Part I

Strategies, Methods and Background

Chapter 1

Why Are There Two Sexes?

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One of the most fascinating aspects of life on earth is the myriad of differences between males and females (Judson, 2002). Children and adults alike are captivated when they first learn that males, rather than females, gestate and give birth to offspring in certain species of seahorse. Role reversal is also observed in the red-necked phalarope, a shorebird in which polyandrous females are more brightly colored than their mates and males alone incubate eggs. People are likewise amazed when they hear that ambient temperature determines the sex of many reptiles. While such unusual phenomena capture our curiosity, there are also practical reasons for studying sex differences. For instance, defects in development of the reproductive tract and genitalia are fairly common in humans. Sex differences in physiology and disease affect virtually every organ system in the human body, including the nervous system. Depression, Alzheimer's disease, and schizophrenia are examples of afflictions that differ in incidence, onset, and/or symptoms between males and females. Understanding of the mechanisms underlying sexual differentiation of the body and mind should lead to novel therapies designed to prevent birth defects and cure devastating neurological diseases.

To fully comprehend sex differences in the brain and behavior in humans and to appreciate how animals can be used to model these differences, we need to examine sexual dimorphisms in an evolutionary context. The basic principle that guides biomedical research is that genetic, developmental, physiological, and behavioral mechanisms are conserved in species that have evolved from common ancestors. The unity of life is seen in our hereditary material: the universal genetic code, the enzymes that synthesize DNA, and the proteins that distribute chromosomes to daughter cells during mitosis and meiosis. This principle also permits significant advances in neuroscience. Hodgkin and Huxley, for example, used the giant axon of squid to elucidate action potentials (Clay, 2005). Our knowledge of the mechanisms underlying long-term potentiation and learning has been furthered by studies

in sea slugs (Kandel, 2004). Research on guinea pigs has been critical in formation of the concept of organization and activation of sexual behavior by gonadal steroids (Pheonix et al., 1959). Consequently, male seahorses giving birth, polyandrous female phalaropes, and reptiles with temperature-dependent sex determination may not be as esoteric as they seem if conserved genes and biological processes have been co-opted for different uses during evolution. Still, these examples highlight an emerging paradox in studies of sexual differentiation. Reproductive traits in general appear to be evolving more rapidly than other characteristics. Here we provide a three-part introduction to sex differences, stressing both the conserved and the unique as part of Darwin's notion of descent with modification (Darwin, 1859).

In the first section, we step back in time and provide a broad perspective on the evolution of eukaryotes. The evolution of meiosis and syngamy (i.e., the fusion of two cells) was a precondition for the evolution of dimorphic gametes and the subsequent evolution of all other sex differences. We then outline general causes of sex differences in animals by focusing on natural and sexual selection. In particular, we illustrate how sex-specific selection can favor different phenotypes in males and females. This pattern of divergent selection ultimately leads to changes in the neural mechanisms that regulate behavior in the two sexes.

In the second section, we explain the mechanisms that underlie sex differences in gene expression as well as the basic developmental mechanisms that produce sex differences. Despite abundant examples of differential selection on males versus females, there is an inherent constraint to the evolution of sex differences. To be precise, the same genes control homologous traits in both sexes. We describe how several mechanisms relieve this genetic constraint. For instance, genetic differences in the form of sex chromosomes and sex-linked genes have evolved independently in many eukaryotic lineages. Another major mechanism is sex-limited (or differential) expression of autosomal loci, as exemplified by hormonal regulation of gene expression. Environmental factors can also have a large impact on the development of sex differences, a phenomenon commonly referred to as phenotypic plasticity.

Finally, we review some elegant research that links evolutionary causes of and proximate mechanisms for sex differences in the brain and behavior. These examples show how sex-specific selection on behavior ultimately drives neural evolution. We bring the chapter to a close by briefly outlining what is known about sexual differentiation of neural mechanisms in humans. These mechanisms are undoubtedly related to sex differences in aggressive and sexual behavior and emotional memory, as well as the incidence of affective disorders, anxiety disorders, schizophrenia, and post-traumatic stress disorder (PTSD).

THE EVOLUTION OF EUKARYOTES, MEIOSIS, AND TWO SEXES

Advances in molecular and cellular biology, along with comparative genomics, are allowing reconstruction of the earliest stages in the evolution of life on earth. The first organisms lacked a membrane-bound nucleus, replicated by binary fission, and are survived by today's prokaryotes. Two groups of extant prokaryotes, the eubacteria and the archaebacteria, appear to be as distinct from one another as they are from eukaryotes (Brown & Doolittle, 1997; Bell & Jackson, 2001; Forterre, 2001; Makarova & Koonin, 2003; Robinson & Bell, 2005). This finding makes it difficult to codify the prokaryote-eukaryote transition (Martin, 2005). Yet, research is beginning to elucidate how the first nucleated cells originated and diversified. Some of the most important events in the evolution of eukaryotes involved symbioses (mutually beneficial associations of different species). For instance, the endosymbiotic theory for the origin of mitochondria is well established, even if the timing is in dispute (Embley & Martin, 2006).

One hypothesis has it that the first eukaryotes lacked endosymbionts (currently represented by diplomonads, parabasalids, and microsporidia) and that endosymbionts were acquired in a separate lineage that gave rise to eukaryotes with mitochondria. An alternative hypothesis suggests that endosymbiotic bacteria were acquired concurrent (or nearly so) with the origin of eukaryotes and that these organisms evolved into mitochondria as well as the more derived organelles called hydrogenosomes and mitosomes in eukaryotes that lack prototypical mitochondria (Embley & Martin, 2006). In either case, this ancient event has direct implications for human health because mutations in mitochondrial DNA, which is maternally inherited, cause a number of diseases (Chen & Butow, 2005; Dimauro & Davidzon, 2005). Mitochondria also play a central role in apoptosis,

a form of cell death that contributes to normal development and to diverse pathological states (Schafer & Kornbluth, 2006; Garrido et al., 2006). It is especially interesting that vertebrates evolved the capacity for a novel class of molecules (i.e., estrogens and androgens) to influence mitochondia-dependent apoptosis in the nervous system (Nilsen & Brinton, 2004; Forger, 2006; Lin et al., 2006).

There are several hypotheses for the origin of the membrane-bound nucleus (Martin, 2005), but two basic categories can be distinguished. The first group of hypotheses suggests direct evolution of this unique structure in the initial forms of life (Woese, 1998), while the second posits a symbiotic origin for the nucleus (Dolan et al., 2002). Whether the nucleus evolved *de novo* or from an archaebacterial-eubacterial symbiont, it is clear that microtubules played a central role in the evolution of eukaryotes. Microtubules are essential for mitosis and are a key component of the cytoskeleton. Moreover, the first split within the eukaryotic lineage involves a basic difference in the assembly of microtubules (Stechmann & Cavelier-Smith, 2003; Richards & Cavelier-Smith, 2005). While animals, fungi, Choanozoa, and Amoebozoa (unikonts) have a single microtubule-organizing center, plants, chromists, and all other protozoa (bikonts) have two microtubule-organizing centers.

In animals, the microtubule-organizing center or centrosome is composed of two centrioles located near the nucleus. Each centriole replicates during interphase to produce two pair of centrioles. In prophase of mitosis, paired centrioles are pushed apart by microtubule polymerization. Microtubules spanning pole-to-pole (i.e., centriole-to-centriole) form the backbone of the mitotic spindle. Another set of microtubules attaches one pole to one side of the centromere of sister chromatids. An opposing set of microtubules links the other side of the centromere to the other pole. Depolymerization of these microtubules during anaphase pulls the sister chromatids to opposite ends of the cell, which then divides to complete mitosis. In plants, spindle fibers form between two microtubule-organizing centers already located on opposite ends of the cell. Otherwise, mitosis is essentially the same in unikonts and bikonts.

Given the basic role that microtubules play in mitosis, it is amazing that mutations in a few genes that interact with microtubules have a highly-specific effect on the size of the mammalian brain (Bond & Woods, 2006). Products of these genes are localized to the centrosome in periventricular cells and are hypothesized to regulate formation and orientation of the mitotic spindle. Proliferation of neural progenitors occurs when spindle fibers run parallel to the ventricular epithelium. In contrast, neurogenesis generally occurs when spindle fibers are perpendicular to the ventricular epithelium. Exactly how orientation of the mitotic spindle relates to commitment to a neuronal fate is unknown, but it is possible that the post-mitotic location of the centrosome (i.e., cell asymmetry and microtubule polarity) is vital, like it is to development of neuronal polarity (de Anda et al., 2005). Again, we see how an ancient event in the evolution of eukaryotes has implications for neural development.

While mitochondria and mitosis are important to human health, the adaptations most salient to our discussion of sex differences are meiosis and syngamy. Three simple molecular changes account for the transition from mitosis to meiosis. The first change was in alignment and crossing over between homologous chromosomes. This process of genetic recombination utilized pre-existing mechanisms for DNA repair found in prokaryotes (Santucci-Darmanin & Paquis-Flucklinger, 2003), further illustrating Darwin's concept of descent with modification. Another change was in attachment of microtubules to sister chromatids. Two kinetochores, which link microtubules to the centromere, are in a bipolar orientation in mitotic cells. The end result of this geometric arrangement is that sister chromatids are attached and pulled to opposite poles. In contrast, kinetochores on sister chromatids are oriented in the same direction during meiosis I (Hauf & Watanabe, 2004). Special proteins also serve to hold sister chromatids together during meiosis I (Kitajima et al., 2004). The natural consequence of unipolar kinetochore geometry, sister chromatid cohesion, and synapsis is that sister chromatids are pulled to the same pole and that homologous chromosomes are pulled to opposite poles. Finally, meiosis II, which is virtually identical to mitosis, completes reduction division. Discussion of the evolution of syngamy is beyond the scope of this chapter (see Cavelier-Smith, 2002), but suffice it to say that alternation between diploid and haploid stages in the life cycle of eukaryotes opened the door for selection to produce sex differences.

The first characteristic that we might broadly consider a sex difference is mating type. Nearly all lower eukaryotes have mating-type loci that prevent syngamy between cells with the same genotype (Charlesworth,

1994; Souza et al., 2003). Yet, most eukaryotic lineages display no other sign of sexual dimorphism (i.e., fungi, Choanozoa, Amoebozoa, chromists and protozoa). The cells that fuse during syngamy in these groups are of the same size, indicating isogamy was the ancestral state in eukaryotes. Since anisogamy (i.e., dimorphic gametes) and more derived sex differences are only found in one lineage on either side of the unikont-bikont split, sexual dimorphism, it is suggested, evolved independently in animals and plants. Until that point, natural selection was the main force driving biological evolution.

Sexual selection only became relevant with the evolution of dimorphic gametes (Levitan, 1996; Levitan & Ferrell, 2006). The key to understanding the evolution of sex differences therefore lies in the fact that each zygote gets half its genome from its father and half from its mother. This means that an individual's reproductive success through male function (i.e., sperm) must be measured relative to the male function of other individuals. The converse applies to fitness through female function (i.e., eggs). Accordingly, traits that benefit one sex can have harmful effects when expressed in the other sex. This pattern of sex-specific selection favors different phenotypes in males and females and the evolution of sexual dimorphism. Elegant experimental work by William Rice (1992) demonstrated that genes with sexually antagonistic effects on male versus female fitness are abundant in fruit flies.

Another important concept is sexual conflict, which occurs when male and female reproductive interests do not coincide. In other words, traits that increase the fitness of the sex expressing the trait can decrease a mate's fitness (Rice, 1996a; Chapman et al., 2003). Male fruit flies, for instance, produce seminal chemicals that induce females to lay more eggs and decrease the likelihood that females will mate again (Wolfner, 1997). These chemicals increase the fitness of polygynous males, but simultaneously decrease the fitness of females by shortening their lifespan (Wigby & Chapman, 2005). Another example of sexual conflict occurs in water striders, a species in which males and females struggle over mating (Rowe et al., 1994; Preziosi & Fairbairn, 2000; Rowe & Arnquist, 2002). Males can prevent their mates from re-mating with other males by clinging to females' backs after copulation. This behavior, while ensuring that a male fertilizes all of his mate's eggs, has a significant energetic cost for females that carry males for a few minutes up to several weeks (Watson et al., 1998). It is not surprising then that males and females physically struggle with each other to control the frequency and duration of mating.

MECHANISMS UNDERLYING SEX DIFFERENCES

Sexual selection occurs in two basic ways: *intrasexual* and *intersexual*. Intrasexual selection results from direct competition for mates or mating opportunities within a sex. For instance, female shore birds, like red-necked phalaropes, spotted sandpipers, and jacanas compete with each other for paternal males (Schamel et al., 2004a,b). Females in these species are physiologically capable of producing two (or more) clutches of eggs in a breeding season, while males can only incubate and care for one clutch. Females able to monopolize two (or more) males therefore have higher fitness than females that are only able to mate with one male or who aren't fortunate enough to mate at all (Andersson, 2005).

Intersexual selection occurs when interactions between the sexes influence reproductive success. A classic example is female mate choice that is based on male characteristics, i.e., the peacock's tail. Conversely, the bright plumage of female phalaropes and the facial ornamentation of female Wattled Jacanas may be a result of male preferences for these traits (Emlen & Wrege, 2004). Exaggerated traits, be they behavioral or morphological, provide a mating advantage in one sex, but are costly to display for both sexes. Asymmetric benefits and costs once more favor the development of sex differences. Yet, there is an inherent constraint to the evolution of such differences because the same genes control homologous traits in the initially monomorphic sexes. How then do males and females develop different phenotypes?

One way is through the evolution of chromosomes passed exclusively from father to son or from mother to daughter, as in mammals (XY males, XX females) and birds (ZZ males, ZW females). Empirical and theoretical studies support the following model for the evolution of sex chromosomes and sex-linked inheritance. A new sex-determining locus initially evolves on an autosome: i.e., a locus with a dominant allele M for maleness, and a recessive allele m for femaleness. There are two possible genotypes with this sex-determining system: Mm individuals develop as males, while mm individuals develop as females. By chance,

genes with antagonistic effects on male versus female fitness may reside on the same chromosome as the novel sex-determining gene. Selection then favors tighter linkage between alleles that benefit males and the male determining allele M. Selection also favors linkage between alleles that benefit females and the female allele m. Recombination between nascent X and Y chromosomes is suppressed, which in turn leads to progressive deterioration of the Y chromosome (Rice 1996b; Lahn & Page 1999). An analogous scenario applies to the evolution of W and Z chromosomes.

Sex chromosomes have evolved independently in diverse groups of animals and are even found in some plants (Bull, 1983; Tanurdzic & Banks, 2004). Nevertheless, the importance of sex linkage as a mechanism for phenotypic differentiation between the sexes varies among groups. For example, just 0.15% of all genes (or 45/30,000) are Y-linked in humans. Roughly 4.5% of all genes (or 1,344/30,000) are X-linked in humans. A much higher percentage of genes are found on the X chromosome in fruit flies (~16% or 2,309/14,449), though the Y chromosome carries proportionately fewer genes (0.06% or 9/14,449) (Carvalho et al., 2001). The difference in gene content between the Z and W chromosomes is lower in chickens: 1.4% of all genes are Z-linked (328/23,000), while 0.2% are W-linked (47/23,000). The degree of sex chromosome differentiation even varies within groups: zebrafish have autosomes, platyfish have genotypic sex determination without any distinction between sex chromosomes, and guppies have morphologically distinct X and Y chromosomes (Traut & Winking, 2001). The potential for sex-linked genes to play a direct role in differentiation of the brain has been under appreciated until recently (Arnold, 2004).

The majority of genes, however, do not reside on sex chromosomes. Moreover, many organisms do not have sex chromosomes at all, but still have dimorphic males and females. How do the sexes come to differ in these species? To answer this question, we need to understand what happens when selection favors different autosomal alleles in males versus females (Rhen, 2000). Imagine, for instance, a gene that induces development of a trait that is favored in females, but disfavored in males. A constitutively expressed allele would be advantageous in females while a null allele would benefit males. Neither sex is able to reach its phenotypic optimum with this type of genetic variation. A simple solution to this dilemma is the evolution of a third allele that is only expressed in females. While sexually antagonistic selection causes the rapid fixation of such sex-limited mutations, other patterns of sex-specific selection can also increase sexual dimorphism (Rhen, 2000).

At least two distinct mechanisms produce differential expression of autosomal loci in males and females. The first involves interactions between sex-linked and autosomal loci (Noonan & Hoffman, 1994; Kreutz et al., 1996; Montagutelli et al., 1996; Paallysaho et al., 2003; Perry et al., 2003; Chase et al., 2005), while the second involves sex steroids (Hughes, 2001; MacLaughlin & Donahoe, 2004, this volume). The first mechanism is not widely recognized, but the latter is well known. In fact, sex steroids, which act independently of sex chromosomes, are the major mechanism regulating the development of sex differences in vertebrates. Despite diversity in the initial trigger for sex determination among amniotic vertebrates, the basic morphogenetic process of gonadal differentiation is conserved. The gonadal anlagen are initially bipotential, consist of a cortical region that gives rise to the ovary, and a medullary region that gives rise to the testis. Moreover, the key somatic cell types in the ovary (granulosa and theca cells) and the testis (sertoli and leydig cells) are conserved, as are the steroids these cells produce: estrogens, progestins, and androgens.

The evolution of this mode of sexual differentiation depended upon the appearance of a receptor that recognized and bound steroidal molecules (Thornton, 2001). Indeed, phylogenetic analyses indicate that the first steroid hormone receptor evolved before the protosome-deuterostome split 600–1000 mya. The ancestral receptor had estrogen receptor-like properties and gave rise to all of the steroid hormone receptors that exist today (Thornton et al., 2003). The putative estrogen receptor co-opted as its ligand the estrogen-like molecules associated with oocyte maturation. This event was significant because estrogen is the terminal hormone in the steroidogenic pathway, thereby making the intermediate hormones, progesterone and androgen, potential ligands. After the first of two genome-wide duplications, one of the duplicated estrogen-receptor genes evolved into a progesterone receptor, which like estrogen, was linked to the ovarian cycle, and in particular ovulation, oviposition, and birth. The second genome-wide duplication occurred after separation of the lamprey lineage from other vertebrates. This event was followed by evolution of the androgen receptor, laying the groundwork for androgen-mediated sex differences. In general,

steroids enter cells, bind to cognate receptors, and induce or suppress transcription of target genes (Rhen & Cidlowski, 2004). Research during the last decade has shown that sex steroids also have non-genomic effects that are mediated by second messenger pathways (Rhen & Cidlowski, 2004). Yet, the importance of nongenomic mechanisms of steroid action for sex differences in the brain is currently unclear.

So far we have only discussed the evolution of the intrinsic genetic and hormonal factors responsible for sex differences. The two sexes, however, do not develop in a vacuum. Many environmental factors, including embryonic, ecologic, and social surroundings, are known to influence sexual differentiation. The pivotal role of the environment in development was recognized at the turn of the twentieth century by Hertwig and Woltereck, whose work on *Daphnia*, an organism that reproduces asexually to produce clones of itself, demonstrated that genetically identical individuals would develop very different phenotypes depending upon their environment (Gilbert, 2002); a human counterpart has recently been described in monozygotic twin studies (e.g., Chakravarti & Little, 2003; Fraga et al., 2005). The general phenomenon in which a single genotype (i.e., individual) can produce more than one phenotype in response to specific environmental stimuli is referred to as phenotypic plasticity (Lewontin, 2000). It is also important that individuals with different genotypes often have different responses to the same environmental stimuli. This means that phenotypic plasticity itself has a genetic basis and can evolve adaptively (Pigliucci, 2005; Gluckman et al., 2005; Fordyce, 2006). Genotype-environment interactions of this sort include the processes underlying neural and behavioral development and learning (Duchaine et al., 2001; Dopazo et al., 2003; Egnor & Hauser, 2004).

Phenotypic plasticity has two important implications for our understanding of sex differences. First, males and females may differ in their level of plasticity (Jonasson, 2004; Cahill, 2006; Sherry, 2006). Second, sex differences may be shaped or caused by experiential differences (McCarthy & Konkle, 2005). It is frequently the same genetic and hormonal factors that we have already introduced that mediate environmental effects on phenotype. For instance, exposure to exogenous (i.e., maternally derived) hormones or xenobiotics (i.e., man-made chemicals) early in life can alter responses to hormones later in life (Crews & McLachlan, 2006). Other factors such as stress and drugs in action during embryogenesis can shape the subsequent behavioral phenotype of the individual, and modify the way the individual responds to adult experiences. The clinical significance of this work resides squarely within the concept identified as the "fetal basis of adult disease." For example, malnutrition in a mother during early pregnancy increases the risk of schizophrenia in the child once the child reaches adulthood (Barker, 2003; Barker et al., 2002; Bateson et al., 2004; Gluckman & Hanson, 2005). These disorders are often precipitated by stress, which alters the endocrine state. Some women who experienced the collapse of the World Trade Center while pregnant developed PTSD. These women and their babies have lower cortisol levels than unaffected mothers and their babies (Yehuda et al., 2005).

Building on a long history of research in developmental psychobiology, Meaney and colleagues (2001; Weaver et al., 2004) have demonstrated that the nature and amount of care a rat pup receives from its mother modulates its reaction to stress later in life through effects on the glucocorticoid receptor (GR) in the hippocampus. This maternal effect can cross generations, but critically depends on the pup's experience in the first week of life. Recently, it was documented by this group that rearing by a high-quality mother results in the expression of the transcription factor NGFI-A, a nerve growth factor-inducible protein, that binds to the first exon of the GR gene, resulting in increased expression of GR. High-quality maternal care during this critical period results in demethylation of the NGFI-A binding site in the GR promoter and increases the acetylation of histones at the promoter. Just as cross fostering pups can reverse these molecular changes, infusion of histone deacetylase inhibitor into the hippocampus can reverse these events. Is there a counterpart in humans? Caspi and colleagues (2002, 2003) have demonstrated how the rearing environment can overcome the influence of genotype in the etiology of violent behavior. It is important to note, however, that this form of epigenetic transmission is not transgenerational, but rather induced in each generation by the parent or the environment.

EXAMPLES OF SEX DIFFERENCES IN THE BRAIN AND BEHAVIOR

Males and females behave differently, and from an evolutionary point of view, this dimorphism results

from the influence of behavior on the fitness of the two sexes. From a mechanistic point of view, this leaves us with two questions: What exactly is different about male and female brains? and How might sex differences evolve through the mechanisms just outlined?

Enormous progress has been made in answering the first question. We now understand that the same steroid and peptide hormones involved in regulating gamete production, pregnancy (gravidity), birth (oviposition), and parental care, if it occurs, are powerful determinants of brain function. These hormones direct the development of sexually dimorphic brain structures and influence reproductive as well as nonreproductive behaviors (Jonasson, 2004; Cahill, 2006). Although less progress has been made on the second question, two success stories involve closely related sexual and unisexual whiptail lizards and monogamous and polygamous voles.

Whiptail lizards (genus *Cnemidophorus*) exhibit an extremely simple pattern of sexually dimorphic behavior (Crews, 2005). Around the time of ovulation, females allow males to mount them in a fashion characteristic of the genus. Outside of this period, there is essentially no interaction between the sexes; no parental behavior, minimal courtship, no territoriality, and as far as is known, very little social behavior. Perhaps the most significant aspect of whiptail lizards is that a number of species consist only of females that reproduce by obligate parthenogenesis. Further, we know that parthenogenetic species arose through hybrid unions of sexual species. For example, the desert-grasslands whiptail (*C. uniparens*, trans. one parent) arose through an initial hybridization between two sexually reproducing species, the rusty rumped whiptail (*C. burti*) and the little striped whiptail (*C. inornatus*, trans. without ornament, referring to this species' lack of spots), and a subsequent backcross of the hybrid with *C. inornatus*. Hence, the relationship among these species is perhaps best viewed as a snapshot of evolution as representatives of ancestral and the descendant species still exist.

Equally remarkable is that each parthenogen displays both male-like and female-like copulatory behavior during the reproductive cycle: since these animals are all female and lack intromittent organs, this behavior has been termed *pseudocopulation* (Crews & Fitzgerald, 1980). Thus, unlike the ancestral species in which mating behaviors are sexually dimorphic, with males mounting females who are receptive to this behavior, *C. uniparens* display both male- and female-typical sexual behaviors in alternating fashion, according to ovarian state. The ovarian cycle is characterized by circulating concentrations of estradiol, gradually increasing during follicular development, and then declining sharply following ovulation; whereas, progesterone titer is low during follicular development and increases dramatically around the time of ovulation.

Androgens are undetectable throughout the cycle in female *C. inornatus* and in *C. uniparens*. Female-like receptive behavior is limited to the preovulatory phase of the cycle whereas male-like mounting behavior is displayed most frequently following ovulation. Thus, the behavioral transition occurs at ovulation when there is a parallel transition from estradiol dominance to progesterone dominance, suggesting that changes in hormone levels could underlie changes in behavior.

Clonal reproduction and the retention of sexual behavior allows the investigator to circumvent major confounds in the study of sexual dimorphisms, namely that males and females differ in several ways, and hence sex differences may be due to genotypic differences, hormonal background, or even experiences particular to each sex. In addition to each parthenogen displaying mounting and receptive behaviors, it is possible to create 'males' to compare with the males of the ancestral sexual species. That is, by treating eggs with an aromatase inhibitor one can induce development of *Virago* males (meaning "a man-like woman"). Virago males are genetically identical to parthenogens yet they have fully developed male genitalia, motile sperm, and only display male-like mounting behaviors. Taken together, these whiptail lizards enable study of the neural substrates underlying sex-typical behaviors from an evolutionary standpoint (comparing the ancestral and descendant species).

Species and sex differences are found in hormonal regulation of steroid receptors in the brain. Females, but not males, of the sexual species respond to exogenous estrogen by increasing progesterone receptor (PR) mRNA in the ventromedial hypothalamus (VMH). Males have higher androgenic receptor (AR) mRNA in the medial preoptic area (POA) than do females of the sexual species or the descendant parthenogens. Androgen treatment also increases the expression of PR mRNA in the periventricular preoptic area (PvPOA) in both males and females of the sexual species as well as in the descendant parthenogens. Exogenous estradiol increases PR mRNA expression

in the PvPOA of the parthenogen, but not in females of the sexual species. This last finding suggests a possible proximate mechanism underlying species differences in behavior. The POA is a conserved brain area involved in the control of mounting behavior and is normally sensitive to androgen. In the parthenogenetic species, the preovulatory surge in estrogen upregulates PR mRNA in this brain region, enabling the postovulatory progesterone surge to activate pseudocopulatory behavior. In contrast, estradiol does not upregulate PR in the PvPOA during the preovulatory phase in females of the sexual species, and these females do not display male-typical mounting behavior in response to the surge of progesterone following ovulation. Finally, despite their male-like morphology and behavior, Virago *C. uniparens* are female-like in characteristics that are sexually dimorphic in *C. inornatus*. Forexample, in Virago males the volume of both the POA and VMH are female-typical, they display estrogen-induced upregulation of PR in the POA, and testosterone regulation of arginine vasotocin (AVT) expression, which is independent of neuroendocrine history or genetic sex (Hillsman et al., 2007).

Insight into the evolution of more complex social behavior comes from comparative studies of prairie voles, which are monogamous, and in montane voles, which are polygamous (Carter et al., 1995; Young et al., 2005; Nair & Young, 2006; Young & Carter, this volume). In the polygamous species, males and females are solitary, except during mating, and only females care for offspring. In contrast, males and females in the monogamous species display long-term social bonds (regardless of reproductive status), biparental care of offspring, and aggression toward unfamiliar con-specifics. Formation of pair bonds that endure beyond mating in monogamous prairie voles depends on oxytocin signaling in females and arginine vasopressin (AVP) signaling in males (Young & Wang, 2004). In fact, central administration of oxytocin to females and AVP to males enhances formation of a pair bond even if the duo is not allowed to mate. Conversely, antagonists for the oxytocin receptor and the AVP receptor 1a block social attachment in mated female and male prairie voles. A nucleus-specific difference in expression of AVP receptor 1a between prairie and montane voles is responsible for the difference in social behavior in these closely-related species (Lim et al., 2004a). In particular, AVP receptor 1a is expressed at a higher level in the ventral pallidum of the prairie vole than in the montane vole. Transgenic overexpression of the AVP receptor 1a in the ventral pallidum of male montane voles results in attachment of males to their mate. An analogous experiment examining the role of oxytocin in the evolution of social attachment in females has yet to be conducted, but there are differences in oxytocin receptor expression between prairie and montane voles (i.e., higher expression in the nucleus accumbens in the monogamous species). A working model for pair bonding has olfactory cues from a sexual partner activating oxytocin and AVP pathways in females and males, respectively. In turn, these pathways converge on a common dopaminergic reward pathway that is activated during copulation in both sexes, which results in a conditioned preference for the sexual partner (Young & Wang, 2004).

Although there are no sex differences in AVP receptor 1a expression in the prairie vole, males have more AVP positive cells in the bed nucleus of the stria terminalis and the medial amygdala as well as denser AVP projections to nuclei involved in social behavior (Bamshad et al., 1993; Laszlo et al., 1993; Lim et al., 2004b). It is particularly intriguing that male-biased expression of AVP (or its non-mammalian homologue arginine vasotocin AVT) appears to be conserved among vertebrates, even though the mechanism underlying this sex difference varies (De Vries & Panzica, 2006). For example, although testosterone induces AVP/AVT expression in adult male rats and Japanese quail, hormonal organization of this male-typical response is different. Testosterone via aromatization to estrogen during early development masculinizes the AVP system in rats. Conversely, early exposure to estrogen feminizes the AVP system in Japanese quail. There is evidence that sex-linked genes contribute to sex differences in AVP expression in mice (De Vries et al., 2002; Arnold, 2004; Gatewood, et al., 2006), but not in whiptail lizards (see previous).

Humans appear to be different from many other vertebrates in not having a gross sex difference in the AVP system (Fliers et al., 1986). Nevertheless, administration of physiologically relevant levels of AVP has sex-specific effects on social perception of and autonomic responses to other humans (Thompson et al., 2006). Men treated with AVP and allowed to view pictures of men with affiliative facial expressions respond with agonistic facial activity and lower ratings of the friendliness of those faces. Women treated with AVP have just the opposite response to pictures of women with affiliative facial expressions.

The conserved function of AVP/AVT as a modulator of social behavior, in conjunction with changes in the regulation of AVP expression in the brain underscores the notion of descent with modification. This general concept is also evident in the function of certain brain nuclei: the amygdala, for instance, plays a key role in behavioral sex differences in humans and other animals (Hamann, 2005; Cahill, 2006). This particular brain region is involved in regulating social behaviors that have an emotional component, including fear, aggression, and sexual motivation, but the socially relevant input varies (i.e., pheromones in rodents, visual stimuli in humans).

There are many other sex differences in brain structure, gene expression, neurochemistry, reproductive behavior, and nonreproductive behavior in humans (Nopoulos et al., 2000; Hamann, 2005; Rinn & Snyder, 2005; Cahill, 2006; reviewed in this volume). While we are unique in many ways, especially with respect to our brain and behavior, we cannot hope to understand why we have these characteristics without understanding our ecological and evolutionary history (Joseph, 2000; Panter-Brick, 2002; Sherry 2004). Our goal in this chapter was to provide a conceptual overview of the ultimate (natural and sexual selection) and proximate (sex chromosomes, sex steroids, and phenotypic plasticity) causes of sex differences and to illustrate how animals can be used to help us understand these differences in humans.

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