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## Sex differentiation and sexually dimorphic disease

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**Sex Differentiation  
and  
Sexually Dimorphic Disease**

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**Emily A. Jefferson**

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This Thesis Submitted in Partial Fulfilment of the Requirements for  
The Award of

**Bachelor of Science (Human Biology) Honours**

In the Faculty of Computing, Health and Science,  
Edith Cowan University, Joondalup, Western Australia

**Date: 24<sup>th</sup> November 2006**

## ABSTRACT

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Sexual dimorphism of the central nervous system is a still widely debated and an area of much research. Conclusive evidence that anatomical and physiological differences in the CNS exist has been reported by post-mortem studies and magnetic resonance imaging (MRI). This present study seeks to contribute to the understanding of the differences in the brain between genders and to ascertain reasons as to why the literature is so varied.

A number of structures such as the cerebral cortex, hypothalamic nuclei and the amygdala have proven to be significantly larger within males as opposed to females. The nuclei of the hypothalamus and the amygdala are involved in a variety of functions all closely related to sexual behaviour. The increase in size of these structures within males may contribute to the increase in psychosexual disorders seen more commonly in males. The anterior commissure and corpus callosum, two grey matter structures, have been shown to be larger in females, enabling females to utilise both hemispheres of the cerebrum when undertaking certain tasks, whereas males are seen to use one hemisphere.

It is known that certain diseases and disorders are more common or appear more severe in one sex compared to the other. Correlations have been found linking disease prevalence and severity with androgens, yet few have reported relationships between brain structure and disease. Neuropsychological disorders such as autism and schizophrenia have been linked to the anatomical differences of the male and female brain, however the pathology of the majority of sexually dimorphic diseases remains

largely unknown. Experimental designs need to be reassessed in order to provide significant evidence of sexual dimorphism in these pathologies. Sex must be seen as an important variable that needs to be accounted for in order to contribute to the understanding of the functional differences exhibited by males and females.

## USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.

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## ABBREVIATIONS

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<b>A<math>\beta</math></b>	Amyloid beta
<b>AChE</b>	Acetylcholinesterase
<b>ACTH</b>	Adrenocorticotrophin hormone
<b>AGP</b>	Adrenogenital primordium
<b>APP</b>	Amyloid precursor protein
<b>BNST<sup>r</sup></b>	Bed nucleus of the stria terminalis
<b>BNSTc</b>	Central division of bed nucleus of the stria terminalis
<b>BNST-dspm</b>	Darkly staining posteromedial component of the bed nucleus of the stria terminalis
<b>CAH</b>	Congenital Adrenal Hyperplasia
<b>ChAT</b>	Choline acetyltransferase
<b>CNS</b>	Central nervous system
<b>CVA</b>	Cerebrovascular accident
<b><i>DAX-1</i></b>	Dosage sensitive sex-reversal adrenal hypoplasia congenita-critical region of the X chromosome
<b><i>Dax-1</i></b>	Mouse dosage sensitive sex-reversal adrenal hypoplasia congenita-critical region of the X chromosome
<b>DHEA</b>	Dehydroepiandrosterone
<b><i>DMRT1</i></b>	Doublesex and mab-3 related transcription factor 1
<b><i>Dmrt1</i></b>	Mouse Doublesex and mab-3 related transcription factor 1
<b>dpp</b>	Days post partum
<b>dpo</b>	Days post ovulation

<b>fMRI</b>	Functional magnetic resonance imaging
<i>Fog2</i>	Feminisation of germline 2 gene
<b>FSH</b>	Follicle stimulating hormone
<b>FSH<math>\beta</math></b>	Follicle stimulating hormone beta
<b>GATA4</b>	Human GATA binding protein 4 gene
<i>Gata4</i>	Mouse GATA binding protein 4 gene
<b>HMG</b>	High mobility group
<b>HRT</b>	Hormone replacement therapy
<b>ICM</b>	Inner cell mass
<b>IFN-<math>\gamma</math></b>	Interferon gamma
<b>IL-1<math>\beta</math></b>	Interleukin 1 beta
<b>IL-6</b>	Interleukin 6
<b>KTS</b>	Lysine-Threonine-Serine amino acid triplet
<b>LH</b>	Lutenising hormone
<b>LH<math>\beta</math></b>	Lutenising hormone beta
<b>MI</b>	Myocardial infarction
<b>MIH</b>	Mullerian Inhibiting Hormone
<b>MPTP</b>	1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine
<b>MRI</b>	Magnetic resonance imaging
<b>NBM</b>	Nucleus basalis of Meynert
<b>NES</b>	Nucleus export signal
<b>NFTs</b>	Neurofibrillary Tangles
<b>NLS</b>	Nuclear localisation signal
<b>PAOH</b>	Preoptic Area of the Hypothalamus
<b>PD</b>	Parkinson's Disease

<b>PET</b>	Positron emission tomography
<b>RA</b>	Rheumatoid Arthritis
<b>SCN</b>	Suprachiasmatic nucleus
<b>SDN</b>	Sexually dimorphic nucleus
<b><i>SF-1</i></b>	Steroidogenic factor-1 gene
<b><i>Sf-1</i></b>	Mouse steroidogenic factor-1 gene
<b>SLE</b>	Systemic Lupus Erythematosus
<b><i>SOX9</i></b>	SRY-box containing gene 9
<b><i>Sox9</i></b>	Mouse Sry-box containing gene 9
<b><i>SRY</i></b>	Human sex determining region Y gene
<b><i>Sry</i></b>	Mouse sex determining region Y gene
<b>TDF</b>	Testis Determining Factor
<b>Tfm</b>	Testicular feminization
<b>TH</b>	True hermaphroditism
<b>TNF-<math>\alpha</math></b>	Tumour necrosis factor alpha
<b>VDB</b>	Vertical limb of the diagonal band of Broca
<b>VMH</b>	Ventromedial hypothalamus
<b>VMPC</b>	Ventromedial prefrontal cortex
<b><i>WT1</i></b>	Wilm's tumour associated gene
<b><i>Wt1</i></b>	Mouse Wilm's tumour associated gene

## **AIM OF STUDY**

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The overall aim of this present study was to critically review gender specific contemporary research into the anatomical and physiological differences in the central nervous system and to ascertain their relationship to disease.

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## CHAPTER ONE

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### Introduction

Since the 1950's, scientists have sought to produce conclusive evidence supporting the theory that anatomical and physiological differences exist within the central nervous system between genders. Evidence has been collected throughout this period in support of this theory, through the use of laboratory animals and the study of hormonal abnormalities within humans (Good et al, 2001).

The development of the human brain in childhood and adolescence is characterised by progressive and regressive processes that determine the size and shape of a particular brain structure. *In vivo* studies using magnetic resonance imaging (MRI) have provided some of the answers that are sought in regards to sexual dimorphism of the brain. MRI studies have enabled researchers to conduct large sample studies on the human brain, providing information that small scale post mortem studies are unable to provide (Good et al, 2001).

The development of two individual sexes within a species is a common trait throughout the animal kingdom and is referred to as sexual dimorphism. Genes and their interaction with specific sex hormones play a pivotal role in determining the gender of an individual (Kalthoff, 2001). Developmental biologists have been able to identify the genetic pathways that control sex determination within many species such as *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (roundworm), yet identifying the same in mammalian development has proved to be more difficult (Goodfellow and Lovell-Badge, 1993).

All female oocytes carry the X chromosome so it is the males' spermatozoa that contributes either an X or Y chromosome and thus determines the sex of an embryo. A normal human male embryo has an XY genotype whilst female embryos' consist of an XX genotype. The Y chromosome can therefore be seen as the critical factor for male sex differentiation (Kalthoff, 2001).

A region on the Y chromosome named the testis-determining factor (TDF), which spans 35kb, has been determined as the region that codes for testis formation. An important gene named the sex-determining region Y (*SRY*) has been located within the TDF and has been linked with the first stage of sexual differentiation. The *SRY* gene has a crucial role within the testes where developing Sertoli cells that will differentiate, nourish and protect spermatogonia (stem cells that differentiate into spermatozoa) are formed. Due to the lack of the Y chromosome, female gonadal ridges differentiate into ovarian cells (Johnson and Everitt, 2000).

## CHAPTER TWO

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### Sex Determination and the *SRY* Gene

The formation of testes occurs due to the presence of the *SRY* gene located on the Y chromosome. *SRY* expression occurs within the pre-Sertoli cells that are located within the male genital ridge of a developing embryo. The *SRY* protein structure provides the only clue as to how this gene encodes testis formation. It is composed of a single exon with a DNA binding high mobility group (HMG) box and therefore is a transcription factor with the ability to alter other genes in the sex determination pathway. The HMG box allows the *SRY* protein to bind to the minor groove within DNA, causing the DNA to bend and expose certain binding sites to other proteins that may be necessary in the developmental pathway (Viger et al, 2005). The majority of *SRY* mutations that occur within humans are located within the HMG box which emphasises the importance of the gene, yet the exact mechanism that causes *SRY* to act as a testis-determining factor still remains unclear (Harley et al, 2003).

The first evidence of the mouse *SRY* gene (*Sry*) occurs at day 10.5 of embryo development and rises to peak levels at day 11.5. By day 14.5 of embryogenesis the *Sry* protein is no longer detected as the *Sry* expression abruptly halts. Within humans, the *SRY* protein can be detected between day 41 and 45 postovulation and can still be present at day 52. Unlike *Sry* in the mouse, human expression of *SRY* is not abruptly stopped and is located within the genital ridge and to a minor extent in the brain (Viger et al, 2005).

Experimental studies have shown that the *Sry* exerts its action on the supporting cell lineage of the developing gonad to direct the differentiation of Sertoli cells rather than the default female follicular cells. Yet *Sry* is not always necessary for Sertoli cell differentiation as knockout mice (mice with the *Sry* gene deleted) have been seen to produce Sertoli cells even without the *Sry* gene. These studies suggest that the *SRY* gene is just one of the genes involved in male sex determination (Goodfellow and Lovell-Badge, 1993).

The study of human XX sex reversal and the use of transgenic mice have been valuable in trying to ascertain how *SRY* expression is controlled. These studies have suggested that there are transactivating factors within the male and female genital ridge that recognises the *SRY* promoter and can express the *SRY* gene. Evidence suggests that these factors are SOX9, SF-1, WT1, DMRT1, GATA4 and DAX-1 (Viger et al, 2005).

## **2.1 Other Genes Involved in Sex Determination**

### **2.1.1 SOX9**

The SOX family of genes share the same homology with the HMG box of the *SRY* gene. Like the *SRY* gene, the HMG box enables *SOX9* to act as a transcription factor and is able to bind and bend specific DNA sequences. A mutation in the *SOX9* gene is responsible for causing Campomelic Dysplasia, a male to female sex reversal disease associated with skeletal malformations and XY gonadal dysgenesis in 75% of individuals. This gene has proven to be an important sex determining gene due to its high conservation amongst vertebrates and the fact that the gene causes sex reversal when mutated. *SOX9* is expressed within bone of both sexes and within the testis of

males which explains the phenotypic effects of this gene when it becomes mutated. (Knower et al, 2003).

The *SOX9* protein is located within the cytoplasm of the undifferentiated gonad in both sexes (Gasca et al, 2002). Yet soon after the expression of *SRY*, *SOX9* expression is upregulated within the male urogenital ridge and downregulated within the females'. *SOX9* upregulation within the male causes movement of the protein into the Sertoli cell nucleus from the cytoplasm. It is believed that this movement of the protein is possible through two nuclear localisation signals (NLS) located in the HMG box allowing the shuttling of *SOX9* from the cytoplasm into the nucleus as sexual differentiation occurs. Once *SOX9* has been shuttled into the nucleus it is then able to activate other genes in the sex differentiation pathway. Once the necessary genes have been activated *SOX9* is exported out of the nucleus and back into the cytoplasm through the nucleus export signal (NES), also located in the HMG box (Knower et al, 2003).

The expression of *SOX9* within the nucleus appears to be all that is required to ensure sexual differentiation of the male gonad. When leptomycin B, an inhibitor of nuclear export signals, is cultured in female gonads the result is the formation of male gonads. This suggests that *SOX9* does not have to be exported out of the nucleus for male sex differentiation to occur. Female transgenic mice expressing *Sox9* have been shown to produce normally functioning Sertoli cells and Leydig cells which suggests *Sry* is not necessary for male sexual differentiation but rather is expressed to upregulate *Sox9* expression. Therefore *SOX9* is most likely the next sex determining gene after *SRY* in the sex determination pathway (Knower et al, 2003).

The targets of *SOX9* in the sex determination pathway have been discovered using both *in vitro* and *in vivo* studies. Two genes, Steroidogenic Factor-1 (*SF-1*) and Mullerian Inhibiting Hormone (MIH) have been shown to be transactivated by the *Sox9* protein and are also expressed within the gonad (Knower et al, 2003).

### 2.1.2 SF-1

*SF-1* (steroidogenic factor 1) is an orphan nuclear receptor due to its unknown activating ligand and is expressed within the gonads and adrenal glands of both sexes, controlling the transcription of genes that are involved in steroidogenesis (Swain et al, 1999). Both the gonads and adrenal glands derive from the adrenogenital primordium (AGP) which is observable at day 9 of embryogenesis (E9) within the mouse. By E13 the gonads and adrenals have separated from one another and are clearly visible as distinct organs. The gonads have differentiated into either ovaries or testes by E12 and by E16 the adrenal glands have formed the cortex, medulla and functional zone of the organ (Val et al, 2003).

The expression of *Sf-1* (mouse *SF1* gene) within the adrenal glands does not change from when it is first detected at E9 until the organ is developed. When the cortex and medulla begin to differentiate *Sf-1* expression is limited to the cortex of the adrenals. From E18 until 6 days post partum (6dpp), steroidogenic factor-1 expression is barely detected. Within undifferentiated gonads *Sf-1* can be detected in high levels until E12.5, around the same time gonads differentiate into male or female genitalia. At this point, expression levels rapidly decrease within the ovary and begin to increase again at E18. Within the testis, expression levels remain high throughout gestation, located within the Sertoli cells and Leydig cells. Human data regarding Steroidogenic factor-1

expression has not yet been obtained yet it is known that *SF-1* expression is detected at 32 days post ovulation (32dpo), which is the same time as the adrenogenital primordium appears within the urogenital ridge, therefore displaying similar expression patterns to those found within the mouse (Val et al, 2003).

Studies using adult mice have shown that *Sf-1* is also detected within the ventromedial hypothalamus (VMH) and the pituitary gland. Homozygous knockout mice possess a structurally abnormal VMH and do not express LH $\beta$  (Lutenising Hormone beta) or FSH $\beta$  (Follicle Stimulating Hormone beta) within gonadotrope cells. These beta subunits of the FSH and LH genes are responsible for the interaction of the hormone with the specific hormone receptor. Regardless of genotype, these mice also exhibit female external genitalia, lack of adrenal glands and the presence of Mullerian ducts. Death occurs by day 8 after birth due to acute mineralocorticoid and glucocorticoid deficiency as a consequence of the absent adrenals. Prior to sexual differentiation, these abnormalities are not present. At E12-12.5, when sexual differentiation normally occurs, the gonads degenerate by apoptosis. This data conveys the necessity of *SF-1* for the differentiation and maturation of cells within the gonads and adrenals, and that its expression is not needed for the actual formation of these organs (Val et al, 2003).

Within humans there are three different *SF-1* mutations that have been reported; the heterozygous G35E mutation, the heterozygous R255L mutation and the homozygous R92Q mutation. The G35E mutation causes complete sex reversal of a genotypically male individual and is also characterised by acute adrenal insufficiency. The R255L mutation causes adrenal insufficiency yet doesn't have any effect on the ovaries of



genotypically female individuals. The R92Q mutation causes agenesis of the right adrenal gland, adrenal hypoplasia of the left adrenal and complete sex reversal. It is clear through these abnormalities that *SF-1* is necessary for the differentiation of the testis as well as the degeneration of the Mullerian ducts (Val et al, 2003).

### 2.1.3 WT1

The Wilm's tumor-associated gene or *WT1* encodes a number of proteins that are involved in kidney and gonad development. It is necessary for the establishment of the bipotential gonad and for the development of the testis once they have differentiated. This gene which is composed of 10 exons is complex, with the capability of forming 24 different isoforms through the use of different start sites, RNA editing and splicing. Through alternative splicing within exon 9, isoforms are formed either with or without the specific amino acids KTS (Lysine-Threonine-Serine). This triplet causes the loss of DNA binding capabilities by the fourth zinc finger. The specific isoforms that are produced are either +KTS which may possibly play a role in RNA processing or -KTS which are themselves transcription factors that activate or deactivate transcription (Morrish and Sinclair, 2002).

*Wt1* expression can be detected as early as E9.5 within the mesoderm, adrenal glands, gonads and kidneys of developing mice. By E10.5 expression is detected within the coelomic epithelium of both sexes and later in development becoming localised in the Sertoli cells of the male and epithelial cells of the female gonad. Homozygous knockout mice do not survive past mid-gestation and characteristically are lacking gonads, adrenal glands, kidneys and have defects within their heart and spleen.

Heterozygous knockout mice who have either the +KTS or –KTS isoforms survive to birth but die shortly after due to kidney abnormalities (Morrish and Sinclair, 2002).

Depending on which isoform is present, these knockout mice have phenotypically different gonads, which conveys a different function for each of the isoforms within sex determination. Mice with only the +KTS isoform present do not develop past the streak stage and degenerate by means of apoptosis. Those expressing the –KTS isoform do develop gonads but all male individuals are completely sex reversed. The expression of *Sry* within those mice with only the –KTS isoforms is markedly decreased, therefore suggesting a role in *SRY* expression. These results propose that the –KTS isoform must be essential for the formation of the bipotential gonad and +KTS has a role in male sex differentiation, possibly by allowing enough *Sry* expression to ensure a male sexual pathway develops (Morrish and Sinclair, 2002).

#### 2.1.4 DMRT1

This *doublesex* and *mab-3* related transcription factor 1 (*DMRT1*) gene contains a DNA binding motif called the DM domain. This domain and its specific expression within the testis led researchers to believe this gene was involved in the sex determination pathway. The *DMRT1* gene is the only gene that is conserved among *Caenorhabditis elegans*, *Drosophila* and mammals. The location of the human *DMRT1* gene has been mapped to the short arm of chromosome 9 which is only 30kb from the mutation site which causes male to female sex reversal (Lei and Heckert, 2004).

The expression pattern of *Dmrt1* within the mouse has provided a clue as to the function of this gene. Before gonads have differentiated the levels of *Dmrt1* are very

high. Expression levels taper off in females once their ovaries have differentiated whereas *Dmrt1* remains high within Sertoli cells of the testis from 11.5dpp (days post partum) right through to adulthood (Morrish and Sinclair, 2002).

*Dmrt1* knockout mice did not produce sex determination or differentiation abnormalities until after birth. Once born, the Sertoli cells of these mice do not complete differentiation and eventually die, producing structurally abnormal testes with minimal seminiferous tubules. These tests show that *Dmrt1* is necessary for Sertoli cell and germ cell differentiation within postnatal testes (Lei and Heckert, 2004).

#### 2.1.5 GATA4

Studies by Lei and Heckert (2004) report that *GATA4* is an essential transcription factor that regulates the expression of *DMRT1*. Its protein is expressed within Sertoli cells and granulosa cells throughout gonadogenesis, being downregulated in the ovary once they have differentiated and remaining high within the testis. A close relative of the GATA family is *Fog2*, which must be present for *Gata4* to function. This gene is mainly located within the brain, heart and testis and directly interacts with *Gata4* to upregulate or downregulate its transcription (Lei and Heckert, 2004).

When the *Fog2* gene is deleted in transgenic mice, death occurs in mid-gestation due to severe abnormalities of the cardiac system. Another characteristic of these mice is that their testes fail to differentiate, which is the same abnormality seen in homozygous *Gata4* knockout mice. These results suggest that the abnormalities in male gonads develop due to the loss of interaction between *Fog2* and *Gata4* rather

than the mutation on their own. The expression of *Sf-1*, *Wt1* and *Gata4* was not detected in the mutant mice beyond 12.5dpc in male mice. *Gata4* binds to three control elements within the promoter of *Dmrt1*. Without the presence of *Fog2*, *Dmrt1* expression is markedly decreased. Therefore *Fog2* must activate *Gata4* which in turn activates the expression of *Dmrt1* (Lei and Heckert, 2004).

#### 2.1.6 DAX-1

Dosage sensitive sex-reversal adrenal hypoplasia congenita-critical region of the X chromosome, gene 1 (*DAX1*) is a gene located on the X chromosome and when duplicated can cause male to female sex reversal. Like *SF-1*, *DAX1* is an orphan nuclear hormone receptor due to its unknown ligand. Once sex determination has occurred, *Dax1* is expressed in Sertoli and Leydig cells of the testis and the somatic cells within the ovary. In late development and throughout adulthood, expression is localised to the Leydig cells within the testis and thecal and granulosa cells in the ovaries (Morrish and Sinclair, 2002).

As both *Sry* and *Dax1* are expressed within the same tissues at the same time, it is thought that *Dax1* antagonises the function of *Sry*. Further evidence for this comes from transgenic mice studies in which the expression of *Dax1* and *Sry* results in a phenotypically and genotypically female mouse. Yet if the *Dax1* gene is deleted leaving just the *Sry* gene, the mouse develops as a male which confirms *Dax1* inhibits *Sry* function so the genotypically female mouse can follow the female developmental pathway (Morrish and Sinclair, 2002).

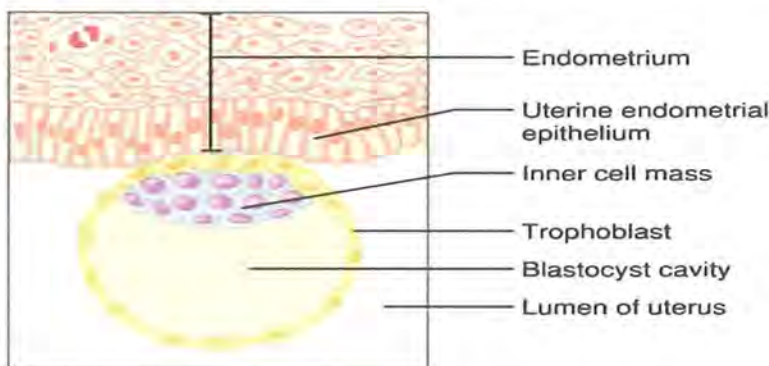
Numerous *in vitro* studies have provided evidence for *Dax1* having an inhibitory effect on *Sf-1* transcription yet the exact mechanism behind this inhibition is unclear.

Proposed mechanisms are protein-protein interaction, DNA binding or through the use of co-repressors. It is possible that a combination of all three mechanisms may be used to inhibit *Sf-1* transcription (Crawford et al, 1998; Nachtigal et al, 1998; Zazopoulos et al, 1997).

### Sex Differentiation of the Reproductive System

#### 3.1 The Bilaminar Germ Disc

Once an ovum has been fertilised the single cell undergoes the process of cleavage, which is a series of divisions that increase the cell number but not the cell size. Between days 7 and 10 of gestation the ovum has become a 32-64 cell blastocyst, consisting of an outer cellular layer named the trophoblast, an inner layer of cells called the inner cell mass (ICM), and a fluid filled cavity termed the blastocyst cavity as seen in Figure 1. The trophoblast differentiates into the embryonic placenta, whilst the ICM divides into two more cellular layers, the hypoblast and epiblast. Once these two layers have formed at the end of the second week, the developing embryo has become the bilaminar germ disc. The trophoblast also undergoes morphogenetic changes as it divides into an outer syncytiotrophoblast layer and an inner cytotrophoblast layer (Larsen, 2001). The syncytiotrophoblast layer forms lacunae, in which the maternal blood vessels can provide nourishment. The cytotrophoblast differentiates into extraembryonic mesoderm that serves to provide nourishment, protection and a means for respiration for the developing embryo (Kalthoff, 2001).



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Figure 1 - *The Bilaminar Germ Disc (Marieb, E., 2006, pp 1115).*

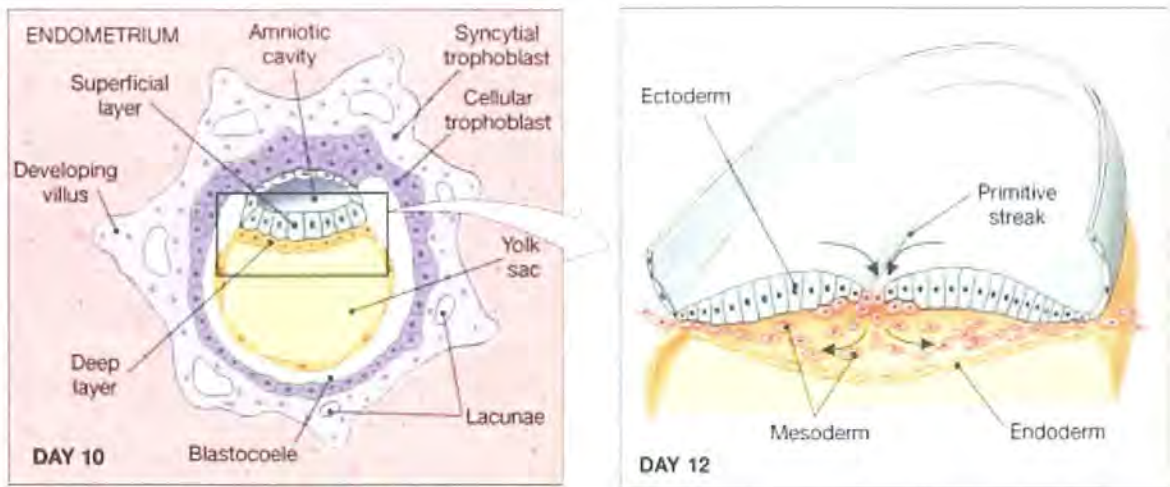
## **3.2 The Trilaminar Germ Disc**

### **3.2.1 The Primitive Streak**

At approximately day 15 of development a longitudinal groove along the midline of the epiblast begins to form on the bilaminar germ disc, becoming the primitive streak. By day 16, an elevated area of epiblast cells forms a mound at the cranial region of the bilaminar germ disc hence forming the primitive node. The primitive streak is an important structure in embryonic development as it outlines the longitudinal axis of the embryo (Larsen, 2001).

### **3.2.2 Layers of the Trilaminar Germ Disc**

The epiblast cells on either side of the primitive streak begin to proliferate and flatten in preparation for migration at approximately day 16. Once these cells are structurally capable, they migrate through the primitive streak to rest between the epiblast and hypoblast. The invasion of numerous epiblast cells into the hypoblast forms what will be called the endodermal layer. Other epiblast cells migrate between the epiblast and endoderm forming another layer of the trilaminar germ disc, the mesoderm. Once the endoderm and mesoderm are formed the epiblast is now termed the ectoderm layer and the structure has become the trilaminar germ disc as seen in Figure 2. These three layers differentiate to become specific organs and tissues within the embryo (Larsen, 2001). The ectodermal layer gives rise to the dermis, brain, spine, neurones and sense receptors. The mesodermal layer forms the notochord, muscles, blood, bone and the sex organs, while the remaining endodermal layer forms the lining of the gut, lungs and bladder and forms the liver and pancreas (Kalthoff, 2001).



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Figure 2 - *The Trilaminar Germ Disc (Martini, 2005, pp1071).*

### 3.3 Germ Cells and Gonad Formation

During the second week of development, cells from the epiblast layer produce the female and male gametes. These cells will eventually detach from the ectodermal layer and become primordial germ cells within the yolk sac. At the 4-6 week stage the primordial germ cells migrate again into the wall of the gut tube and attach to the midline of the body wall at the 10th thoracic vertebral level. These cells will then differentiate into the future gonads (Larsen, 2001).

### 3.4 Hormonal Regulation of Sex Differentiation

Hormone activity within the ovaries is not a crucial element in sex differentiation for females, whereas males require testosterone and Mullerian Inhibiting Hormone (MIH) in order to produce the male Wolffian ducts. The Wolffian system constitutes the vas deferens, epididymis and seminal vesicle of the male reproductive tract. The Mullerian system is the reproductive tract of females and includes the oviducts, uterus, cervix and part of the upper vagina. Between the 8<sup>th</sup> and 10<sup>th</sup> weeks of development the Sertoli cells within the testes of the male embryo begin to secrete



MIH. This hormone is of extreme importance as it causes the degeneration of the Mullerian ducts so development can follow the male pathway. If this hormone is not released, the Mullerian ducts will remain within the embryo producing a child with both male and female genitalia. The Mullerian ducts do not require the presence of ovaries in order to differentiate and develop. When a female embryo has its ovaries removed, the internal genitalia still develop as a female despite the absence of ovarian activity (Larsen, 2001).

Homozygous deletions within the coding region of the MIH gene produces normal testes in transgenic mice, yet they are sterile due to the presence of Mullerian ducts that interfere with the transfer of sperm. Therefore it is clear that MIH is not necessary for testis determination as testes still develop when MIH is deleted from the genome. Rather it is essential for ensuring a genetically male individual has only the male Wolffian ducts (Morrish and Sinclair, 2002).

MIH expression appears to be tightly regulated, with varying expression levels throughout development and adulthood. Within male rats, MIH is expressed in Sertoli cells as soon as testes have differentiated at approximately 13dpc. After birth levels remain high until day 5 where it drastically decreases to a low level at which it remains throughout adulthood. Expression of MIH in female rats can be detected at low levels within foetal ovaries and is expressed at higher levels after birth within developing follicles. Many factors have been suggested to be involved in the regulation of MIH expression such as *SOX9*, *SRY*, *WT-1*, *GATA4* and *DAX-1*. All except *DAX-1* and *WT-1* are able to bind to a region -180bp upstream from the MIH

start site and expression of all of these factors occurs before MIH is expressed (Watanabe et al, 2000).

Many studies have concluded that SOX9 is the number one candidate for the upregulation of Mullerian inhibiting hormone. In vitro, SOX9 binds to the MIH promoter that has a HMG box binding site. Mice carrying homozygous mutations in the Sox9 binding site in the MIH gene produce a phenotype the same as those carrying deletions in the MIH coding region. Other evidence derives from human mutations in the SOX9 gene itself. These XY individuals have Mullerian-like structures due to the proposed lack of MIH protein. It is believed that the absence of SOX9 means MIH is not upregulated and the Mullerian structures remain (Morrish and Sinclair, 2002). The over-expression of MIH in female transgenic mice produces what is called the freemartin effect. If a female mouse is exposed to the blood of a male twin whilst developing, the female mouse somewhat develops as a male due to the MIH exposure. Ovarian germ cells degenerate and Sertoli cells form within seminiferous tubules (Lane and Donahoe, 1998).

### Sex Differentiation Abnormalities

#### 4.1 Hermaphrodites

It is evident that the presence of the *SRY* gene is the first step in sex differentiation towards a male phenotype, its absence producing a female phenotype. In rare cases this mechanism can fail and individuals can be born with male and female internal and/or external genitalia due to a mutation associated with the *SRY* gene. Such cases are termed true hermaphrodites and are genotypically a mosaic of XY, XX or XO cells. Due to the ambiguous external genitalia, sex is hard to determine at birth which has led to many individuals being raised as the wrong sex (Queipo et al, 2002). Secondary hermaphrodites can arise when there is a communication failure between the requisite hormones, such as testosterone and MIH and the internal or external genitalia. This abnormality also produces an individual with a mix of female and male tissue types. As this form of hermaphroditism is due to a hormone abnormality, females tend to become more virilised and males more feminised (Johnson and Everitt, 2000).

True hermaphroditism (TH) is the major form of sex reversal that produces an individual with both ovarian and testicular tissue regardless of genotype. This occurs in the form of either an ovotestis (single gonad) or a testis on one side and an ovary on the other. Depending on the amount of testicular tissue that is present and therefore the amount of MIH that is secreted, affected individuals will have varying development of both Wolffian and Mullerian structures (Queipo et al, 2002). Many studies on true hermaphrodites have shown that 60% of individuals have a 46XX karyotype, 33% are mosaics with a Y chromosome in a second cell line and the

remaining 7% have a 46XY genotype (Hadjiathasiou et al, 1994; Krob et al, 1994; Salas-Cortez et al, 2000).

Only a small number of 46XX true hermaphrodite's have Y-DNA sequences therefore the mechanism leading to testicular tissue in these individuals is unknown. It has been hypothesised that mutations in X-linked or autosomal genes may cause a gain of function in the gene which could explain the development of male characteristics with the absence of *SRY*. In a study by Queipo and colleagues (2002), only 14% of testicular cells were found to have the *SRY* protein. It is thought that this low percentage of *SRY* is not enough to produce male genitalia and therefore leads to the production of ovotestes (Queipo et al, 2002).

#### **4.1.1 Testicular Feminisation**

Testicular feminisation (Tfm) also known as Androgen Insensitivity Syndrome, is an example of abnormal sex differentiation that can produce secondary hermaphrodites. These individuals have a 46XY karyotype yet are phenotypically female due to a lack of virilisation during development. This abnormality is a result of mutations occurring in the androgen receptor gene located on the X chromosome which causes the receptors themselves to be insensitive to the effects of testosterone. Endocrine research has shown that testosterone levels are twice as high in the testicular vein as opposed to the peripheral blood, signifying that testosterone biosynthesis is normal (Regadera et al, 1999).

Testicular feminisation can occur in two forms, complete and incomplete. The complete form produces an individual with an XY genotype and is completely

feminised. The incomplete form produces varying degrees of external genital ambiguity and feminisation, depending on the level of compromise of androgen receptor function (Holterhus et al, 2003). A number of studies have focused on the testes of children affected with Tfm (Regadera et al, 1999, Salas-Cortes et al, 2000). Their findings show that the number of spermatogonia are normal until the individual reaches puberty. At this stage the numbers dramatically decrease and the spermatogonia become hypertrophied (Regadera et al, 1999).

#### 4.2 Congenital Adrenal Hyperplasia

Congenital Adrenal Hyperplasia (CAH) categorises a group of recessive disorders that affect the biosynthesis of cortisol by the adrenal gland (Figure 3). The severity and symptoms of the disorder depend on the enzyme that is affected within the cortisol biosynthesis pathway. The impairment in cortisol synthesis causes chronic stimulation of Adrenocorticotrophin hormone (ACTH) as there is no negative feedback to stop its production. This in turn causes an excess of steroid hormone precursors and affects the production of glucocorticoids and mineralcorticoids from the adrenal cortex (New, 1998).



Figure 3 – Early human adrenal function and androgen biosynthesis implications (Goto, M. et al, 2006, p958).

CAH can occur in classical and non-classical forms which produce varying symptoms. Classical forms are apparent in childhood with an extreme overproduction of cortisol precursors and sex steroids. If an individual has a severe case of the classical form, they suffer from excessive salt loss due to an inhibition of aldosterone preventing reabsorption of sodium within the kidneys. Female children are born with ambiguous external genitalia due to the excess exposure of androgens in utero as seen in Figure 4. The clitoris is enlarged resembling a penis and the labia majora tend to be fused together. Internally these individuals have normal ovaries, uterus and fallopian tubes without any Wolffian structures. Male infants do not present with obvious symptoms at birth and are only diagnosed when the individual begins to lose salt excessively between 7-14 days after birth. Salt wasting presents with vomiting, dehydration, hyponatraemia (low blood sodium concentration), hypokalaemia (low blood potassium concentration) and shock. Most female infants do not reach this stage of the illness as the ambiguous external genitalia leads to early diagnosis. (Merke and Bornstein, 2005).



*Figure 4 – Ambiguous genitalia of female infant due to excessive androgen exposure (Nelson, C. and Gearhart, J., 2004).*

The non-classical form of CAH presents without a cortisol deficiency yet individuals have hyperandrogenism in childhood or early adulthood. Pubarche begins early with 60% of affected females presenting with excessive hair growth (hirsutism), 54% with amenorrhoea and 33% with polycystic ovaries and acne (New, M., 1998).

#### **4.2.1 21 Hydroxylase Deficiency**

The most common cause of CAH arises from a 21-Hydroxylase deficiency. The 21-hydroxylase gene is located on chromosome 6p21.3 and has an active gene (CYP21A2) and a non-active gene (CYP21AIP) that are highly homologous. Most mutations that cause this form of CAH arise from recombination between the active and non-active gene which generates non-transcribing alleles on the active gene (Merke and Bornstein, 2005).

#### **4.2.2 17 $\beta$ -Hydroxysteroid Dehydrogenase 3 Deficiency**

Another Congenital Adrenal Hyperplasia disorder that results in the development of secondary hermaphrodites is 17- $\beta$  Hydroxysteroid Dehydrogenase 3 Deficiency (17[ $\beta$ ]-HSD3). This abnormality arises from a mutation that affects the conversion of androstenedione to testosterone within the testes of the male embryo. Affected males more often than not are born with testes, the vas deferens, epididymis, ejaculatory ducts and female external genitalia. At birth the sex of the child is usually determined to be female, yet at puberty changes occur that can alter the individual's perceptions of their own gender. Androstenedione levels within the blood dramatically increase causing an increase in serum testosterone. Many affected individuals change their gender role from female to male after puberty due to the masculinization caused by testosterone (Mendonca et al, 2000).

### **4.3 Role of Sex Differentiation Abnormalities**

Defects in androgen production or function can lead to genital ambiguity at birth. Over-expression or under-expression of important sex hormones can have extreme side effects. The study of such disorders thus provides scientists with evidence of the strength of androgens and their effects on the body (Witchel, 2002).

### **4.4 Sex Differentiation Abnormalities in Lab Animals**

Rat studies have proven to be an asset in the study of brain structure, hormones and gender behaviours. The rodent's brain does not completely develop until after birth therefore enabling scientists to manipulate certain brain structures or hormones before the brain has fully developed and observe their effects (Moir and Jessel, 1991).

#### **4.4.1 Hormone Manipulation in Rodents**

Castration of a male rat soon after birth causes the rodent to act in a characteristically female manner. The later the rat is castrated the less obvious the female behaviour as the brain has had time to develop along the male pathway and so adapt to the influence of testosterone. Administration of testosterone to the rodent at certain stages of development following castration has varying effects on its behaviour. If the testosterone is administered too early there is little effect on the developing brain, since it has not yet reached the stage in which it is sensitive to androgens. Once the brain of the rodent has completely developed, hormone manipulation has no effect on the brain or behaviour as it has already been fixed into a male or female pattern (Moir and Jessel, 1991).



#### **4.4.2 Non-Human Primates**

Many studies have been conducted on rhesus monkeys in relation to androgens and behavioural aspects. Female monkeys that have been exposed to high levels of testosterone prenatally exhibit behavioural patterns of male rhesus monkeys. These androgenised females are capable of performing a mature mounting pattern that only male rhesus monkeys otherwise can achieve. Studies show that, despite this mounting pattern and male-like rough behaviour whilst interacting with other monkeys, androgenised females do not have a completely male patterned brain. They exhibit successful interaction with males and are able to become pregnant. Compared to rodents, it is clear that non-human primates do not undergo complete androgenisation, suggesting there is a critical androgen-sensitive period during foetal life that has yet to be determined (Graves, 2006; Johnson and Everitt, 2000; Keefe, 2002; Rehman et al, 2004).

## CHAPTER FIVE

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### **Variations in the Sexual Differentiation of the Central Nervous System**

Sex differentiation within the brain has been a topic of controversy for decades. Numerous experiments on animals have proved sexual dimorphism within certain structures of the brain, yet similar studies on human brains have produced mixed results. Different laboratory techniques, sample sizes and study methods are all attributed to the varying results that are published in regards to brain sexual dimorphism. Despite the negative aspects of this research topic the positive results cannot be ignored (Sommer et al, 2004).

#### **5.1 Cerebral Cortex**

The greater size and weight of the male cerebral cortex in comparison to the female cortex is a sexually dimorphic trait that has been well established for over three decades (Kretschmann et al, 1979). Rabinowicz and colleagues (2002) reported a neuronal density that is 15% higher in males than females with a greater difference occurring in the right hemisphere. This higher neuronal density could be a factor contributing to the larger size and weight of the male cortex. The greater number of neuronal cells present in the male cortex would suggest a corresponding greater number of axons. This is thought to be the reasoning behind the finding that males have an increased volume of white matter as opposed to females (de Courten-Myers, 1999).

The different cognitive abilities of males and females are attributed to the varying volumes of grey and white matter within the cerebrum. Females excel in verbal

memory tasks, speed of articulation and verbal fluency tasks whilst males perform better at visuospatial tasks (Sommer et al, 2004). The higher volume of grey matter within the female cortex is thought to contribute to the bilateralism of the female brain and the ability to use both hemispheres for language functions (Gur et al, 1999).

Stroke victims have provided evidence for the bilateralism of the female brain. After lesions to the left hemisphere, the area associated with verbal tasks, females show less impairment in their verbal abilities than males. Functional activation studies have shown that the right hemisphere compensates for the impairment of the left hemisphere enabling females to regain their speech abilities earlier than males and to be less severely affected (Sommer et al, 2004).

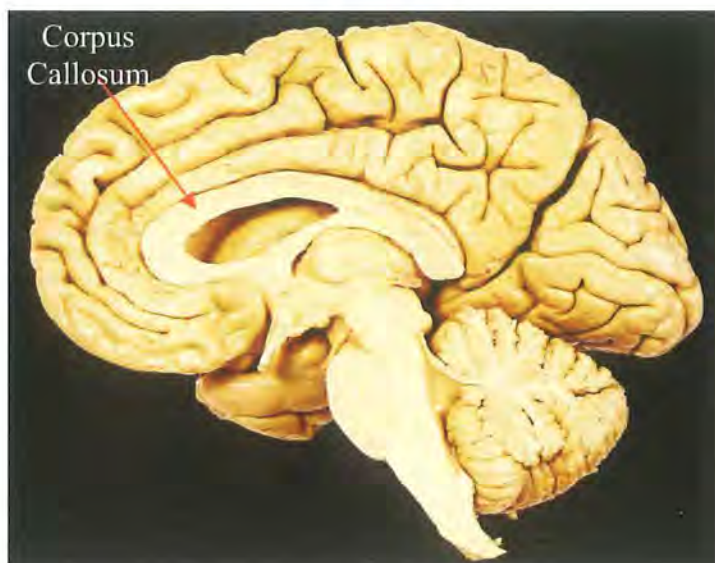
A morphometric study conducted by Rabinowicz and colleagues (1998) demonstrated that the female cerebral cortex consists of more neuropil than males. The neuropil is a conglomeration of glial processes, synaptic neurons, axons and dendrites within nerve cells of gray matter. This increase in neuropil volume within females suggests more connections between cells and a more extensive dendritic network which could account for the bilateralisation of both hemispheres (de Courten-Myers, 1999).

The right ventromedial prefrontal cortex (VMPC) is another structure of the cerebrum that appears to be sexually dimorphic. This area is associated with emotional processing, social functioning, personality and decision making. In a recent study using functional imaging, decision making predominantly activated the right VMPC in males whilst the left side was activated in females. As expected, male individuals with lesions in the right VMPC produced severe impairments in the normal

functioning of this area, whilst lesions in the left produced minor results. Lesions in the left VMPC in females produced the same defects in social functioning whereas lesions in the right produced only minor disturbances (Tranel et al, 2005).

### 5.1.1 Corpus Callosum

The corpus callosum (Figure 5) is a network of over 200 million nerve fibres that allows the two cerebral hemispheres of the brain to communicate with each other (Narr et al, 2000). This structure is divided into three main parts, the anterior portion or rostrum, the central portion known as the isthmus and the posterior portion, the splenium. There has been much debate as to whether there are definitive sexual differences in the size or shape of the corpus callosum. It is understood that the overall size of the corpus callosum is larger in females than males and that the splenium region is of a more bulbous shape (Rabinowicz et al, 2002).



*Figure 5 – Midsagittal section of the brain showing the corpus callosum and other surrounding structures (Marieb, 2006, pp458).*

The function of the larger corpus callosum in females is thought to be related to the bilateralisation of the two cerebral hemispheres. This increased volume of fibres may permit the activation of both hemispheres via the crossing over of information through this dense network (Rabinowicz et al, 2002).

## 5.2 Hypothalamus

The hypothalamus contains numerous nuclei that have been reported to be sexually dimorphic (Figure 6). These sex differences are thought to be related to reproductive behaviour, gender identity and sexual orientation, as well as differences in prevalence of certain disease states (Swaab et al, 2003).

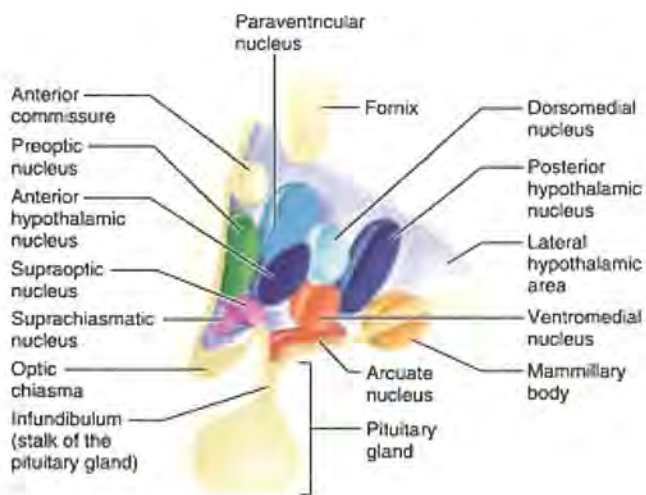


Figure 6 – Main Hypothalamic Nuclei (Marieb, 2006, pp 446).

### 5.2.1 Preoptic Area of the Hypothalamus

The preoptic area is a portion of the anterior hypothalamus that is divided into four nuclei, the lateral, medial and median preoptic nuclei and the pariventricular nucleus, all of which are larger in the male. The median preoptic nucleus, as well as the lateral

and medial divisions, are 50-70% larger in total volume in males. The lateral preoptic nucleus also contains 28% more neurons in males and the medial preoptic nucleus contains 21% more neurons. Rat studies have shown that the increase in size is due to an increase in neuronal numbers. The median preoptic nucleus of the male contains 80% more neurons than females due to the sexually dimorphic nucleus found within this region (Madeira et al, 1999).

### 5.2.2 Sexually Dimorphic Nucleus (SDN)

This division of the medial preoptic area has been an important finding in this field as most studies convey the same results. Sexual dimorphism of the rat SDN was first described by Gorski and colleagues (1978) as being three to eight times larger in male rats than female rats (Figure 7). The human sexually dimorphic nucleus homologous with the rat SDN has been discovered and revealed to be twice as large in young adult males with twice as many cells when compared to females. The SDN is associated with the sexual behaviour of both males and females, and with sexual identity (Swaab et al, 2003).

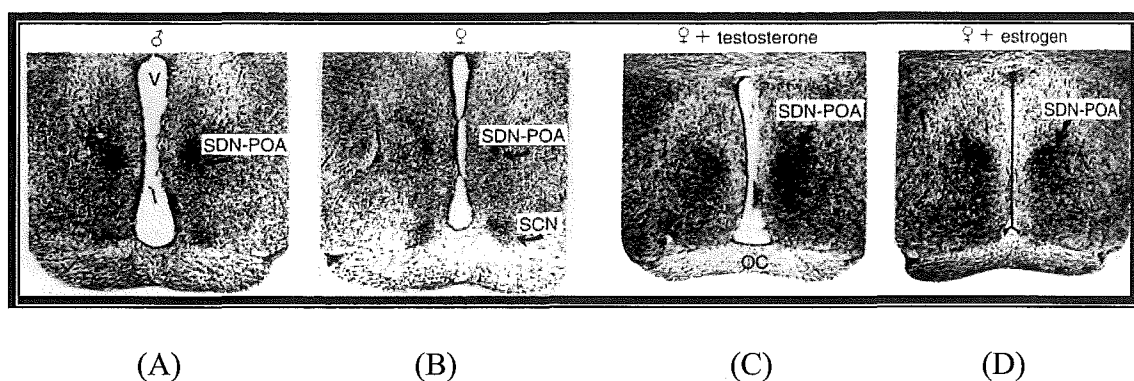


Figure 7 – Sexually Dimorphic Nucleus of the preoptic area in (A) male rat, (B) female rat, (C) female rat perinatally treated with testosterone and (D) female rat treated with oestrogen (Kalthoff, 2001, p726).

Swaab and Hofman (1995) found that lesions within the SDN caused the male rats to become less masculine. However they believed the change in behaviour was so minute that gender identity is not a main function of the SDN, otherwise the changes observed would have been more significant. De Vries' (2004) study supports that of Swaab and Hofman (1995), reporting that lesioning of the SDN does not produce significant changes in the male rats' behaviour.

In humans the age at which the SDN sexually differentiates and takes on a male or female pattern is between two and four years. Before this period there are no apparent sexual dimorphisms and the cell numbers are only 20% of the adult size. Once a female has reached four years of age, the cell numbers rapidly decrease whereas the male SDN remains at a constant until approximately 50 years of age, when the cells begin to die via apoptosis. Females experience their second phase of cell loss at approximately 70 years of age where cell numbers reduce to 10-15% of childhood values (Swaab et al, 2003).

In an attempt to ascertain the effects of testosterone on the size and cell number of the SDN, Von Esenwein and Silke (2005), manipulated female rat brains by introducing testosterone propionate. The results of this proved that testosterone is important in sexual differentiation of the brain, in particular the SDN, as the female rats exhibited a decrease in lordosis (copulatory posture), increased mounting and an increase in cell numbers similar to that of the male rat.

### **5.2.3 Suprachiasmatic Nucleus (SCN)**

The function of this nucleus is to co-ordinate circadian and seasonal rhythms in locomotor activity, sleep, endocrine function and sexual behaviour. It is sexually dimorphic in rodents and humans, but not in the same way. The male rat SCN is larger than the females yet it is the shape of the nucleus that differs between the genders in humans. In females the SCN is more elongated whilst in males it is of a more spherical shape (Abizaid et al, 2004; Hofman et al, 1996; Swaab, 1995).

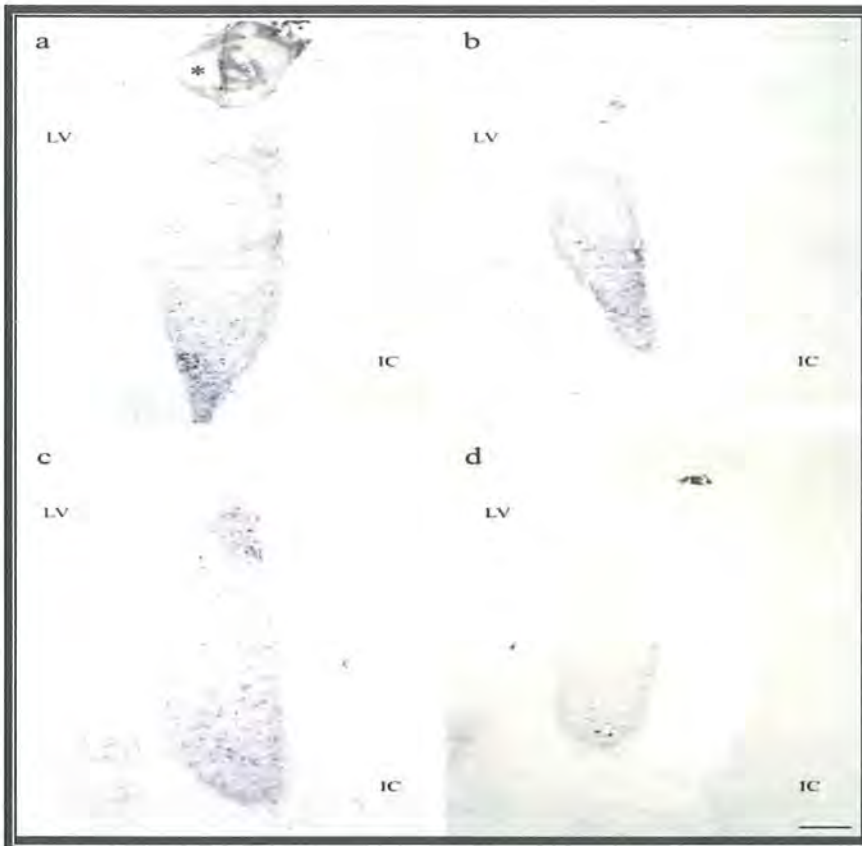
Studies of some homosexuals have provided evidence for the function of the sexual dimorphism in the SCN. Morphometric analysis showed that the volume of the SCN in a group of homosexual males was 1.7 times larger with 2.1 times as many cells than the group of heterosexual men. Programmed cell death of the SCN cells occurs between 13 and 16 months after birth. As homosexuals have the same number of cells as 1-2 year old children it is thought homosexuals do not undergo the same apoptotic mechanisms within the SCN as heterosexuals (Swaab et al, 2003). Proliferation of rat SCN cells occurs between E13 and E17, peaking at E15. This proliferation occurs when aromatase activity (conversion of testosterone to estradiol) is at a high within the hypothalamus, suggesting that estradiol can alter the number of cells that proliferate within the SCN. Estradiol has been confirmed as being a neuroprotective agent by decreasing programmed cell death which could account for the increased cell numbers located within the male rat SCN (Abizaid et al, 2004).

### **5.2.4 Bed Nucleus of the Stria Terminalis (BNST)**

The stria terminalis is a long mass of grey matter fibres that conveys information from the amygdala to the hypothalamus (Chung et al, 2000). Two regions within the BNST



have been found to be sexually dimorphic, the darkly staining posteroventral component of the BNST (BNST-dspm) as well as the central division (Allen and Gorski, 1990, Chung et al, 2002, Garcia-Falgueras, 2005). The central division of the BNST (BNSTc) has been linked with transsexuality as studies show that the size of BNSTc in male to female transsexuals is similar to that of the control group of females as seen in Figure 8 (Kruijver et al, 2000).



*Figure 8 – Immunocytochemical staining of somatostatin neurons in BNSTc of: (a) heterosexual male, (b) heterosexual female, (c) homosexual male, (d) male-to-female transsexual. The transsexual has a BNSTc similar in size to the female (Kruijver et al, 2000 p 2037).*

Sexual dimorphism in the rat BNST occurs within the first week of birth as a result of differences in testosterone levels. Within humans, sex differences don't reach significance until adulthood when it is clear the BNST is larger in males than females. In female rats once the BNST has fully differentiated, no amount of circulating testosterone can increase its volume. The bed nucleus of the stria terminalis in gonadectomised male rats does not decrease in volume due to a decrease in testosterone levels, suggesting that once this structure is differentiated, testosterone is not needed to maintain its volume (Chung et al, 2002).

It is believed that sex differences in the size and cell number of the bed nucleus of the stria terminalis within humans occurs due to increased programmed cell death (apoptosis) in females. A study by Chung et al (2000) has reported there is an increase in apoptosis within females which accounts for the smaller BNST size and cell number. Gonadal steroids appears to be the cause of this difference in apoptosis as castrated males and female rats treated with testosterone experience a decrease in apoptotic cell death (Chung et al, 2000).

#### **5.4 The Anterior Commissure**

This structure is a small bundle of nerve fibres between the two cerebral hemispheres much like the corpus callosum. As females appear to use both hemispheres more often than males it is thought that the anterior commissure would therefore be larger in females than males. Many studies have concluded that the anterior commissure is on average 12% larger in females than in males (Allen and Gorski, 1992; Moir and Jessel, 1991; Swaab and Hofman, 1995), whilst others have reported no sex differences in rats or humans (Bishop and Wahlsten, 1999; Highley et al, 1999; Jones

et al, 1997). The anterior commissure is not present in all individuals but is present in approximately 78% of females and 68% of males, again more evidence for the bilateralisation of the female brain (Swaab et al, 2003). More studies need to be conducted on the anterior commissure, with larger sample sizes and more thorough statistical analysis in order for a conclusion to be made on the sexual dimorphism of this structure (Lasco et al, 2002).

### **5.5 Amygdala**

The amygdala is an almond shaped structure comprised of numerous nuclei located within three regions, the basolateral nuclei, corticomедial nucleus and the central nucleus (Bear et al, 2001). Once total brain size has been taken into account the human amygdala is much larger in males than females (Cahill et al, 2004; Durston et al, 2001; Goldstein et al, 2001). Within rats, the posterodorsal component of the medial amygdala has been found to be 50-80% larger in males (Cooke et al, 1999), with an increased volume of neurons in the posteromedial region (Rasia-Filho et al, 2000).

The amygdala's role within the brain is to receive afferent signals from the olfactory bulb and project them to the hypothalamus, stria terminalis, preoptic area and the remainder of the limbic system (Cooke et al, 1999). It is also implicated in sexual arousal, reproductive behaviour, emotional memory and fear (Hamann, 2005). Females and males process emotionally arousing events differently as revealed by positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Long term memory for arousing material was found to be located within the right hemisphere of the amygdala in males and the left hemisphere in females (Cahill

et al, 1996, 2001, 2004; Canli et al, 2002). In a study by Cahill and colleagues (2001), males and females watched either highly aversive or neutral films whilst their brain activity was measured with PET. When asked about these films at a later date (long term memory), the same results were produced, the left hemisphere in females was activated whilst the right hemisphere was activated in males.

Psychological studies have shown that there are sex differences in emotionally related behaviour. Females are said to retain more vivid memories for an emotional event and are able to recall them at a quicker rate. The greater prevalence of anxiety and depression in females has been postulated to be due to this enhanced ability to remember details of certain events (Hamann, 2005). Bilateral lesions in the amygdala reduce long term memory of emotional events but do not affect memory for neutral events. This provides evidence that the amygdala's role in memory is for events that are emotionally arousing as opposed to neutral events (Cahill et al, 2004).

Klüver and Bucy (1939) recognised that rhesus monkeys with damaged temporal lobes had a distinct behavioural pattern. Normal fear and anger responses were lost, they were unable to visually recognise objects and exhibited an increase in sexual behaviour. This was later called the Klüver-Bucy syndrome and has been recognised in humans with temporal lobe damage. Humans also experience a somewhat dulling of all the emotions, not just fear and anger as in rhesus monkeys. As the entire temporal lobe was removed, structures other than the amygdala could account for some of these odd behavioural characteristics. The inability of the rhesus monkeys to recognise objects, would be due to the loss of the visual areas within the temporal

lobes, yet the emotional and sexual behavioural characteristics are believed to be attributed to the loss of amygdaloid function. (Bear et al, 2001).

Appetitive sexual behaviour, which is the motivation and enthusiasm exhibited to receive a sexual reward, is a sexually dimorphic trait within rats. The amygdala is essential for male appetitive sexual behaviour but does not play a role in female appetitive sexual behaviour. Lesions to the medial amygdala in male rats decreased their ability to respond to sexual cues from the female whereas these lesions within the female did not affect her sexual behaviour (Hamann, 2005).

### **Sexually Dimorphic Distribution of Disease**

Throughout the long history of human disease, certain diseases and disorders have more commonly affected one sex than the other. The question is whether this pattern of disease distribution is due to sexual dimorphism or to another as yet undiscovered mechanism. A number of correlations have been found between certain diseases and the levels of circulating androgens yet few workers have reported a relationship between brain structure and the incidence of disease.

#### **6.1 Schizophrenia**

Schizophrenia is a psychiatric disorder in which individuals experience episodes of hallucinations, delusions, psychosis and paranoia leading to social withdrawal, impaired attention and cognitive dysfunction (Cyr et al, 2002). A study by Angermeyer and Kuhn (1988) examined 36 research papers on schizophrenia and the age of onset between genders. All except three showed that males develop schizophrenia at an earlier age than females. More recent studies have published similar results and conclude that not only are the incidence rates for the disease lower in females but they also respond better to treatment, exhibit better social functioning and develop less severe symptoms than males (Bryant et al, 1999; Cyr et al, 2002; Nopoulos et al, 1997; Takahashi et al, 2000).

It has been proposed that the reason females are not affected as severely and as early as males is due to the protective role of oestrogen (Cyr et al, 2002; Fink et al, 1998; Hafner et al 1991; Hafner, 2003; Sumner et al, 1999). It is known that relapse rates

increase when oestrogen levels are low in the female menstrual cycle, and decrease when oestrogen levels are high (Cyr et al, 2002). Short-term applications of oestrogen in rats produces a weak neuroleptic effect by reducing the affinity of dopamine receptors. A reduction in dopamine receptor sensitivity has been shown to have neuroprotective and antipsychotic effects which may be the reason female schizophrenics have a better prognosis in terms of age of onset and disease severity (Hafner, 2003)

Many studies have found that the parietal lobe may be a factor in the cause and severity of schizophrenia (Frederikse et al, 2000; Kaplan et al, 1993; Wigal et al, 1997). The parietal lobe is associated with perception, attention, recognition and visuospatial processing, all of which are abnormal in schizophrenic patients. The volume of the inferior parietal lobe is much larger in healthy males than in females yet in male schizophrenics the volume is significantly smaller when compared to female schizophrenics. Therefore abnormalities in inferior parietal volume appear to be limited to male schizophrenics. The left side of the brain, which normally dominates in healthy male individuals, tends to lack in function in male schizophrenics, whereas no such finding has been made in female schizophrenics. It is believed that the bilateral brains of females' enables those affected with schizophrenia to cope better with the disease, leading to different disease expression between genders (Frederikse et al, 2000).

Wright and colleagues (2000) conducted a meta-analysis on 58 studies that had aimed to determine the influence of brain morphology in schizophrenia. Of the 58 studies from 1988 to 1998, nine of them referred to schizophrenia and gender, all of which

did not prove significant differences in brain structure between male and female schizophrenics. Ventricular enlargement was slightly greater in male schizophrenics but was not of great statistical significance (Wright et al, 2000). Despite the large number of studies that were reviewed by Wright and his colleagues (2000), the data that was used is between 8 and 18 years old. This must be kept in mind when reviewing this study as many advances in experimental techniques and data analysis have been made since these studies were conducted, therefore the results obtained in these 58 studies may not be reliable.

## 6.2 Autoimmune Disease

Autoimmune diseases occur as a result of immune responses being generated against self-antigens due to a breakdown in the mechanism that allows the immune system to recognise 'self' from 'non-self'. All autoimmune diseases such as systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis, Sjogren syndrome and scleroderma are significantly predominant in females as seen in Table 1. (Rubin, 2001).

<u>AUTOIMMUNE DISEASE</u>	<u>FEMALE TO MALE RATIO</u>
Hashimoto Thyroiditis	26:1
Graves Disease	4 to 8:1
Systemic Lupus Erythematosus	9 to 13:1
Sjogren Syndrome	9:1
Juvenile Onset of Myasthenia Gravis:	
- White patients	2 to 14:1
- Black Patients	1:1
Scleroderma	3 to 4:1

Table 1 – Female to male ratio's of common autoimmune diseases (modified from Ahmed et al, 1999).



### 6.2.1 Systemic Lupus Erythematosus (SLE)

SLE is a multisystemic inflammatory disease which predominantly affects the joints, kidneys, skin (Figure 9) and serous membranes. Although these are the most common sites affected in SLE, almost any organ can be affected as immune complexes can deposit in any tissue or organ. An important feature of SLE is the development of autoantibodies against nucleic acids which are able to cause damage via numerous mechanisms. Antinuclear antibodies bind to free DNA, forming complexes that are deposited in glomeruli, walls of arterioles and joint synovia causing glomerulonephritis, arteriole fibrosis and arthritis respectively. Autoantibodies to platelets, red blood cells, muscles and skin are also produced causing the multisystemic disease (Tizard, 1995).



*Figure 9 – Female adolescent with facial lesions as a result of Systemic Lupus Erythematosus (Tizard, 1995, p498).*

The immune system in females is more enhanced than that of males with a better B-cell mediated immunity, higher immunoglobulin levels, stronger antibody responses and an increased resistance to certain infections. It is believed that sex hormones play a vital role in systemic lupus erythematosus as the disease worsens at particular

periods of hormone changes when oestrogen levels are high (Osman, 2003). According to Yacoub Wasef (2004), before puberty the female to male ratio of SLE is 3:1, during childbearing years it ranges from 10:1 to 15:1 and after menopause the ratio is 8:1. Male and female SLE patients have abnormal hydroxylation of oestrogen which produces 16 alpha-hydroxyesterone. This compound covalently bonds with proteins such as erythrocytes and lymphocytes, resulting in antibody production (Wasef, 2004).

Female SLE patients have lower androgen levels than healthy females and an increased oxidation of testosterone whereas male patients have normal androgen levels and normal oxidation of testosterone. Gonadectomised female and male mice have been reported to have an enhanced immune response to endotoxins. Testosterone treatment reverses this immune response suggesting a protective role of testosterone in autoimmune diseases (Gaillard et al, 1998).

### **6.2.2 Rheumatoid Arthritis (RA)**

This autoimmune disease differs between sex as well as age. Prevalence rates in females increase from menarche reaching a peak at menopause, whereas it is rare for males to have RA under the age of 45, with numbers steadily increasing in older males before reaching numbers similar to that of females. Androgens are thought to play a major role in this autoimmune disease as male patients exhibit low testosterone levels. Dehydroepiandrosterone (DHEA), an adrenal gland product which is the major androgen in females has also been found to be in low levels in RA patients. Between 20 and 30 years of age, DHEA levels reach a peak and decrease thereafter (Wilder, 1996). Despite evidence for a role of DHEA in rheumatoid arthritis, oestrogen and

progesterone deficiency are also thought to be involved due to the menopausal peak of the disease and the fact that oral oestrogen contraceptives can alter the disease onset and severity (Olsen et al, 2002).

### **6.3 Autism**

Autism is a neurodevelopmental disorder characterised by deficits in social interaction, stereotyped repetitive behaviours, language impairments and diminished cognitive abilities which must be apparent by three years of age (American Psychiatric Association, 1994). This disorder is divided into three categories depending on the level of cognitive dysfunction. Low-functioning autism has an IQ of less than 70, high-functioning autism has an IQ above 70 and Asperger syndrome which is similar to high-functioning autism, without the language deficits (Powell, 2004). It has been clearly established that autism is four times more prevalent in males than females and Asperger syndrome is ten times more common in males, yet the underlying mechanisms are largely unknown (Gillberg et al, 1999; Hertz-Picciotto et al, 2006; Powell, 2004; Stone et al, 2004).

Few studies have referred to autism and the effects of gender, other than the higher male to female prevalence rates. Thompson et al (2003) conducted a literature review of autism to ascertain the number of studies that analysed data on female and male autistic patients. During the period 1990 and 1992 (see Table 2), there were 392 studies found within the Psychlit database, 119 of which provided information on prevalence rates among males and females. Of these 119 articles, 57 were conducted using just one sex with small sample sizes, 20 analysed variables separately for each

gender and 3 out of these 20 analysed intellectual differences between males and females with autism (Thompson et al, 2003).

<b>LITERATURE SEARCH FROM 1990-1992</b>	
<b>Search for “Autism” produced = 392 Articles</b>	
<b>Article information</b>	<b>Percent of 392 Articles</b>
Information about males VS females	30%
Single sexed articles with small sample size	15%
Analysis of variables separately for genders	5%
Female and male IQ considered	<1%

*Table 2 – Number of articles with gender specific information with regard to autism from 1990-1992 (adapted from Thompson et al, 2003).*

The same search was conducted for the period 2000-2002 which produced 563 articles on autism (see Table 3). Of these articles, 134 contained information on the number of males and females within the study, and 76 of these consisted of small sample sizes of just one sex. The separate analysis of variables for males and females was only 12 as opposed to the 20 found in 1990-1992. Over this 10 year period there was only an increase of 171 articles and a 3% decrease in the number of studies that analysed variables separately for each gender (Thompson et al, 2003). This analysis of articles by Thompson and colleagues suggests that the information available on autism has a bias towards males. This needs to change in order to obtain a clear understanding of the mechanisms leading to the development and symptoms of autism.

**LITERATURE SEARCH FROM 2000-2002**

**Search for “Autism” produced = 563 articles**

<b>Article Information</b>	<b>Percent of 563 Articles</b>
Information about males VS females	24%
Single sexed articles with small sample size	13%
Analysis of variables separately for genders	2%

*Table 3 - Number of articles with gender specific information with regard to autism from 2000-2002 (adapted from Thompson et al, 2003).*

A theory behind the behavioural aspects of the autistic brain is one that involves an extreme masculinisation of the brain. Baron-Cohen (2002) has suggested that there are two types of brains; the empathising brain and the systemising brain. The empathising brain or female brain, is able to identify thoughts and behaviours of others and is therefore able to respond accordingly. The systemising brain or male brain, analyses certain aspects of a system to determine the behavioural consequence of an action. There is evidence to suggest the autistic brain is a highly masculinised systemising brain with an impairment in empathising. Behaviours such as ‘mind reading’ (ability to understand and predict behaviour of others), reading facial expressions, eye contact, language development and social interaction are all superior behaviours in females. Males score lower in all such behaviours, with autistic patients scoring even lower (Baron-Cohen, 2002).

Some individuals with Asperger syndrome or high functioning autism have special abilities in mathematical calculations, music and memory of statistics and numbers, all of which are characteristics of a systemising brain common in males. Attention to detail, preference for factual and structured information, collecting and organising

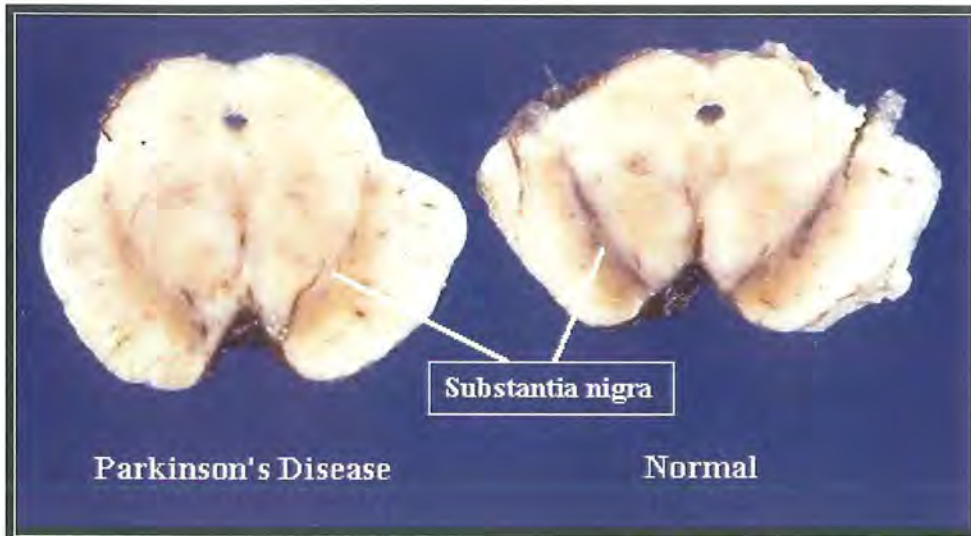
objects as well as hobbies such as train spotting due to the structured formatted timetables are all common in autistic individuals as well as being characteristic of a systemising male brain (Baron-Cohen, 2002).

Due to a lack of gender based studies on autism, a definitive answer can not be made with regard to sexually dimorphic brain structures in accounting for the difference in male-female prevalence rates. Several structures such as the amygdala, hippocampus, cerebellum and cerebrum have all been implicated in the pathogenesis of autism (Herbert, 2005; Schumann et al, 2004). Macrocephaly occurs in 20% of autistic children compared to 3% of the normal population (Dementieva et al, 2005; Deutsch et al, 2003). This enlargement of the brain appears to be apparent in children yet is not maintained in adulthood (Aylward et al, 2002; Curchesne et al, 2001). Further research is needed on the gender differences in autism to create a better understanding of the disease and its development. A male-female prevalence ratio of 4:1 is a significant difference and the reason for this should be ascertained (Stone et al, 2004).

#### **6.4 Parkinson's Disease**

This neurological disease is between 2 and 3 times more common in males than females (Baldereschi et al, 2000; Bower et al, 1999; Czlonkowska et al, 2005; Milanov et al, 2001; Van Den Eeden et al, 2003). Parkinson's disease (PD) is characterised by selective degeneration of dopamine neurons within the substantia nigra pars compacta producing a decrease in dopamine levels and a loss of neuromelanin (the dark pigmentation found in dopaminergic neurons) as seen in Figure 10 (Fahn et al, 2004). The substantia nigra inhibits the function of the cerebral nuclei with the release of dopamine. A decrease in dopamine production causes the

cerebral nuclei to become overactive, producing an increase in muscle tone and other characteristic symptoms of Parkinson's disease such as stooped posture, slowed voluntary movements, rigidity and muscle tremors (Cotran et al, 1999).



*Figure 10 – Midbrain section of the substantia nigra showing the loss of pigmentation in Parkinson's disease (WebPath,2006).*

An accepted theory behind the lower prevalence of PD in females is again the neuroprotective role of oestrogen. Studies in which rodents were treated with neurotoxins such as 6-hydroxydopamine or 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) and subsequently treated with oestrogen showed a decrease in dopaminergic neuron loss (Czlonkowska et al, 2005). Females are reported to have greater dopamine neuronal density within the caudate nucleus which could be another explanation as to why females do not develop PD as often and as severely (Becker, 1999; Walker et al, 2000).

Numerous studies have reported increased cytokine levels within cerebrospinal fluid and the striatum of Parkinson's patients (Czlonkowska et al, 2005; Hunot et al, 1997; Le et al, 1999). Pro-inflammatory cytokines such as interferon-gamma (IFN- $\gamma$ ), interleukin-1 beta (IL-1 $\beta$ ) and tumour necrosis factor-alpha (TNF- $\alpha$ ) are evident within the striatum of PD patients, suggesting a role of the inflammatory response in the pathogenesis of Parkinson's disease (Nagatsu et al, 2000; Nagatsu, 2002). Evidence from oestrogen studies have shown that it decreases production of certain cytokines such as IL-1 $\beta$ , TNF- $\alpha$  as well as interleukin-6 (IL-6) and that these pro-inflammatory cytokines also increase after menopause or if oestrogen levels are reduced (Bernard-Poenaru et al, 2001; Cantatore et al, 1995; Rogers et al, 2001).

The dopaminergic toxin MPTP (1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine) has also been used to establish inflammatory responses within the CNS. Mice injected with the toxin exhibited an increase in the pro-inflammatory cytokine TNF- $\alpha$  within the striatum yet there was a higher expression of this factor in the male mice than the female. TNF- $\alpha$  was found 6 hours after administration of MPTP whereas it took 1 day for the protein to be located within female mice (Cesielska et al, 2003). TNF- $\alpha$  is a powerful neurotoxin and may initiate and sustain the inflammatory response in Parkinson's disease leading to degeneration of dopaminergic neurons (Czlonkowska et al, 2005).

The majority of Parkinson's cases occur sporadically with unknown causes yet 10% of cases are said to be familial (Fahn et al, 2004). The *SRY* gene found on the Y chromosome has recently been reported to influence the production of dopamine in the substantia nigra. Studies in which the *SRY* gene was deleted from one side of the



substantia nigra in male mice showed that in that portion, there was a 38% decrease in dopaminergic neurons. These mice also exhibited Parkinson's like motor functions in the one side controlled by the substantia nigra that had been altered (Gramling, 2006).

## **6.5 Cardiovascular Disease**

In general, premenopausal females appear to have a lower prevalence of cardiovascular disease and show a better prognosis than males. Between the ages of 45 and 64, 39% more males die from heart disease than females yet after 65 years of age, the rate of death for women exceeds that of males by 22% (Leinwand, 2003). The diastolic function of the heart, i.e. the phase in which the chamber fills with blood preparing for contraction (systole) is more efficient in females than in males. Systolic function is also superior in females due to increased thickness in the heart wall (Leinwand, 2003). Men show poorer contractility, myocardial thinning and inferior chamber dilation when compared to females (Adams et al, 1999; Legget et al, 1996; Leinwand, 2003).

Hypertension, which is classified as having a constant elevated blood pressure, is more common in men of 30-45 years than age matched women. Male rats have greater vascular contraction than female rats and ovariectomised females have a greater vascular contractility than intact females, suggesting a role of oestrogen in the physiological control mechanisms of hypertension (Khalil, 2005). Support for this theory arises from statistics that show postmenopausal women suffer from hypertension at the same rates as men (Czubryt et al 2006; Khalil, 2005).

Myocardial infarction (MI), in which necrosis of an area within the heart occurs due to ischaemia, is another cardiovascular disease that occurs in greater numbers in men than premenopausal women. Atherosclerosis is a common cause of myocardial infarction yet as this occurs in women much later in life; women experiencing MI's are also significantly older than males (Czubryt et al, 2006). Although women experience MI's less often than men, it is reported that young or middle aged women who suffer a MI have an increased mortality rate compared with men (Czubryt et al, 2006; Vaccarino et al, 2000). Stromberg and Martensson (2003) have reported that myocardial infarctions in women are more severe and heart failure is more likely to occur as a consequence.

Before puberty the number and size of cardiac cells, or myocytes, are approximately the same in both genders yet it is reported that the absolute mass of the male heart is 15-30% larger than that of post-pubertal females. This suggests that some hormonal influence which occurs during puberty causes male myocytes to enlarge producing a heart of greater mass (Leinwand, 2003).

Although a significant volume of evidence has supported the role of oestrogen in protecting the cardiovascular system, studies of hormone replacement therapy (HRT) regimes has suggested otherwise. Post-menopausal women taking oestrogen and progesterone supplements have shown that these hormones provided no additional protection against cardiovascular disease, contradicting earlier reports on HRT (Harman et al, 2005; Manson et al, 2001). Paradoxically HRT has been associated with an increase in certain cardiovascular disease such as Cerebrovascular Accident (CVA) commonly known as stroke. A timing hypothesis has been proposed to

account for the results of HRT that contradict the beneficial results of oestrogen in pre-menopausal women and to account for the positive results of HRT in previous studies. It is believed that HRT needs to be administered during or soon after menopause in order for the hormones to have an effect. A delay in treatment is thought to allow atherosclerotic lesions to become too advanced for hormone replacement to have a beneficial effect (Harman et al, 2005; Mendelsohn et al, 2005).

The use of rodents in the study of cardiovascular disease has not been highly successful as studies have produced conflicting results and are not comparable, making it difficult to draw appropriate conclusions. Various studies have been conducted on the papillary muscles of rats, yet the age of the rats and methods for measurement all differed, making comparisons difficult. The papillary muscles of 6 month old rats had been studied and it was concluded that males had a slower contraction and relaxation rate. Another study using the papillary muscle of an isolated working heart preparation concluded that males had an increased cardiac output. Experiments on rats younger than 6 months old produced no varying differences in the cardiovascular system between genders. It is clear that experiments need to be conducted in a systematic manner, using the same strain of rodent, the same sampling technique and rats of the same age, in order to reach suitable conclusions (Leinwand, 2003).

The age of the rodents at the time of cardiovascular testing is especially important if the results are to be compared with the human cardiovascular system. As shown by Leinwand (2003), complete sexual differentiation of the human cardiovascular system may not occur until adulthood. In rodents the comparable age would be 10 to 12

months yet most studies are performed when the rodent is 6 months of age or younger, which may explain why experiments have produced results that have indicated no sexual differentiation in heart structure or function.

## **6.6 Alzheimer's Disease**

This neurodegenerative disease is the leading cause of dementia worldwide, characterised by extracellular deposition of amyloid plaques and the presence of intracellular neurofibrillary tangles (NFTs) within the brain (Bates, 2005; Gandy, 2005; Spires et al, 2005). Memory loss is the first symptom of Alzheimer's disease, followed by a decline in all cognitive abilities. In the end stage of the disease, motor functions are affected and patients become bedridden (Spires et al, 2005).

The formation of amyloid plaques mainly occurs within the hippocampus, neocortex and amygdala of affected individuals. Neurofibrillary tangles (NFTs) are found within the hippocampus, neocortex, amygdala and within several thalamic and hypothalamic nuclei. The degree of neuronal loss within the brain is directly correlated with the extent of NFT formation. In the end stages of the disease approximately 90% of prefrontal cortex neurons have died and 70% of the neurons within the hippocampus suffer the same fate, causing the severe dementia seen in Alzheimer's patients (Spires et al, 2005).

Although a majority of Alzheimer's cases are sporadic, approximately 1% of cases occur due to an autosomal dominant inheritance. The first gene associated with Alzheimer's disease was found in the amyloid precursor protein (*APP*) gene yet genetic linkage studies have discovered mutations in the presenilin 1 and 2 genes on

chromosome 14 and 1 respectively. The presenilin genes are involved in the production of amyloid  $\beta$  by forming the active site of the  $\gamma$ -secretase complex that in turn cleaves the amyloid precursor protein. Once *APP* is cleaved it forms the amyloid  $\beta$  that aggregates to form the characteristic plaques of Alzheimer's disease (Spires et al, 2005).

Oestrogens have a profound role within the central nervous system in protecting neuronal cells. Some studies of oestrogen replacement therapy and Alzheimer's disease have shown that oestrogen decreases the presence of amyloid  $\beta$ , in turn decreasing plaque development. *In vivo* and *in vitro* studies have shown that testosterone increases the presence of amyloid  $\beta$  and increases its toxicity within the hippocampal region of the brain. Once women enter menopause and oestrogen levels decrease, the neuroprotective role of oestrogen is diminished, which may lead to the higher prevalence of Alzheimer's seen in women (Bates et al, 2005).

Two major cholinergic nuclei within the basal forebrain, the vertical limb of the diagonal band of Broca (VDB) and the nucleus basalis of Meynert (NBM), have been implicated in the pathology of Alzheimer's. The cholinergic neurons within these nuclei are significantly decreased in male and female Alzheimer patients. As memory and other cognitive functions are a main role of the cholinergic system, it is believed that this decrease in neurons causes many of the symptoms seen in Alzheimer's disease (Ishunina et al, 2002). Within humans (Donahue et al, 2000; Ishunina and Swaab, 2001), and animals (Ishunina et al, 2002; Mufson et al, 1999), the function of the cholinergic neurons is largely influenced by sex hormones. The number of androgen receptors within the VDB and NBM are significantly lower in female

Alzheimer patients as opposed to males, which may account for the increased risk of Alzheimer's in females (Ishunina et al, 2002).

In general, studies report a higher incidence and prevalence rate of Alzheimer's disease in women (Bonsignore et al, 2002; Yue et al, 2005) yet when examined closely, several variables have not been accounted for. There are a variety of explanations that may account for the inconsistencies in research results with regard to disease incidence and prevalence between genders. The fact that females are known to survive longer than males and are therefore more susceptible to diseases associated with ageing is an important variable that needs to be assessed when reporting data. Females with Alzheimer's disease are reported to survive longer compared to males, therefore there may be more females alive to participate in studies than there are males. These two factors produce a selection bias due to the under-representation of males within Alzheimer's disease studies (Bonsignore et al, 2002).

## CHAPTER SEVEN

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### Methods

This literature review involved the analysis of journal articles that were accessed through the Edith Cowan University online database collection. Numerous databases were scrutinised to ensure this review was based on the latest and most current information regarding this topic. The main database engines used for this study were:

- Biomed Central
- Pubmed Central
- ProQuest
- Oxford Journals
- Science Direct
- Ovid Online
- Google

Other databases such as BMJ Journals Online and Cochrane were excluded from this study as initial searches were unproductive in relation to this topic. All journal articles used in this study were chosen for their relevance to sex determination, sex differentiation, sex abnormalities, brain structure between genders or gender-related diseases. The date of publication was a critical element when choosing articles for inclusion in this review as the topic is continually progressing. Papers from 2006 were initially reviewed but few existed so it was necessary to widen the search between the years 2000 and 2006. Reference lists from relevant journal articles were investigated and used to collaborate results of current studies.

The search terms used that produced the most useful articles included: sex determination, sex differentiation, SRY gene, SOX9 gene, SF-1 gene, WT-1 gene, DMRT1 gene, GATA4 gene, DAX-1 gene, sex abnormalities, hermaphrodites testicular feminization, androgen insensitivity syndrome, Congenital Adrenal Hyperplasia, 21 Hydroxylase Deficiency, 17 $\beta$ -Hydroxysteroid Dehydrogenase 3 Deficiency, brain sex, brain structure and gender, brain differentiation, hypothalamus and gender, amygdala and gender, corpus callosum and gender, cerebral cortex and gender, brain structure and hormones, Schizophrenia and gender, Alzheimer's and gender, Autism and gender, Autoimmune disease and gender, sexually dimorphic disease.



### Results

#### 8.1 Cerebral Cortex

Males have a greater volume and weight of the cerebral cortex than females, with a neuronal density that is 15% higher (Reiss et al, 1996; De Courten-Myers, 1999; Rabinowicz et al, 2002; Witelson et al, 2006). White matter volumes are on average larger in males and grey matter volumes are larger in females (De Courten-Myers, 1999). The female cerebral cortex has been found to consist of more neuropil than males, producing more cell to cell connections and a more extensive dendritic network (Rabinowicz et al, 1998). Activation of the ventromedial prefrontal cortex also differs between males and females. Males tend to use the right hemisphere of the ventromedial cortex for emotional processing and decision making, whereas females use the left hemisphere (Tranel et al, 2005).

#### 8.2 Corpus Callosum

The literature regarding sexual dimorphism of the corpus callosum is controversial but the majority of studies agree that the corpus callosum is larger in females and of a more spherical shape than that of the male as seen in Table 4 (De Lacoste-Utamsing et al, 1982; Rabinowicz et al, 2002; Smith, 2005). This increase in fibres connecting the two hemispheres is one theory underlying the bilateralisation of the female brain (Rabinowicz et al, 2002).

### 8.3 Hypothalamus

There are several nuclei that are sexually dimorphic within the hypothalamus (Swaab et al, 2003). The preoptic area of the anterior hypothalamus contains four nuclei, the lateral, medial, median preoptic nuclei and the pariventricular nucleus which are larger in male brains. The lateral, median and medial preoptic nuclei are 50-70% larger in males (Table 4). The male lateral preoptic nucleus contains 28% more neurons whilst the medial preoptic nucleus contains 21% more neurons accounting for the increase in size. The male medial preoptic nucleus contains 80% more neurons due to the highly sexually dimorphic nucleus within this region (Madeira et al, 1999).

The sexually dimorphic nucleus (SDN) of the medial preoptic area is 3-8 times larger in male rats than female rats. The human SDN is approximately twice as large in young males as females, with twice the number of cells (Gorski et al, 1978; Madeira et al, 1999; Swaab et al, 1992; Swaab et al, 2003; Von Esenwein et al, 2005). Before the age of two years, there are no sexual differences in the size or neuronal number of the sexually dimorphic nucleus (Swaab et al, 2003).

The suprachiasmatic nucleus (SCN) of the hypothalamus is larger in the male rat yet in humans it is only the shape that differs. The female SCN has an elongated shape whilst the males' is more spherical (Swaab, 1995; Hofman, 1996; Abizaid et al, 2004). In male homosexuals the volume of the SCN is 1.7 times larger with 2.1 times as many cells as heterosexuals (Swaab et al, 2003).

The Bed Nucleus of the Stria Terminalis (BNST) has two sexually dimorphic regions, the darkly staining posteromedial component of the BST (BST-dspm) and the central

division (Allen and Gorski, 1990, Chung et al, 2002, Garcia-Falgueras, 2005). The BNST as a whole is larger in males compared to females (Table 4) yet these differences do not reach significant values until adulthood (Chung et al, 2002).

#### **8.4 Anterior Commissure**

The majority of studies report that the anterior commissure is 12% larger in females than males (Moir and Jessel, 1991; Allen and Gorski, 1992; Swaab and Hofman, 1995). Some have reported no sexual dimorphism in rat or human studies (Jones et al, 1997; Bishop et al 1999; Highley et al, 1999). This structure is only present in 78% of females and 68% of males (Swaab et al, 2003).

#### **8.5 The Amygdala**

This structure is significantly larger in males than females (Goldstein et al, 2001, Durston et al, 2001, Cahill et al, 2004). Rat studies show that the posterodorsal region of the medial amygdala is 50-80% larger in males (Cooke et al, 1999), with a larger number of neurons in the posteromedial region (Rasia-Filho et al, 2000).

**Table 4– Summary of Sexually Dimorphic Brain Structures in Humans.**

Study	Year	Brain Structure	Sexual Dimorphism	Size Difference
Reiss et al.	1996	Cerebrum	Yes	10% > in human males
De Courten-Myers.	1999	Cerebrum	Yes	≈ 10% > in human males
Rabinowicz et al.	2002	Cerebrum	Yes	15% > in human males
Witelson et al	2006	Cerebrum	Yes	9-12% > in human males
De Lacoste-Utamsing et al.	1982	Corpus Callosum	Yes	> in human females
Allen et al.	1991	Corpus Callosum	No	-
Denenberg et al.	1991	Corpus Callosum	Yes	> posterior region in human males
Holloway et al.	1993	Corpus Callosum	No	-
Rabinowicz et al.	2002	Corpus Callosum	Yes	> in females
Smith	2005	Corpus Callosum	Yes	> in females
Madeira et al	1999	PAOH	Yes	> in human males
Gorski et al.	1978	SDN	Yes	> in males
Swaab et al.	1992	SDN	Yes	> in males
Swaab et al.	2003	SDN	Yes	> in males
Von Esenwein et al	2005	SDN	Yes	> in males
Swaab.	1995	Suprachiasmatic Nucleus	Yes	difference in shape
Hofman et al.	1996	Suprachiasmatic Nucleus	Yes	difference in shape

Abizaid et al.	2004	Suprachiasmic Nucleus	Yes	difference in shape
Allen et al.	1990	BNST	Yes	> in males
Chung et al.	2000	BNST	Yes	> in males
Chung et al.	2002	BNST	Yes	> in males
Garcia-Falgueras	2005	BNST		> in males
Moir and Jessel	1991	Anterior Commissure	Yes	12% > females
Allen and Gorski	1992	Anterior Commissure	Yes	12% > in females
Swaab and Hofman	1995	Anterior Commissure	Yes	12% > in females
Jones et al.	1997	Anterior Commissure	No	-
Bishop et al.	1999	Anterior Commissure	No	-
Highley et al.	1999	Anterior Commissure	No	-
Goldstein et al.	2001	Amygdala	Yes	> in males
Durston et al.	2001	Amygdala	Yes	> in males
Cahill et al.	2004	Amygdala	Yes	> in males

PAOH = Preoptic Area of the Hypothalamus

SDN = Sexually Dimorphic Nucleus of the Hypothalamus

BNST = Bed Nucleus of the Stria Terminalis

## CHAPTER NINE

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### Discussion

Although there are a number of brain structures that are universally accepted as being sexually dimorphic, various studies report no significant differences in structure or no correlating function to such differences. Cerebral size has been found to be larger in males (De Courten-Myers, 1999; Rabinowicz et al, 2002; Reiss et al, 1996; Witelson et al, 2006) yet this does not produce a corresponding increase in intelligence or higher function of the cerebral cortex in men. The corpus callosum and anterior commissure are bundles of nerve fibres that connect the two cerebral hemispheres. These fibre networks are larger in females and are directly correlated with an increase in cerebral function (Denenberg et al, 1991; Rabinowicz et al, 2002; Smith, 2005). The larger interhemispheric connection in females is believed to contribute to the bilateralisation of the female brain (Rabinowicz et al, 2002) and may be a factor behind the decreased prevalence and severity associated with diseases such as schizophrenia, mental retardation and stroke.

Observations of the nuclei within the hypothalamus have consistently reported sexual dimorphism with regard to size of certain nuclei. The preoptic area of the hypothalamus (Madeira et al, 1999), the sexually dimorphic nucleus (Gorski et al, 1978; Swaab et al, 1992; Swaab et al, 2003; Von Esenwein et al, 2005), suprachiasmatic nucleus (Abizaid et al, 2004; Hofman et al, 1996) and the bed nucleus of the stria terminalis (Allen et al, 1990; Chung et al 2000; Chung et al, 2002; Garcia-Falgueras, 2005) have been identified as being larger in males as opposed to females. The most significant sexual dimorphism has been found within the sexually dimorphic nucleus (SDN). This structure is almost twice as large within human adult

males and 3-8 times larger in the male adult rat. The function of the SDN has been associated with sexual behaviour and identity within both sexes yet the larger nucleus in males is thought to cause a corresponding increase in sexual appetitive behaviour common to the male sex (Swaab et al, 2003).

The amygdala, which is involved in emotional processing, memory, fear responses and reproductive behaviour, is significantly larger in both human and rat males (Hamann, 2005). PET imaging has revealed that the male amygdala is stimulated to a higher degree with regard to arousing material and is also linked to the increased sexual appetitive behaviour exhibited by the male gender (Hamman et al, 2005). A variety of sexual disorders, all of which are more common in males, have been linked to an abnormality within the amygdala (Gomez, 1991). Lesions within the amygdala support the role of the amygdala in abnormal sexual behaviours as these individuals exhibit a failure to recognise social norms in regards to sexual behaviour and are prone to a range of paraphilia's such as voyeurism, exhibitionism, paedophilia and fetishism (Bezeau et al; 2004; Kafka et al, 2002).

The presence of sex differences in morbidity and mortality rates are clear as seen in this present study (Bren, 2005; Gesensway, 2001; Pinn, 2003; Rieker et al, 2005; Wizemann et al, 2001). However the mechanism(s) behind these differences needs more thorough research. Sex hormones play an important role in sex differentiation both in development and throughout life, forming a basis behind the sexually dimorphic disease patterns that are becoming evident (Rieker et al, 2005; Wizemann et al, 2001). The significance of hormone production and maintenance can be shown through sex differentiation abnormalities in which the lack of a hormone or its loss of

function can produce devastating effects (Witchel, 2002) such as testicular feminisation (Holterhus et al, 2003) and congenital adrenal hyperplasia (Merke and Bornstein, 2005).

Primary hermaphrodites arise due to a mutation which causes an individual to be born with internal and/or external genitalia of both sexes. Due to ambiguous external genitalia, individuals may not be raised according to their chromosomal sex. Once puberty arises, these individuals begin to think according to the sex of their brain and not necessarily their external genitalia, causing serious mental anguish and identity problems (Salas-Cortes et al, 2000). Testicular feminisation is an example of a secondary hermaphrodite that arises due to abnormal androgen receptors. These individuals exhibit ambiguous genitalia as well as feminisation, both of which are directly correlated with the degree of androgen receptor function (Holterhus et al, 2003).

Although it is evident that many sex differences are due to the action of hormones, it is unlikely they are the sole cause. There are genes located on the X chromosome that are expressed at increased levels within females despite X chromosome activation. Genes that are located on the Y chromosome, such as the SRY gene, may also play a role in sexual dimorphism leading to differences in the central nervous system and affecting disease patterns (Leinwand, 2003).

From conception, it appears males are disadvantaged with a greater risk of brain damage, congenital deformities, still birth and cerebral palsy. After birth, developmental disorders such as autism, attention deficit hyperactivity disorder and



stuttering are all more common in males (Hertz-Picciotto, 2006; Kraemer, 2000; Powell, 2004; Stone et al, 2004), as well as other diseases such as schizophrenia (Cyr et al, 2002), cardiovascular disease (Leinwand, 2003) and Parkinson's disease (Czlonkowska et al, 2005). Although a majority of diseases appear to be more common in males, there are some diseases more prevalent in females such as autoimmune diseases (Rubin, 2001) and Alzheimer's disease (Ott et al, 2001).

This study has shown that females fare better in terms of prevalence and severity of numerous diseases due to the neuroprotective role of oestrogen. Evidence to support the role of oestrogen arises through oestrogen knockout mice and human studies in which the incidence of disease increases for females once they have reached menopause and a corresponding decrease in oestrogen occurs (Hafner et al, 2003; Khalil, 2005; Rogers et al, 2001).

Researchers have recognised that the majority of organs within the body, not just those of the reproductive system, are sexually dimorphic in structure and in some cases function (Kraemer, 2000). These dimorphisms lead to a different prevalence of disease states, different symptoms and different pharmacological treatments. Females and males differ in regards to drug absorption, distribution and excretion. Therefore studies on drug treatments need to use gender as an important variable in their analysis that must be accounted for (Bren, 2005).

A majority of research has failed to produce data on females and males as a separate population despite the evidence that females and males differ significantly in normal physiology and pathological functions. In 2001, the Institute of Medicine published a

report concerning gender differences in medicine and the importance of gender related studies (Wizemann et al, 2001). Since this report, the number of studies that include sex as a variable have dramatically increased providing a better understanding of the basis behind certain mechanisms and disease pathologies. The report also states that journals should encourage authors to publish sex related data, whether it be of significance or not, in order to create a better understanding of sex differences (Kreeger, 2002).

The approach to comparing males and females, whether it be anatomical or physiological, is in need of more efficient study parameters. The knowledge of the test subject's biology as well as the environment in which the study is occurring are important factors when studying sex related differences. As sex steroids can have a dramatic affect on sexually dimorphic traits it is not sufficient to measure females at random times within the ovarian cycle. Comparing males with two or more groups of females where the oestrous cycle is known would show whether males and females differ in a certain trait and at what time of the ovarian cycle the differentiation occurs. Sex differences may be overlooked in simple studies consisting of one male and one female group, as certain traits may only differ at certain times of the oestrous cycle. If tested at the wrong time of the oestrous cycle, sex differences may not be seen, therefore producing negative results (Becker et al, 2005).

An individual's sex does not just determine their physical appearance but can influence their cognitive abilities, behaviour and all aspects of disease ranging from susceptibility, to drug treatments. It is for these reasons that research on normal structure and functioning as well as disease states should include sex as a key

variable, therefore producing data on both genders instead of males and females being included in the same category (Wizemann et al, 2001). This study has concluded there are several sexual dimorphic structures within the brain as well as the reproductive system, and that many disease states differ with regard to prevalence and severity between genders. It is clear that males are no longer the superior sex but rather just one half of the bigger picture.

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