of non-OCP using females as compared with OCP using females. This hypothesis is consistent with sex differences observed in the relative volume of the corpus callosum (female > male) during adolescence (Chapter 4) and would explain why females have larger corpus callosum as compared with males during this period. Previous studies on postmenopausal women using hormone replacement therapy also demonstrate a positive association between female sex steroids and white-matter volume (Ha

et al. 2007, Greenberg et al. 2006, Low et al. 2006). Similarly, female sex steroids are also known to exert a positive influence on degree of myelination of white matter (Baulieu et al. 2000, Marin-Husstege et al. 2004).

When examining the association of cycle phase with the relative volume of the total corpus callosum and its segments, it is important to consider the freely cycling women separate from the pill using women. This is because the variation in the sex steroid levels across the cycle phases is different for the two groups of women, as explained in detail above. Regarding the effects of cycle phase on the volume and microstructural properties of the corpus callosum the most significant effect observed is an *increase* in the relative volume of the total corpus callosum during the luteal phase as compared with the ovulatory phase, in only the freely cycling group of women (see Figure 7.4.3). Further investigation reveals that the central and anterior segments are the main sub-regions of the corpus callosum to experience this effect. The relative volume of the central segment increases during the luteal versus menstrual and follicular phase, in freely cycling women only; whereas that of the anterior segment increases in both groups of women during the luteal versus menstrual and ovulatory phase. The increase in the relative volume of the anterior segment, however, is considerably greater in freely cycling women (4.2%) as compared with the pill using women (0.6%). In contrast MTR value of the total corpus callosum is seen to *decrease* during the luteal phase of the cycle as compared with the ovulatory phase. The luteal decline observed in MTR value of total corpus callosum is far greater in the freely cycling women (1.5%) as compared with the OCP using

women (0.5%). This effect of luteal phase on the MTR of the corpus callosum is significant for its mid-posterior segment and shows a trend (0.05<p<0.1) for its mid-anterior segment. The observation of luteal increases in the relative volume of the total corpus callosum of freely cycling women, on the background of corresponding decreases in their MTR values suggests that the effect of luteal phase on the CC volume is not brought about by increases in myelination. The possible microstructural change that may lead to a decrease in MTR value includes increase in axonal calibre. Increase in axonal calibre can contribute to an increase in the volume, while causing a reduction in the number of axons per unit volume, which results in an apparent decrease in MTR. The effect of luteal phase on the volume and microstructure (MTR) of the CC may be predicted, at least in part by its hormonal milieu. The level of progesterone is significantly higher in the luteal phase as compared with the ovulatory phase whereas the level of estrogen is not statistically different between these two phases. Based on these

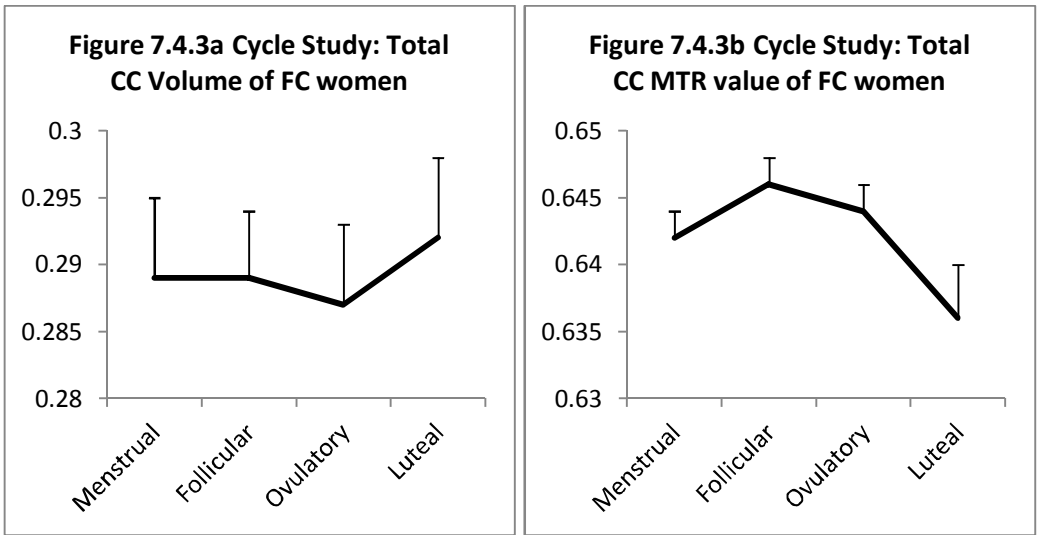

Figure 7.4.3 *Cycle Study: This figure demonstrates the variation in freely cycling women in the (a) relative volume (%) and (b) MTR value of the total corpus callosum across the menstrual cycle phases. Error bars represent standard error of mean.*

findings it may be speculated that the increased production of progesterone during the luteal phase predicts an increase in the relative volume of the corpus callosum, particularly its anterior and central segment; and that this action of progesterone may be underlined by increases in the axonal calibre.

It is important to note that limited changes are also observed in the structure of the corpus callosum of women using contraceptives across the cycle phases, particularly during the luteal phase. These changes mainly include a small increase in the relative volume of the anterior CC segment in the luteal versus menstrual and ovulatory phase, with a corresponding decrease in MTR value. Oral contraceptive users, as previously explained, have lower level of female sex steroids during the menstrual phase when they are not consuming any synthetic steroid contraceptives versus the remaining phases. Moreover their average daily levels of female sex steroids demonstrate moderate increases with continued consumption of oral contraceptives over 21 days. It may be speculated that these changes in female sex steroid levels of OCP using women contribute towards the luteal versus menstrual and ovulatory increase observed in the relative volume of the corpus callosum. Once again, suggesting a positive influence of female sex steroids on the CC.

This is the first study to demonstrate a possible positive influence of sex steroids, particularly testosterone and progesterone, on the structure of the corpus callosum. It is important to be cautious when interpreting these results, however, because they are obtained using relatively less stringent statistical criteria as compared with the remaining studies of this thesis. In particular, two

factors were not corrected for, inter-segmental relatedness and multiple comparisons of post-hoc analysis (two of the post-hoc findings could have been obtained by chance). Moreover, when examining MTR changes corresponding to the volumetric changes observed across the menstrual cycle, trends were considered ($0.05>p>0.10$) along with significant findings ($p<0.05$). This relatively lenient statistical approach is used so as to ensure that any genuine effect is not concealed due to the rigid statistical standards set, which is particularly important in preliminary studies of this sort investigating novel areas. Further research is required in this direction to elucidate the biological basis of the effect of cycle phase on the relative volume of the total corpus callosum of freely cycling and pill using women. Areas in which this study can improve include: 1) having a larger sample of adult women in each group; 2) using a longitudinal study design, which examines women prior to use of oral contraceptives with follow-ups at regular intervals; 3) measuring the level of both endogenous and exogenous (synthetic) female sex steroid-hormones (refer to Timmer et al. 2000 for assays sensitive to synthetic sex steroids), along with other hormones, such as LH, FSH, sex hormone binding globulin and testosterone that are influenced by OCP use (Graham et al. 2006); and 4) confirming the reason for use of oral contraceptives, specifically hormonal disorders such as chronic ovulation, menstrual irregularities and polycystic ovarian syndrome. The possible impact of sex steroids on callosal function and diseases involving the corpus callosum are discussed in Chapter 8, Section 8.5.

There is no significant association of oral contraceptive use or menstrual cycle phase with bimanual performance on the alternating finger tapping task. Thus, the effect of cycle phase and OCP use on the structure of the corpus callosum is not seen to result in corresponding changes in its function. Bimanual coordination is a functional correlate of the microstructural integrity (FA and MD value) of the corpus callosum (Muetzel et al. 2008, Johansen-Berg et al. 2007); callosal fibres connecting the primary motor areas representing the hand pass through its central segment (Pandya et al. 1971). Although the MTR value of the central segment is reduced in OCP using women and its volume varies across the cycle phases, there are no consequences of these structural changes on its function. This is consistent with the findings of Lacreuse et al. (2007). They compared bimanual motor coordination in ovariectomized female rhesus monkeys (which were incapable of producing female sex steroids) with healthy intact female rhesus monkeys and observed no difference. Moreover, Hampson (1990) demonstrated that bimanual motor performance on the Purdue pegboard task did not vary across the menstrual cycle. On the basis of these findings it appears that female sex steroids do not influence bimanual motor coordination.

8 DISCUSSION

8.1 CHAPTER INTRODUCTION

This final chapter discusses the principal findings of this thesis, the relevance of these findings with respect to existing literature and the possible implications of these findings with regards to improving mental health. It also highlights future direction of research in view of methodological limitations of this thesis.

8.2 SUMMARY OF THESIS AIMS AND ADVANTAGES

Sexual dimorphism in the relative volume of the corpus callosum of adults is well established (see Section 1.6.3). Does this sexual dimorphism exist during adolescence and what are the factors responsible for its development? These are the two main questions addressed by this thesis.

Although the current literature examining sex differences in the volume of the corpus callosum, during childhood and adolescence is extensive, it has yielded inconsistent results. As such, it fails to provide conclusive evidence of sexual dimorphism in callosal volume during adolescence. This thesis re-examines and successfully demonstrates sex differences in the volume of the total corpus callosum and its segments, specifically during adolescence. Next, the role of sex hormones in bringing about these sex differences is investigated. This is done by examining variations in CC volume associated with the variation in the level and duration of endogenous sex hormones exposure and pubertal development.

Additionally, and most importantly, this thesis has investigated changes in CC volume associated with alteration of female sex hormone milieu predicted by use of oral contraceptives and female menstrual cycle.

There are various advantages of the studies of this thesis in comparison with previous studies examining sex differences in the corpus callosum. These include:

- Sample Size: Existing studies examining sexual dimorphism in the volume of the corpus callosum have a maximum sample size of 400 participants (see Section 1.6). This thesis examined callosal sex differences in two considerably larger samples (n=737 and n=1,979).
- Sample Age Range: Existing studies examining sex differences in CC volume, prior to adulthood, were usually conducted on samples with the age-range that spanned both childhood and adolescence (Lenroot et al. 2007: 3-to-27 years, Giedd et al. 1999: 5-to-18 years; see Section 1.6.2). This thesis, in contrast, examined sex differences in a sample of adolescents, having a narrower age range (SYS: 12-to-18 years, IMAGEN: 13-to-15 years). As a result, this thesis is able to demonstrate sex differences in the volume of the corpus callosum during adolescence, specifically.
- Volumetric Techniques: Existing studies estimated callosal size by measuring its 2D area on a single mid-sagittal slice. This thesis, in contrast, estimates the 3D volume of the corpus callosum across multiple mid-sagittal slices. The volume, being a 3D measure can be normalized more appropriately with the total brain volume because normalizing 2D

area with 3D brain volume causes disproportionate reduction in the size of the CC for larger brains.

- Statistical Methods: Statistical models used by this thesis accounted for many potential confounding factors neglected by previous studies. Firstly, to prevent any bias caused by overall brain size, all analyses are conducted on brain-size corrected, that is, relative volumes of the corpus callosum. Moreover, the relative volumes of the CC segments are significantly positively inter-correlated and therefore, controlled for inter-segmental dependence. Few previous studies, if any, controlled for this inter-segmental dependence.
- Serum Levels of Sex Hormones: Unlike previous studies, this thesis is the first to measure serum levels of sex steroid-hormones, both male (SYS: serum testosterone level) and female (Cycle Study: serum estrogen and progesterone level), in order to study the influence of sex hormones on the structure (relative volume and MTR value) of the corpus callosum.
- MTR value of the CC: In addition to volumetric estimates of gross CC anatomy, this thesis also measures the MTR value of the corpus callosum (in Cycle Study) to determine whether the volumetric changes observed may be related to variations in the content of myelin, which is the prime determinant of MTR (Schmierer et al. 2004).

8.3 REVIEW AND DISCUSSION OF PRINCIPAL FINDINGS

Table 8.3.1 Summary of Effects observed on the CC regions						
	Sex	**Age**	**Cycle Phase**		**OCP use**	
	Volume	Volume	Volume	MTR	Volume	MTR
Total	+	+	+	-	-	~
Anterior	+	~	+	~	-	~
MidAnterior	-	~	~	-	-	~
Central	+	~	+	~	-	-
MidPosterior	~	~	~	-	~	~
Posterior	+	+	~	~	~	~

Table 8.3.1: Effects of age, sex, menstrual cycle phase and use of oral contraceptive pills (OCP) on the relative volume (%) and MTR value of the total corpus callosum and its segments. Note: ~ denotes no effect observed; For Sex Effects positive denotes F>M and negative denotes M>F; For Age Effects positive denotes increase with age; For Cycle Phase effects positive denotes progesterone rich phase (luteal)>progesterone poor phase (ovulatory, follicular or menstrual) and negative denotes the opposite pattern of results; and for effects of OCP use negative denotes OCP<FC.

Sex differences in the relative volume of the corpus callosum have been explored extensively (see Section 1.6). Sex differences in callosal volume are rare during early childhood and reports of sexual dimorphism become more frequent during late childhood and adolescence (for details of studies see Section 1.6). Many researchers have investigated sex differences in the relative volume of the corpus callosum in samples comprised of children and adolescents (Section 1.6). Results of these studies, however, are very variable and fail to yield definite conclusions (Section 1.6). Variations in sample size and methodological approaches could be responsible for these inconsistent results. The importance of this topic and inconsistencies in existing literature provided the impetus for this thesis to re-examine sex differences in the relative volume of the corpus callosum, during adolescence. Sex differences are examined specifically in the brain size corrected, that is, relative volume of the CC. This is because brain size

is a major confounding factor that affects the size of the corpus callosum and its segments, irrespective of sex. Correlations of up to 0.40 (Johnson et al. 1994) and 0.45 (Bermudez et al. 2000) have been reported between the corpus callosum and total brain volume. Moreover total brain volume was highly correlated with the volume of the CC in both the SYS study (Pearson's *r*=0.382) and the IMAGEN study (Pearson's *r*=0.407). This thesis successfully demonstrates that sex differences in the relative volume of the corpus callosum are apparent during adolescence in two large samples, namely the SYS sample (n=737, 12-to-18 years) and IMAGEN sample (n=1,979, 13-to-15 years*). The relative volume of the total corpus callosum is slightly but significantly greater in girls as compared with boys (SYS: effect size r=0.30; IMAGEN; r=0.20). The main sub-regions of the corpus callosum where this difference exists are the anterior (SYS: r=0.08; IMAGEN: r=0.10), central (SYS: r=0.16; IMAGEN: r=0.14) and posterior segments (SYS: r=0.10; IMAGEN: r=0.12). In the case of age, the relative volume of the posterior segment of the corpus callosum demonstrates small but significant age-associated increases during adolescence (SYS: r=0.12; IMAGEN: r=0.05).* This is consistent with the report of Giedd et al. (1999) who examined the influence of age on the relative volume of CC segments via a longitudinal study design and demonstrated that growth was most robust and significant in the posterior most region of the corpus callosum during adolescence (n=139, 5 to 18 years). Reliability of these findings is further justified by their consistent occurrence in two large samples (SYS and IMAGEN). This area of research is not new; nevertheless, it is investigated with various new improvements (see above) which make this thesis a significant addition to existing literature. *This thesis*

highlighted, for the first time, the importance of correcting for inter-segmental dependence when examining arbitararily segmented CC segments. This is done by demonstrating the differences in the results before and after the correction is done. The mid-anterior and mid-posterior CC segments, for example, are seen to be greater in girls versus boys before controlling for inter-segmental dependence but afterwards sex differences in the former became greater in boys versus girls, whereas in the latter became equivocal. Similarly, age is seen to be positively correlated with most CC segments before correcting for inter-segmental dependence but after the correction only the relation with the posterior segment remained significant. The inter-dependency between segmental volumes is demonstrated by the correlation between their sizes and is probably due to the fact that they share axons having similar topographic origins. It is important to note that there are no significant differences in the inter-segmental correlations of males and females.

Like the structure, sex differences are also observed in the function of the corpus callosum. Intelligence (IQ) and bimanual motor coordination are the principal brain functions examined by this thesis for a possible association with the CC, based on consensus provided by both acallosal and MRI studies (Section 1.4). The choice of behavioural taska was also opportunistic since the study was carried out on an existing dataset. The association of the corpus callosum has been examined with performance in two main areas of intelligence, verbal intelligence (VIQ) and performance intelligence (PIQ). *This thesis is the first to examine this "performance intelligence-CC volume" relationship in further detail, and*

demonstrates that processing speed and not perceptual reasoning is the principal component of performance intelligence associated, albeit weakly, with the corpus callosum of girls but not boys (SYS: variance ΔR^2=0.012, effect size r=0.11). This suggests that the corpus callosum in girls plays a supportive (excitatory) role in interhemispheric processing associated with processing speed tasks. *The sex difference observed in the callosal association with processing speed is not influenced by performance. This is evidenced by the fact that boys with higher scores for processing speed (scores above median: n=174, Mean ±SD: 116 ±9.6), unlike girls, do not demonstrate any correlation between the CC volume and processing speed IQ (β=0.010, p=0.900).* Literature examining the role of the corpus callosum on inter-hemispheric processing of cognitive tasks and menstrual variation in CC associated functional asymmetry of these cortical tasks is explained in detail in Chapter 5 (Section 5.1) and Chapter 7 (Section 7.1), respectively.

Several DTI studies demonstrate that the microstructural integrity (FA value) of the corpus callosum is associated with bimanual motor coordination (Sullivan et al. 2006, Johansen-Berg et al. 2007, Meutzel et al. 2008). Moreover, studies of individuals with agenesis or transection of the CC demonstrated that bimanual motor coordination is compromised in individuals lacking the CC (Sauerwein et al. 1994, Moes et al. 2008, Mueller et al. 2009). Based on these studies, this thesis examines whether the volume of the corpus callosum (like its microstructure) is also associated with bimanual motor coordination in healthy participants of the SYS study. No correlation is observed between bimanual

motor coordination and CC. We, therefore, speculate that the organization of callosal fibres that determines their FA value influences bimanual coordination skills but the volume constituted by these callosal fibres does not. It is important to note that the functional correlates of the CC are examined in the SYS participants only because bimanual motor coordination is not assessed in the IMAGEN study and only few of the WISC tests for IQ have been conducted on adolescents from the IMAGEN sample as a result their IQ scores could not be assessed.

The factors responsible for sex differences observed in the structure (relative volume) of the corpus callosum during adolescence remain unexplained. This thesis explores the role of sex hormones, as one of the possible factors involved in bringing about these sex differences. The reason for studying the role of sex hormones specifically in generating these sex differences is because increasing production of sex hormones from the gonads has been identified as one of the leading factors responsible for the development of sexually dimorphic physical characteristics of the human body during adolescence (Guyton et al. 2000). This suggests that perhaps sex differences observed in the relative volume of the corpus callosum during adolescence may also be attributed, at least in part, to sex hormones. There are three main findings of this thesis that highlight a possible "sex hormone-corpus callosum" relationship. Firstly, pubertal stage of adolescent boys (above and beyond chronological age) is negatively associated with the relative volume of the anterior CC segment (SYS: ΔR^2=0.01, r=0.11) and positively associated with the relative volume of the mid-anterior CC segment

(SYS: ΔR^2=0.01, r=0.15). This is consistent with the sex differences observed in the relative volume of the anterior (F>M) and mid-anterior (M>F) segment of the corpus callosum, during adolescence. The negative "puberty-anterior CC volume" relationship may contribute towards the male versus female *disadvantage* in anterior CC volume (0.067% ±0.01 vs. 0.071% ±0.01) and the positive "puberty-mid anterior CC volume" relationship may contribute towards the male versus female *advantage* in mid-anterior CC volume (0.037% ±0.01 vs. 0.035% ±0.01). *Thus, suggesting that sex hormones that are responsible for inducing pubertal development of boys, mainly testosterone, dihydrotestosterone, dehydroepiandrosterone (DHEA) and androstenedione (Shirtciff et al. 2007, Guyton et al. 2000) may play a role in making the volume of the corpus callosum sexually dimorphic, in a region-specific manner.* In case of girls, no correlation is observed between pubertal stage and the corpus callosum. This may be because the relationship between female sex hormones and the corpus callosum is not robust enough to survive correction for chronological age, unlike that of boys. *Chronological age is well correlated with the volume of the corpus callosum and with pubertal stage. The linear regression models used by this thesis correct for chronological age and therefore form very strict criteria for determining the relationship between the corpus callosum and pubertal stage.* It is important to note that pubertal stage of IMAGEN boys, unlike SYS boys, is not associated with the relative volume of any CC segment. This is probably due to limited variance in pubertal stage of IMAGEN boys as compared with SYS boys.

Following pubertal stage, the second approach to demonstrate a relationship between sex hormones and the corpus callosum is the association of oral contraceptives with the CC. *Use of oral contraceptives constitutes a pharmacological intervention that has a profound effect on the normal endocrinology of the body and it, therefore, provides a more effective means of examining the role of sex hormones on CC volumes as compared with studying CC volume measures associated with pubertal stage of the general population.* Moreover, association of contraceptive use is not only assessed with gross volumetric measures of the CC but also with microstructural properties of the CC as indexed by MTR (measured in Cycle study alone). This enables determination of whether the association between OCP use and CC volume is related to variations in the content of myelin, which is the prime determinant of MTR (Schmierer et al. 2004). Relative volume of the total corpus callosum (SYS: ΔR^2=0.05, r=0.22), particularly its anterior (SYS: ΔR^2=0.06, r=0.25), mid-anterior (SYS: ΔR^2=0.07, r=0.26; IMAGEN: ΔR^2=0.05, r=0.22) and central segment (IMAGEN: ΔR^2=0.04, r=0.19), is significantly lower in females using oral contraceptives as compared with females not using oral contraceptives. Regarding MTR value, there is a trend observed for corresponding differences in the MTR value of the central segment of the corpus callosum, that is, these values are lower in OCP using versus non-using females (Cycle Study: ($F_{1,22}$ =3.714, p=0.086). *Based on these findings it may be speculated that oral contraceptives exert a negative influence on the volume of callosal white matter by altering the normal sex hormonal milieu, and that these changes may be underlined by reduction in the degree of myelination.* Females using oral

contraceptives are shown to have lower level of natural (body-produced) sex steroids, both male (testosterone: SYS, p=0.046) and female (estradiol: SYS, p=0.008; Cycle, p=0.001 and progesterone; Cycle, p=0.001), as compared with females not using oral contraceptives. It is important to note that the difference in the level of female sex steroids of the two groups should be viewed with caution because the assays were not very sensitive to synthetic contraceptive steroids (Timmer et al. 2000 for examples of assays sentive to synthetic steroids); nevertheless, previous studies have shown that mean daily level of female sex steroids are lower in OCP using versus non-OCP using females (Van Den Heuvel et al. 2005, Kuhnz et al. 1992, Stricker et al. 2006). Thus, suggesting a positive association between sex steroids (both male and female) and the structure of the CC (volume and MTR). This hypothesis is consistent with the findings of previous studies that have demonstrated a positive role of male and female sex steroids in the relative volume of white matter (male: Perrin et al. 2008; female: Ha et al. 2007, Greenberg et al. 2006, Low et al. 2006) and in the myelination of white matter in tissue cultures prepared from rodent brains (male: Levy et al. 1996, Stocker et al. 1994; female: Baulieu et al. 2000, Marin-Husstege et al. 2004). It is important to note that contraceptive use in cycle study, unlike SYS and IMAGEN study is not associated with relative volume of the CC. This is most likely due to its limited sample size (13 OCP vs. 13 non-OCP females) and older age range (18-to-30 years) in comparison to SYS and IMAGEN study (52 OCP vs. 52 non-OCP females); age range being relevant because the effect of contraceptives on CC volume might be greater during adolescence when the corpus callosum is

growing than during adulthood when there is little development in the size of the corpus callosum (Johnson et al. 1994, Lee et al. 2009).

The third and final approach to demonstrate a possible effect of sex hormones on the structure of the CC is based on variations induced by menstrual cycle phase. Changes in the structure (relative volume and MTR) of the CC across the phases of the menstrual cycle are explored using a *longitudinal study design* (in Cycle Study alone). The most significant effect of cycle phase on CC observed is an increase in the relative volume of the total corpus callosum of freely cycling women, particularly the anterior and central segment, from the ovulatory to the luteal phase. On the other hand, MTR value of the total CC decreases from the ovulatory towards the luteal phase, thus suggesting that the effect of luteal phase on CC volume is not associated with increases in myelination. The possible microstructural change that may lead to a decrease in MTR value includes increase in axonal calibre. Increase in axonal calibre can increase the volume while reducing the number of axons per unit volume, thus resulting in an apparent decrease in MTR. The effect of luteal phase on the volume and microstructure (MTR) of the CC may be predicted, at least in part by its hormonal milieu. Robust but short-lived variations are observed in the levels of female sex steroids across the menstrual cycle phases. The level of progesterone is significantly higher in the luteal phase as compared with the ovulatory phase whereas the level of estrogen is not statistically different between these two phases. *Based on these findings it may be speculated that the increased production of progesterone during the luteal phase predicts an increase in the*

relative volume of the corpus callosum, particularly its anterior and central segment; and that this action of progesterone might be mediated by increasing the axonal calibre. This is the first study to predict a possible influence of female sex steroids, in particular progesterone, on the volume of the corpus callosum. There are no studies to our knowledge that have investigated the effects of menstrual cycle phase or contraceptive use with the relative volume of any white-matter region of the brain. Previous studies on postmenopausal women using hormone replacement therapy, however, have been conducted, which demonstrate a positive *association* between female sex steroids and white-matter volume (Ha et al. 2007, Greenberg et al. 2006, Low et al. 2006).

The relationship of sex hormones with the structure of the CC (volume) failed to be demonstrated by callosal association with the level of sex hormones (testosterone in boys of SYS sample) or the duration of exposure to sex hormones (duration since menarche in girls of SYS and IMAGEN sample), above and beyond chronological age. This is most likely due to correcting for chronological age that is well correlated with the volume of the corpus callosum and with both the indices of sex hormones mentioned above. Perrin et al. (2008) demonstrate a correlation between the level of bioavailable testosterone and the relative volume of white matter in male adolescents, particularly those with an ‘‘efficient” variant of the androgen-receptor gene (with respect to transcriptional activity). This effect, however, also fails to survive correction for chronological age. As mentioned previously, the linear regression models used by this thesis correct for chronological age and therefore, form very strict criteria for

determining the relationship between the corpus callosum and all measures of sex hormones.

In addition this thesis validates the use of FreeSurfer as an automatic volumetric technique to measure the volume of the corpus callosum. The current gold standard for computational volumetric analysis of the corpus callosum is manual delineation of its mid-sagittal area by a trained expert. FreeSurfer is a modern volumetric analysis tool available that enables fully automated segmentation of the corpus callosum and subsequent calculation of its volume. FreeSurfer is being increasingly used, particularly for volumetric analysis of the CC (and other brain structures) in large samples, on account of various advantages in comparison to the gold standard that include: 1) faster processing time; 2) no requirement of an experienced tracer and, therefore, less variation based on tracer-dependent skills; and 3) segmentation across multiple mid-sagittal slices versus a single slice, thus, providing a more comprehensive 3D estimate of callosal size. Several researchers have used FreeSurfer as a means to segment the corpus callosum and study the effects of various pathologies on its size (Francis et al. 2011, Tartaglia et al. 2009, Vatta et al. 2011). No one has, however, validated the use of this automated method against the gold-standard, manual tracing method. *This thesis is the first to validate the fully automated FreeSurfer estimates of CC mid-sagittal area and volume with the semi-automated, Display-assisted manual ones.* Display is the visualization tool used for computer-assisted but essentially manual delineation of the corpus callosum (http://www.bic.mni.mcgill.ca /ServicesSoftware/HomePage). This thesis successfully demonstrates that

FreeSurfer estimates of mid-sagittal area of the corpus callosum measured on T1W scans from 617 adolescents of the SYS study are closely correlated with its manual estimates (intra-class correlation=0.9) and therefore, provides an accurate alternative means of volumetric analysis. Factors that ensured this high correlation include, measuring CC area in the same mid-sagittal slice using both techniques to ensure a good comparison. *Moreover, visual inspection of all the automatic FreeSurfer segmentations is seen to be of vital importance to ensure consistency between the two methods. This quality check removes all poorly segmented CCs, thus, ensuring that all segmentations are accurate and comparable to the manual ones.* Visual inspection revealed that FreeSurfer overestimates the corpus callosum slightly at the junction of the CC midbody and fornix. The border at this junction is seen to impinge onto the fornix. This may be responsible for the slight overestimation of the CC mid-sagittal area by FreeSurfer as compared with manual means (difference of 11 mm^2).

8.4 METHODOLOGICAL CONSIDERATIONS AND FUTURE DIRECTIONS

The findings of this thesis regarding "the effects of sex steroids on the structure (volume and MTR) of the corpus callosum" need to be considered cautiously due to certain methodological issues. Firstly, the association of puberty with the volume of the corpus callosum is assessed using cross-sectional data, similarly comparison of OCP using and non-OCP using females of the SYS, IMAGEN and Cycle study is also conducted via cross-sectional means; this cross-sectional

design does not allow causal inferences to be made regarding the effects of puberty and OCP use on the CC. Secondly, these effects are subtle and the negative effects of OCP use observed on CC segments would be lost if correction for inter-segmental dependence is performed. Additionally, the effects of OCP use are not seen consistently across all studies; use of oral contraceptives is negative associated with CC volume in the SYS and IMAGEN study but not in the Cycle study. Lastly, the methods used to assay the levels of sex steroids are not sensitive to synthetic steroids; as a result previous studies had to be relied upon, to some extent, in order to gauge the possible sex steroidal milieu of OCP using females.

Future research is required to elucidate better the relationship between sex steroids and the corpus callosum. Areas in which future studies may improve include; 1) using a longitudinal study design to examine the changes in the structure of the corpus callosum induced by pubertal development and OCP use; 2) using improved means of measuring sex steroid-hormones, in particular both the natural and synthetic level of sex steroids should be assayed (refer to Timmer et al. 2000 for assays sensitive to synthetic steroids); and 3) other possible sex hormones and sex-hormone associated bio-chemicals that are clearly likely to be associated with pubertal development and to be influenced by OCP use, such as dihydrotestosterone, dehydroepiandrosterone (DHEA) androstenedione, LH, FSH and sex hormone binding globulin should also be measured. Further research is also required to establish better the microstructural basis of the “sex steroid-CC volume” relationship. Diffusion Tensor Imaging (DTI) can be employed along with

MTR imaging to examine the changes in CC white matter in more detail. Another possible approach that may be used by future studies to examine the influence of sex steroids on the corpus callosum includes studying the brains of normal or pathological population samples that experience altered levels of sex hormones, such as pregnant women (elevated estrogen and progesterone levels), patients with hypogonadism (reduced sex steroid levels), Klinefelter syndrome (reduced testosterone levels) or polycystic ovary syndrome (elevated testosterone levels).

8.5 POSSIBLE IMPLICATIONS

The corpus callosum is the largest cerebral commissure in the brain and, as such, is comprised of approximately 190 million axons that allow transfer of neural impulses across the two hemispheres. The inter-hemispheric connectivity provided by the corpus callosum is essential for smooth functioning of multiple neural networks. This is demonstrated by the correlation between structural properties of CC white matter and various cortical functions. For example, the area of the corpus callosum is associated with cognition (verbal intelligence: Hines et al. 1992; non-verbal intelligence: Hutchinson et al. 2009). Similarly, the microstructural integrity (FA value) of the CC is associated with cognition (working memory: Voineskos et al. 2010; processing speed: Kennedy et al. 2000) and bimanual motor coordination (Muetzel et al. 2008). Animal and agenesis studies have also demonstrated a possible association of the CC with depth perception (animal: Milleret et al. 2001; agenesis: Jeeves et al. 1991) and sound localization (agenesis: Lessard et al. 2002); although there are no human studies,

to our knowledge, demonstrating a relationship between these functions and structural properties of the CC in healthy samples. Subtle variations in the structural properties of CC white matter have also been observed in various neuropsychiatric disorders. For example, the area of the corpus callosum is reduced in patients with schizophrenia (Bersani et al. 2010), multiple sclerosis (Pozzilli et al. 1991), attention deficit hyperactivity disorder (ADHD; Hutchinson et al. 2008) and autism (Kilian et al. 2008). Similarly, the microstructural integrity of the corpus callosum is compromised in patients with schizophrenia (FA and MTR value; Kubicki et al. 2005), multiple sclerosis (FA value: Hassan et al. 2005; MTR: Gass et al. 1994, Rocca et al. 1999) and autism (FA value: Alexander et al. 2007, Keller et al. 2007). Moreover, the degree of cognitive and behavioural impairment in these conditions is seen to depend on the extent of involvement of callosal structure. Examples of such behavioural traits include: verbal fluency in multiple sclerosis (associated with CC area; Pozilli et al. 1991), non-verbal intelligence (PIQ) in autism (associated with CC FA and MD value; Alexander et al. 2007) and degree of hyperactivity and impulsivity in ADHD (associated with CC size; Giedd et al. 1994). Studying the structure of the corpus callosum, therefore, has widespread implication with regards to understanding neural underpinnings of normal cognitive & mental health and neuropsychiatric psychopathologies.

This thesis demonstrates sexual dimorphism in the structure (relative volume) of the corpus callosum during adolescence (F>M), with corresponding differences in its cognitive function (processing speed: F>M). Previous studies have also reported sex differences in other cognitive domains (verbal fluency and memory

F>M; Ankney et al. 1996) that have been shown by some authors to be related to the CC (Hines et al. 1992). Moreover, brain disorders involving the corpus callosum, such as schizophrenia (M>F; Hafner et al. 2003), multiple sclerosis (F>M; Whitacre et al. 1999 and 2001), autism and ADHD (M>F; Hutchinson et al. 2008, Gershon et al. 2002) are also known to be sexually dimorphic. The neurobiological processes contributing to the development of sex differences in the structure of the corpus callosum are poorly understood. This thesis is the first report to highlight sex steroids as one of the possible factors responsible for the development of sexual dimorphism in the structure (volume and MTR value) of the corpus callosum of humans. Studying the role of sex steroids in the development of sexual dimorphism, using the corpus callosum as a model structure, may help us to understand better the neural basis of sexually dimorphic brain functions (mentioned above) and may provide insight into the pathophysiology of sexually dimorphic neurological disorders (mentioned above). Interestingly, research into the possible effects of sex steroids on many of these sexually dimorphic brain functions and brain disorders is already underway. For example, sex steroids (estrogen in hormone replacement therapy) have been shown to be associated with better verbal cognitive performance in postmenopausal women (Shaywitz et al. 2003, Wroolie et al. 2011). High level of (prenatal) testosterone is associated with autistic traits (Auyeung et al. 2010). Similarly, testosterone level has also been highlighted as one of the possible neuroendocrine markers for development of psychosis (van Rijn et al. 2011). Production of sex steroids (testosterone and estradiol) has also been proposed as a possible mechanism contributing to the development of ADHD and major

depressive disorder (Martel et al. 2009). Taken together these studies highlight the fact that understanding the biological basis of the effects of steroids on white matter may pave the way towards developing improved means for prevention and treatment of these sexually dimorphic neuropathologies. Moreover, they also suggest a possibility for the use of sex steroids as neuroprotective agents in the future, in view of their protective effect on cognition.

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APPENDIX A

SYS: Correlations between Total Brain Volume (mm^3) and Absolute CC Volumes (mm^3)			
CC Volume	**Total**	**Boys**	**Girls**
Total	*r=0.382, p<0.0001, df=704*	*r=0.331, p<0.0001, df=341*	*r=0.452, p<0.0001, df=363*
Anterior	*r=0.407, p<0.0001, df=706*	*r=0.350, p<0.0001, df=342*	*r=0.444, p<0.0001, df=364*
MidAnterior	*r=0.352, p<0.0001, df=707*	*r=0.325, p<0.0001, df=344*	*r=0.463, p<0.0001, df=363*
Central	*r=0.198, p<0.0001, df=702*	*r=0.215, p<0.0001, df=340*	*r=0.304, p<0.0001, df=362*
MidPosterior	*r=0.218, p<0.0001, df=706*	*r=0.202, p<0.0001, df=342*	*r=0.308, p<0.0001, df=364*
Posterior	*r=0.300, p<0.0001, df=704*	*r=0.222, p<0.0001, df=340*	*r=0.362, p<0.0001, df=364*

Appendix A Table I SYS: *Pearson's correlation between the absolute volume of the total brain (mm^3) and the absolute volume of the corpus callosum and all its segments (mm^3).*

IMAGEN: Correlations between Total Brain Volume (mm^3) and Absolute CC Volumes (mm^3)			
CC Volume	**Total**	**Boys**	**Girls**
Total	*r=0.407, p<0.0001, df=1879*	*r=0.363, p<0.0001, df=897*	*r=0.361, p<0.0001, df=982*
Anterior	*r=0.397, p<0.0001, df=1873*	*r=0.354, p<0.0001, df=893*	*r=0.382, p<0.0001, df=981*
MidAnterior	*r=0.345, p<0.0001, df=1856*	*r=0.277, p<0.0001, df=879*	*r=0.318, p<0.0001, df=977*
Central	*r=0.190, p<0.0001, df=1873*	*r=0.151, p<0.0001, df=892*	*r=0.163, p<0.0001, df=981*
MidPosterior	*r=0.284, p<0.0001, df=1875*	*r=0.218, p<0.0001, df=893*	*r=0.245, p<0.0001, df=982*
Posterior	*r=0.319, p<0.0001, df=1876*	*r=0.289, p<0.0001, df=894*	*r=0.296, p<0.0001, df=982*

Appendix A Table II IMAGEN: *Pearson's correlation between the absolute volume of the total brain (mm^3) and the absolute volume of the corpus callosum and all its segments (mm^3).*

APPENDIX B

SYS: Inter-Segmental Correlations			
	Boys	**Girls**	**p-value for difference**
Anterior-MidAnterior	*0.465*	*0.496*	*0.609*
Anterior-Central	*0.322*	*0.206*	*0.113*
Anterior-MidPosterior	*0.429*	*0.445*	*0.802*
Anterior-Posterior	*0.542*	*0.573*	*0.568*
MidAnterior -Central	*0.668*	*0.609*	*0.206*
MidAnterior-MidPosterior	*0.578*	*0.573*	*0.924*
MidAnterior-Posterior	*0.377*	*0.439*	*0.345*
Central-MidPosterior	*0.599*	*0.533*	*0.217*
Central-Posterior	*0.276*	*0.251*	*0.733*
MidPosterior-Posterior	*0.462*	*0.530*	*0.252*

Appendix B Table I SYS: Correlations between the relative volumes (%) of the segments of the corpus callosum in both boys and girls and the significance (p value) of the sex difference in these inter-segmental correlations.

IMAGEN: Inter-Segmental Correlations			
	Boys	**Girls**	**p-value for difference**
Anterior-MidAnterior	*0.390*	*0.524*	***0.000***
Anterior-Central	*0.245*	*0.375*	***0.002***
Anterior-MidPosterior	*0.427*	*0.463*	*0.339*
Anterior-Posterior	*0.522*	*0.523*	*0.979*
MidAnterior -Central	*0.656*	*0.659*	*0.910*
MidAnterior-MidPosterior	*0.565*	*0.500*	*0.055*
MidAnterior-Posterior	*0.447*	*0.451*	*0.915*
Central-MidPosterior	*0.507*	*0.528*	*0.541*
Central-Posterior	*0.265*	*0.32*	*0.200*
MidPosterior-Posterior	*0.550*	*0.547*	*0.927*

Appendix B Table II IMAGEN: Correlations between the relative volumes (%) of the segments of the corpus callosum in both boys and girls and the significance (p value) of the sex difference in these inter-segmental correlations.