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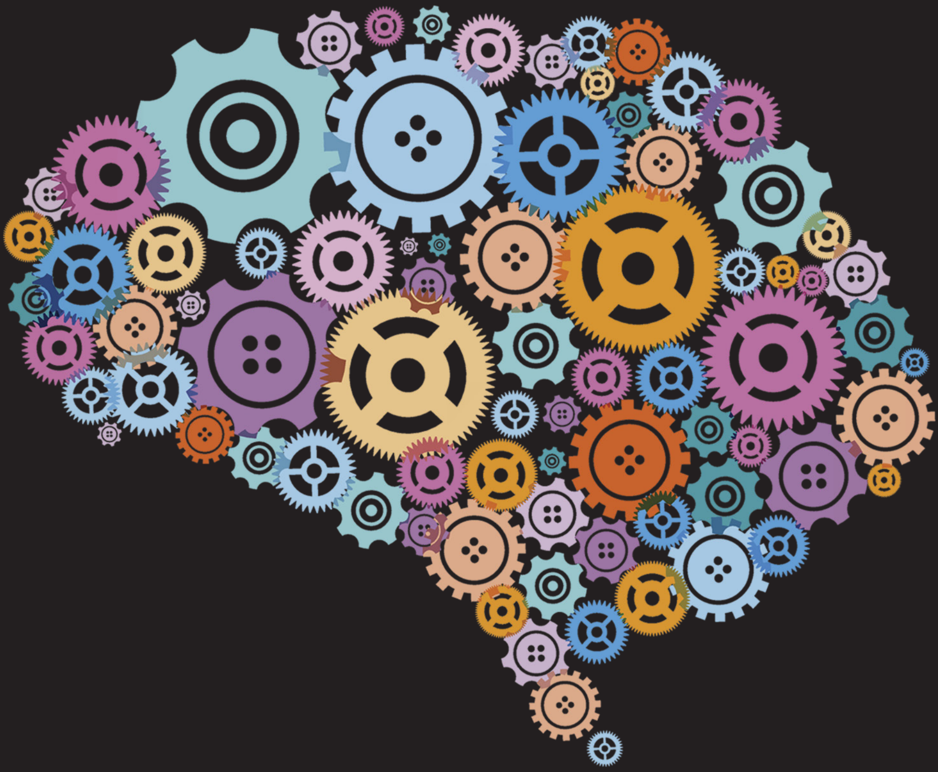
SEX AND THE DEVELOPING BRAIN

SECOND EDITION



Margaret M. McCarthy, PhD

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Colloquium Series on The Developing Brain

Editor

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The goal of this series is to provide a comprehensive state-of-the-art overview of how the brain develops and those processes that affect it. Topics range from the fundamentals of axonal guidance and synaptogenesis prenatally to the influence of hormones, sex, stress, maternal care, and injury during the early postnatal period to an additional critical period at puberty. Easily accessible expert reviews combine analyses of detailed cellular mechanisms with interpretations of significance and broader impact of the topic area on the field of neuroscience and the understanding of brain and behavior.

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Sex and the Developing Brain, Second Edition
Margaret M. McCarthy
www.morganclaypool.com

ISBN: 9781615047284 paperback

ISBN: 9781615047291 ebook

DOI: 10.4199/C00152ED1V01Y201703DBR014

A Publication in the

COLLOQUIUM SERIES ON THE DEVELOPING BRAIN

Lecture #14

Series Editor: Margaret M. McCarthy, University of Maryland School of Medicine

Series ISSN

ISSN 2159-5194 print

ISSN 2159-5208 online

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COLLOQUIUM SERIES ON THE DEVELOPING BRAIN #14

MORGAN & CLAYPOOL LIFE SCIENCES

ABSTRACT

The brains of males and females, men and women, are different, that is a fact. What is debated is how different and how important are those differences. Sex differences in the brain are determined by genetics, hormones, and experience, which in humans includes culture, society, and parental and peer expectations. The importance of nonbiological variables to sex differences in humans is paramount, making it difficult if not impossible to parse out those contributions that are truly biological. The study of animals provides us the opportunity to understand the magnitude and scope of biologically based sex differences in the brain, and understanding the cellular mechanisms provides us insight into novel sources of brain plasticity. Many sex differences are established during a developmental sensitive window by differences in the hormonal milieu of males versus females. The neonatal testis produces large amounts of testosterone which gains access to the brain and is further metabolized into active androgens and estrogens which modify brain development. Major parameters that are influenced by hormones include neurogenesis, cell death, neurochemical phenotype, axonal and dendritic growth, and synaptogenesis. Variance in these parameters results in sex differences in the size of particular brain regions, the projections between brain regions, and the number and type of synapses within particular brain regions. The cellular mechanisms are both region and endpoint specific and invoke many surprising systems such as prostaglandins, endocannabinoids, and cell death proteins. Epigenetic modifications to the genome both establish and maintain sex differences in the brain and behavior. By understanding when, why, and how sex differences in the brain are established, we may also learn the source of strong gender biases in the relative risk and severity of numerous neurological diseases and disorders of mental health. Boys are much more likely to be diagnosed with autism spectrum or attention and hyperactivity disorders, as well as speech and language deficits, compared to girls. By contrast, women are more likely to suffer from affective disorders, such as depression, anxiety, compulsion, and eating disorders and more likely to experience autoimmune and neurodegenerative disorders. Schizophrenia with an early onset is more common in males but a late-onset version is markedly more frequent in females. Male biased disorders have origins in development while female biased disorders are almost exclusively post-puberty. This remarkable shift in disease risk demands our attention. Novel insights into the biological origins of disease are also gained by comparing and contrasting the same processes in different sexes.

KEYWORDS

sex differences, androgen, estrogen, hypothalamus, preoptic area, hippocampus, sensitive period, synaptogenesis, neurogenesis, apoptosis, neuroepigenetics, neuroinflammation, sexual differentiation, gender bias in disorders of the brain, steroid hormones, estradiol, GABA, prostaglandins, vasopressin, kisspeptin, anteroventral periventricular nucleus (AVPV), astrocytes, microglia

Contents

Preface

The second edition of *Sex and the Developing Brain* includes the addition of 17 new figures, and modifications or updates to many existing figures, plus one new video. Additional highlights include discussions of the NIH mandate on sex as a biological variable; sex differences in neuropsychiatric disorders with origins in development; microglia; endocannabinoids; sex differences in play behavior; FoxP2 and ultrasonic vocalizations, and an entire new section on the development of the cerebellum. The text is expanded by 16 pages and 2 to 3 dozen additional references, and some outdated or since debunked references have also been removed.

ACKNOWLEDGMENTS

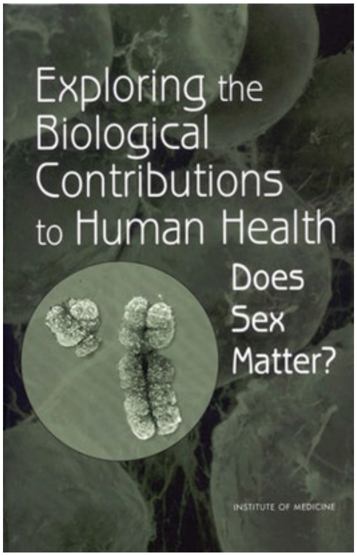
My deepest gratitude to N. Shalon Edwards for her expert editing of this volume.

CHAPTER 1

Introduction

Sex—a small word with big meaning. Sex can be a transitive verb, as in to perform the act of sex, or it can be an adjective, as in sex-limited, or it can be a noun, as in the fairer sex. These multiple meanings pervade and often confuse scholarly discussion of the topic, as I experienced recently in a conversation with two colleagues. The first did not know me well and asked, "what do you study?" and before I could answer the second responded with enthusiasm "she studies sex!" Which is true, but not in the way the first colleagues wide eyes and raised brows would imply. I study sex differences, in particular sex differences in the brain. This can evoke yet another reaction, such as, "oh are you trying to understand why girls are not good at math?" And again, the answer is no, not only because that statement is not true but also because I do my work using laboratory rodents, and rats cannot do math. The reason I study sex differences in the brain is to seek understanding of why boys are so vulnerable. There are more male fetuses conceived than female but so many die in utero that the sex ratio is close to parity by the time of birth. Boys are more likely to be born prematurely and to suffer a birth injury, and if they do they, will fare worse than their sisters. Boys are also much more likely to be diagnosed with autism spectrum disorders, stuttering, reading, speech and language deficits, attention and hyperactivity disorders, and even early-onset schizophrenia. This broad-ranging spectrum of vulnerabilities so early in life compels us to understand how the brain develops and is framed by the overarching question, are males more sensitive or are females more resilient? Or, perhaps, both are true, but at this moment, the answer is unknown.

Although sex differences are self-evident, they have also been largely ignored outside the context of reproductive biology. This began to change in 2001 when the Institute of Medicine published a report *Exploring the Biological Contributions to Human Health: Does Sex Matter?*, in which the overwhelming impact of sex on almost every biological endpoint was examined (Wizemann and Pardu, 2001). This report also provided the current distinction between sex and gender (Figure 1). Sex is how we codify the reproductive capacity of individuals based on genetic, gonadal, and somatic characteristics with which we are all familiar. All sexually reproducing species on the planet have two sexes, male and female. There can be some slippage in the constraints of sex, such as sex changing fish, parthenogenic female lizards, and hermaphrodite flatworms, but for the overwhelming majority of species, there are only two sexes. Gender on the other hand, combines both an individual's perception of self and societies perception and response to that individual as either male or



Exploring the
Biological
Contributions
to Human Health
Does
Sex
Matter?

INSTITUTE OF MEDICINE

FIGURE 1: Cover of the Institute of Medicine report: Sex Matters: Assessing the Biological Contributions to Health. This report was commissioned by the Institute of Medicine, an arm of the National Academy of Sciences of the United States, and after a multi-year and highly contentious process was released in 2001. The report concluded that the effects of gender or sex were pervasively important to all aspects of human health, and the importance of sex differences in the brain was particularly noted as an area requiring further research.

female. Thus, only humans are considered to have gender. But how can we understand gender in all its complexity and conflict in the human animal? The impact of experience, environment, culture, and society on human gender is overwhelming, even beginning before birth with sex-typic colors chosen for the nursery. Both parent and strangers speak to and physically interact with babies differently, depending on their perceived gender, cooing softy and gently rocking girls while merrily jostling boys. Thus, it is impossible to parse out the impact of the pervasive and infinitely variable influences that shape the gender of any one individual. But sex is a biological variable with relevance to far more than just reproductive capacity and about which appreciation is growing exponentially. Recent years have seen the development of a scientific society, the Organization for the Study of Sex Differences (http://www.ossdweb.org) and a new journal, *Biology of Sex Differences*, which reflects the increasing awareness of the importance of sex to virtually all aspects of human health, including the brain, be it the developing, mature, or aging brain. But separating out the confounding influences of being human from the purely biological requires that we turn to animal models.

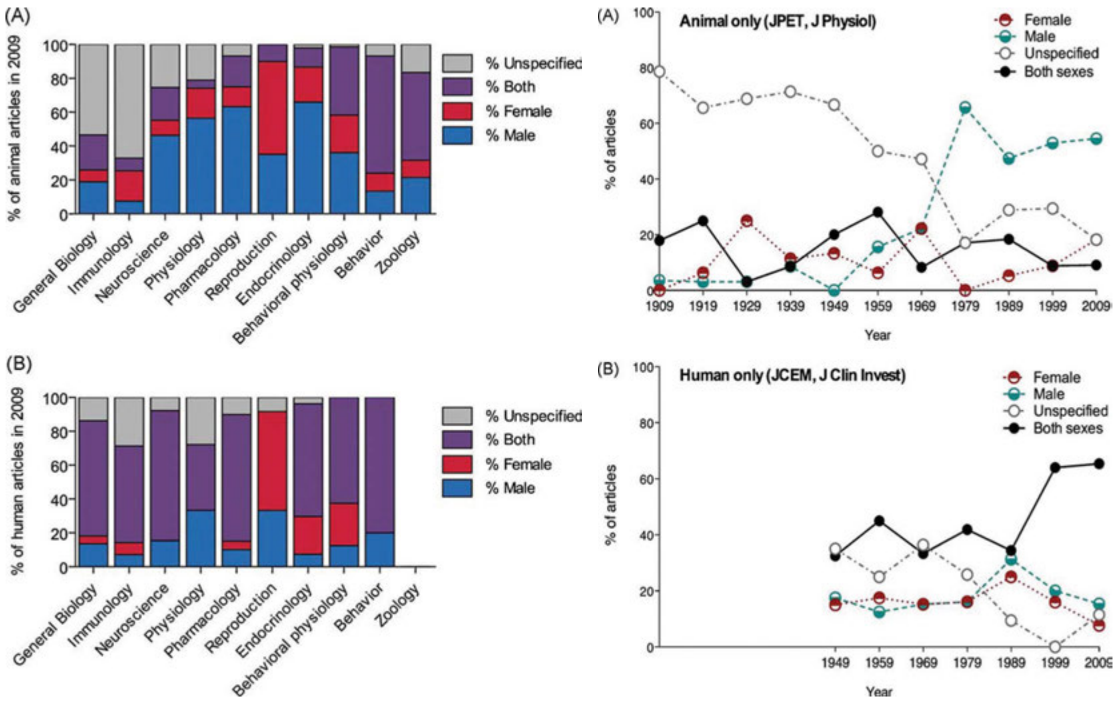


FIGURE 2: Female subjects are under represented in animal research. Top left panel, analyses of a random selection of papers published in 10 biological fields in 2009 revealed a strong bias in the exclusive use of male animals in eight of those disciplines. Neuroscience and Pharmacology (with the journals selected being focused on neuropharmacology) were two of the most heavily male biased disciplines. Lower left panel, the same analyses done in studies involving human subjects revealed that the majority involved both sexes. This can be attributed to a mandate by NIH in 1993 that all clinical trials include woman, children and minorities unless there are strong scientific justifications for exclusion. The historical analysis depicted in the lower right panel demonstrates the effectiveness of this mandate with a major uptick in studies using both sexes between 1989 and 1999. However, the same historical analysis of animal studies, shown in the upper right panel, shows the opposite with a major uptick in the number of studies using exclusively male animals (reprinted with permission from Beery and Zucker, 2010).

The use of animal models to study sex differences in brain and behavior raises another interesting policy issue that has recently impacted biomedical research in the United States, Canada, and the European Union. Funding agencies in these countries have mandated that all preclinical research (meaning mostly on animal models and cell lines) include equal representation of males and females. This might seem like an obvious thing to occur and one could wonder why it was required, but the reality was that the lion's share of research on animals was being done only on male animals (Beery and Zucker, 2010). The field of neuroscience was particularly egregious, with over five times as many studies on male animals as female animals (Figure 2). In many cases, the sex of the subject was not even reported. In the United States, the new policy went so far as to require that all data be disaggregated by sex and analyzed statistically for similarities and differences. Again, this apparently obvious step was needed because of lessons learned from a previous policy regarding sex. In 1993, the US Congress mandated that all clinical research (meaning on humans) had to have equal representation of both sexes, minorities, and, when appropriate, children. The result of the mandate was an increase in the inclusion of women in clinical trials, but sadly, in many cases, the data were never analyzed for the effect of sex on a given treatment, condition, or intervention. In hindsight, we see that much valuable information about sex differences in health, and disease was left unexplored.

A wide array of animal models have proven informative about the processes by which males and female are distinguished from each other, ranging from *Drosophila* and *C. elegans* to a variety of rodents and ultimately nonhuman primates, particularly *Rhesus macaques*. The genetic versatility combined with unparalleled fecundity of laboratory rats and mice has naturally led to the predominance of these species for in depth analyses of the process of sexual differentiation in mammals. The advantages to using rodents are obvious, and although the shortcomings are equally obvious, we set them aside when the goal is to understand brain development at the cellular and molecular levels.

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CHAPTER 2

Sex Differences in Brain and Behavior in Context

When considering sex differences in brain and behavior, it helps to have a conceptual framework for interpreting the nature and impact of particular observations. Four overlapping operationally defined categories help place findings in context (Figure 3).

The first category is "sex dimorphisms." "Di" means two and "morph" means form, so this refers to instances when the two sexes have two forms of an endpoint. Most often, these are endpoints directly associated with reproduction. The best example is sex behavior in rodents, where males show mounting and females lordosis, a sexually receptive posture. These two behaviors have different forms and the neural circuits that control them are correspondingly different, although this does not mean there is one circuit for male sex behavior and one circuit for female sex behavior. Instead, the same circuit controls sex behavior but aspects of the circuit are different in males versus females in terms of size of projections, density of synapses, etc. Other examples include maternal behavior of female mammals and the control of luteinizing hormone secretion from the anterior pituitary which controls ovulation in females and spermatogenesis in males.

The second category is "sex differences," and this is probably the most common because it refers to when males and females have the same endpoint but the measure of that endpoint lies along a continuum and the average is different between the sexes. Although not a brain measure, the best example is height. Everybody has a height, and on average, men are taller than women, but you cannot predict an individual's sex by measuring their height because some men are shorter than some women and vice versa. Many endpoints in the brain and behavior exhibit sex differences, but they may only be apparent during a certain phase of life, under certain circumstances or in specific contexts. This is another important aspect of sex differences, they are not always sex determined, they might just be influenced or "affected" by sex.

The third category is a catchall called "sex divergence and convergence" and refers to the phenomenon that some sex differences only appear under certain circumstances, such as during stress, whereas in other cases, a sex difference may exist for the purpose of bringing the sexes closer together, an effect referred to as compensation. Let us explore further. Examples of divergence are the impact

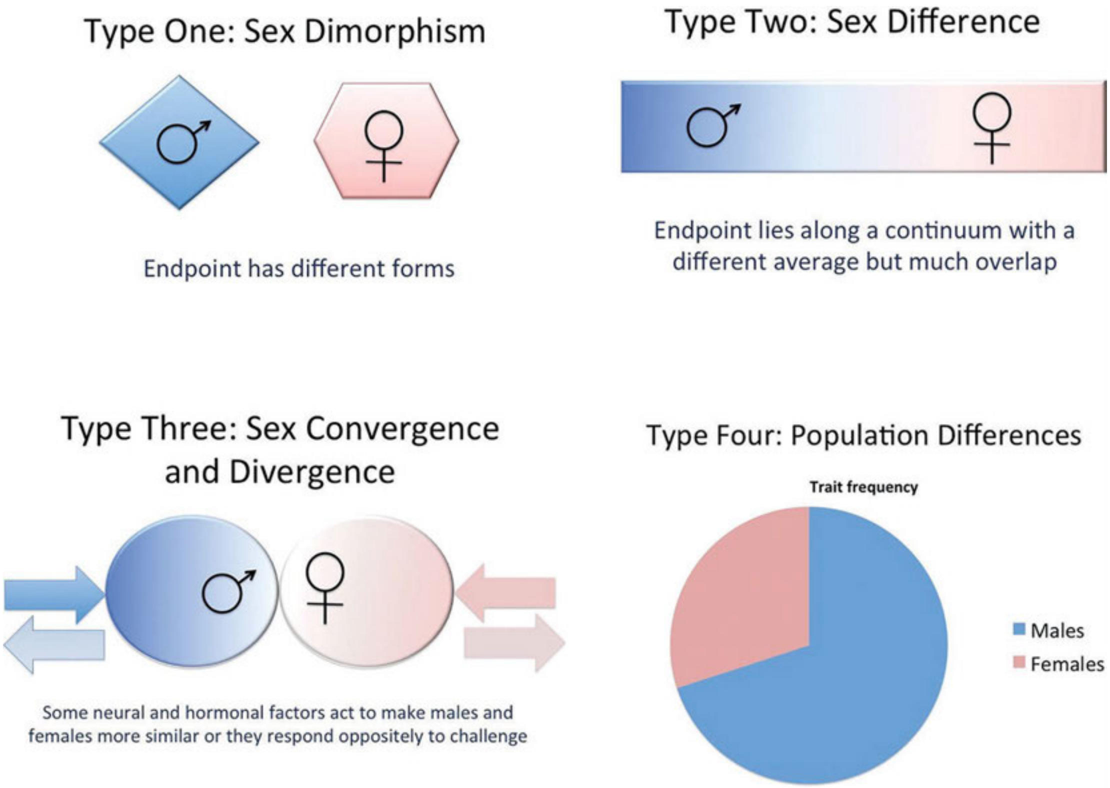

FIGURE 3: Sex as a biological variable. There are multiple ways in which the two sexes can differ from each other. Type 1, sex dimorphism refers to when an endpoint has two (di) forms (morph) and one is almost exclusively present in one sex versus the other. Examples of this include stereotypical sex behaviors seen in many animals or the control of gonadotropin secretion from the anterior pituitary which is pulsatile in males but exhibits a surge in females. The SDN (sexually dimorphic nucleus) of the POA was named for the large size difference in this small collection of neurons which shows little to no overlap in distribution between males and females. Type 2 is much more common and refers to sex differences in which males and females have an average response that varies along a continuum, but there is overlap in the distribution. In this instance, the sex of an individual cannot be predicted based on the endpoint because both males and females may fall along a portion of the range of responses. Type 3 is a codification of sex differences that occur in context, meaning that only under certain conditions do males and females differ. For instance, during stress, males may show improved learning while females show impaired learning or vice versa. This is an example of divergence. By contrast, there are some instances in which the sexes appear to be the same on a particular endpoint, say behavior, but the underlying neural mechanisms are different. This is likely due to the inherent constraints in biology of sex, that is, males do not get pregnant, etc. This concept is also referred to as compensation as first noted by the neuroendocrinologist Geert de Vries. Type 4 refers to when the response on a particular endpoint is the same in males versus females but the frequency with which it occurs differs. This is often the case of disease endpoints such as the gender bias in autism spectrum disorders, with males experiencing four to five times the incidence of females.

of stress on learning and the synaptic profile of hippocampal pyramidal neurons. Under normal conditions, both sexes learn a task equally well, but If stressed prior to learning male rats respond with an increase in dendritic spine synapses, which are excitatory, and show improved learning compared with unstressed males. Conversely, females show reduced numbers of synapses and their performance on the learning task degrades (Shors, 2006). Thus, the two sexes start in the same place but diverge under stress. Convergence, on the other hand, is when the two sexes start at a different place but evolve mechanisms to get to the same point. The most celebrated example of this was articulated by the neuroscientist Geert DeVries and refers to the parenting behavior of males. In most mammals, only females show parental behavior because they have experienced the hormonal milieu of pregnancy and lactation. In some species, however, males have evolved a novel neural circuit that induces them to behave in an identical manner towards their offspring as females, with the exception of lactation of course. Thus, in this instance, the two sexes converge to the same place.

The fourth category is called "population sex differences" and refers to the fact that some endpoints are the same in males and females but are more likely to occur in one sex versus the other. For instance, Alzheimer's disease is the same disease in men and women, but it occurs more frequently in women. A sex difference such as this might impact health policy or research priorities. In all cases, the existence of an effect of sex can be a valuable wedge tool for exploring why one sex is vulnerable and the other resilient.

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CHAPTER 3

Sex Determination versus Sex Differentiation

The production of a male versus a female is the product of two phases, sex determination and sex differentiation. In mammals, sex determination is a function of the sex chromosomes, XY for male and XX for female, a fact startlingly recent in its discovery in the 1950s. Even more startling is that it was not until the 1990s that the gene on the Y chromosome coding for male development was finally identified after an exhaustive search (Sinclair et al., 1990) – classic reference 1. Termed "Sry," for sex-determining region of the Y chromosome (Figure 4A), this single gene codes for a small transcription factor protein called testis-determining factor (tdf), which initiates a cascade of gene expression that directs the development of the bipotential gonad to become a testis, as opposed to an ovary, at the beginning of the sex differentiation process. One of the earliest steps in testis development is the regulation of steroidogenesis, with a downregulation of estrogen production and increased androgen synthesis. An additional hormone, anti-Mullerian hormone (AMH), produced by the immature testis compliments the actions of androgens so that the Wollfian duct system will survive and differentiate into the vas deferens and epididymis of the male, whereas in the female duct system, the Mullerian ducts, will degenerate in response to AMH. (Figure 4B) Androgens will further the process of differentiation by promoting the formation of a penis and scrotum to form male genitalia, as well as increased bone and muscle mass and later secondary sex characteristics, such as beard growth in men and racks of antlers in deer.

If there is no functional tdf, either due to the absence of Sry or a mutation that leads to a dysfunctional protein, the gonadal anlage will develop as an ovary, the Mullerian duct will survive and develop into the oviducts, uterus, and upper portion of the vagina, the Wollfian duct will degenerate due to lack of androgens and the genitalia form a clitoris, labia, and the remaining portion of the vagina. Thus, in both males and females, the gonadal, or somatic sex, is secondary to and dissociable from genetic sex. An XX individual with the Sry gene translocated onto an autosome will develop testis, and likewise, an XY individual with a mutated or deleted Sry gene will develop ovaries. So what about the brain?

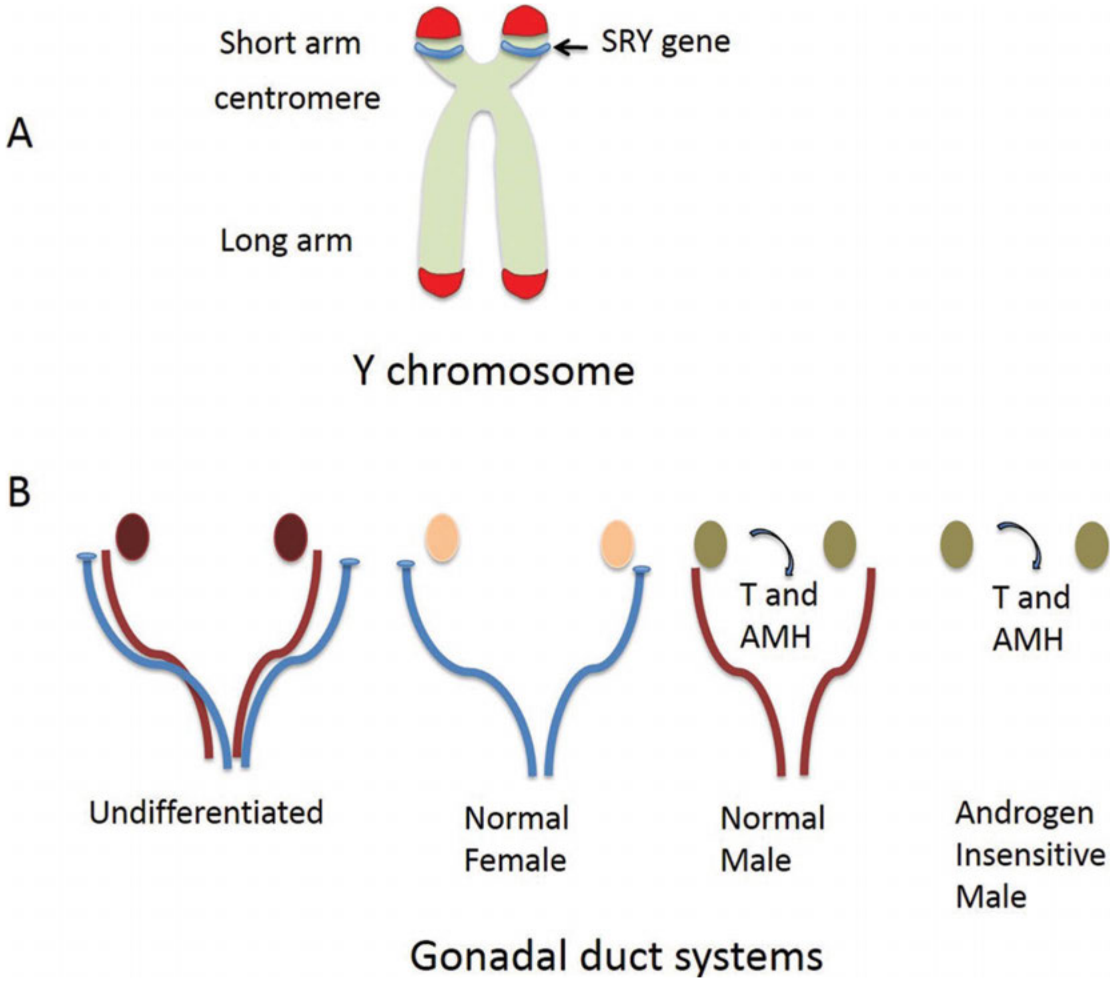

FIGURE 4: Sex determination. (**A**) Sex is determined by the Sry gene (for sex determining region of the Y chromosome) which directs the undifferentiated gonad to become a testis. (**B**) The reproductive ductal systems are present in both males and females initially, with the Wolffian duct system slated to become the epididymis and vas deferens of the male and the Mullerian duct system slated to become the oviduct, uterus and upper third of the vagina in females. The Wolffian duct is retained in males in response to androgens produced by the testis, and the Mullerian duct degenerates in response to AMH, also produced by the testis. In females, the Wolffian ducts degenerate due to the lack of androgen and the Mullerian ducts are retained due to lack of AMH. If a male has a dysfunctional androgen receptor, the Wolffian ducts degenerate but so do the Mullerian ducts because the testes still produce AMH.