Sex Differences in the Brain

From Genes to Behavior




edited by Jill B. Becker
Karen J. Berkley
Nori Geary
Elizabeth Hampson
James P. Herman
Elizabeth A. Young

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OXFORD
UNIVERSITY PRESS

2008

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Oxford University Press, Inc., publishes works that further
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Oxford New York
Auckland Cape Town Dar es Salaam Hong Kong Karachi
Kuala Lumpur Madrid Melbourne Mexico City Nairobi
New Delhi Shanghai Taipei Toronto

With offices in
Argentina Austria Brazil Chile Czech Republic France Greece
Guatemala Hungary Italy Japan Poland Portugal Singapore
South Korea Switzerland Thailand Turkey Ukraine Vietnam

Published by Oxford University Press, Inc.
198 Madison Avenue, New York, New York 10016
www.oup.com

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Library of Congress Cataloging-in-Publication Data
Sex differences in the brain : from genes to behavior /
edited by Jill Becker . . . [et al.].
p. cm. Includes bibliographical references.
ISBN 978-0-19-531158-7
1. Sex differences.
2. Sex differences (Psychology)
3. Brain—Sex differences.
4. Human behavior—Physiological aspects.
I. Becker, Jill B.
[DNLM: 1. Brain—physiology.
2. Behavior—physiology.
3. Central Nervous System Diseases—physiopathology.
4. Mental Disorders—physiopathology.
5. Mental Processes—physiology.
6. Sex Factors. WL 300 S5185 2008]
QP81.5.S484 2008 612.8'2—dc22 2007021143

1 3 5 7 9 8 6 4 2

Printed in the United States of America
on acid-free paper

This book is dedicated to Florence P. Haseltine, Ph.D., M.D., founder of the Society for Women's Health Research. Her unstoppable energy and commitment in support of sex differences research is inspirational to us all.
Florence—thank you for leading the way.

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Foreword

MENTAL DISORDERS ARE BRAIN DISORDERS: WHY SEX MATTERS

There seems to be no end to the debate over sex differences in the brain. When people finally agree that differences exist, there is an even more intense debate over what these differences mean. Do more neurons mean more computing power? Do more connections mean more communication between neurons? Do structural differences correlate with functional differences?

In fact, there are clear, reproducible mean differences in many neuroanatomical variables when groups of male and female brains are compared. But understanding these differences runs directly into a central quandary in neuroscience: How do we link form and function? We are now able to define form at the molecular level by identifying individual cells by their RNA transcripts. In addition, we are able to detect function in individual neurons by measuring physiological signatures of identified cells. Similarly, we have been able to image physiological changes in brain systems associated with behavior and cognition. However, we have not been able to build the bridge from individual cells to brain systems in a way that allows a seamless understanding that spans from molecules to behavior.

This is one of the ways in which the study of sex differences can make a difference: by understanding how chromosomal sex confers genomic differences, how gonadal hormones and their transcription factor receptors lead to developmental changes in brain systems, and how systems in the brain become associated with differences in cognition and behavior. The study of sex differences is a unique opportunity to elucidate the entire trajectory from genes to behavior, or, as more frequently stated in the clinical realm, from genotype to phenotype.

Why is this important? Aside from answering fundamental questions for neuroscience, the study of sex differences is important for public health. According to the World Health Organization, mental

disorders are the leading source of disability in Americans between the ages of 15 and 44. We now understand mental disorders as brain disorders, but we do not understand how brain circuits become abnormal. Part of finding this answer will reside in being able to identify the risk factors for disease and, more importantly, defining the mechanisms by which these factors confer risk.

Among the various risk factors for mental disorders, gender is preeminent. Relative to males, females are at least three times as likely to have anorexia nervosa, twice as likely to have depression, and one fourth as likely to have autism. For schizophrenia and obsessive-compulsive disorder, with roughly equivalent prevalence in males and females, the onset is earlier in males. Moreover, there are gender differences in the clinical features: females with major depressive disorder are more likely to express sadness whereas males present with irritability.

We do not understand the mechanisms for any of these gender differences, but patterns of gonadal hormone action are major candidates. We know that many mental disorders emerge with hormonal transitions at puberty, parturition, and menopause. We also know that the brain is a target organ for gonadal hormones. As we define the mechanisms by which these hormones alter brain function at the molecular, cellular, and systems levels, we should begin to define how gender and hormonal transitions increase risk for mental disorders.

This book results from the visionary leadership of the Society for Women's Health Research and specifically the staff who have sponsored the Isis Fund Network on Sex, Gender, Drugs, and the Brain. By exploring a range of sex differences from genes to behavior, the chapters herein review the latest insights into how sex and gender matter. The findings promise to alter our approach to mental disorders, leading initially to a better understanding of pathophysiology and ultimately to better treatments. Of course sex differences exist, but what really matters for public health is how these differences lead to vulnerability for some individuals and resilience for others.

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Preface

Differences in the brain between males and females have been observed in behavioral traits, in the anatomy of the brain, and in the physiological responses of the nervous system to outside stimuli and internal perturbation. The brain is sensitive to the effects of gonadal hormones, beginning in fetal development and continuing throughout the lifespan, and there is mounting evidence that some sex differences may result from differences in gene expression that are independent of the effect of gonadal hormones. In humans, these differences are reflected in the differential impact of neurological and mental illness on men and women, including conditions as diverse as multiple sclerosis, major depression, dementia, and chronic pain disorders. This book brings together an international group of experts on sex differences in the brain, writing about critical methodological issues in sex differences research as well as the most recent developments in this rapidly moving field. It is the culmination of the work of many individuals, and has its origins in a meeting at the Cosmos Club in Washington, DC, in 1990.

At that meeting, a group of researchers, clinicians, and activists began work that led to the founding of the Society for Women's Health Research (SWHR) to "advance the health of women through research." This group identified the paucity of women participants in medical research studies as a major barrier to such advancement. By 1993, SWHR had brought about changes in grant guidelines at the US National Institutes of Health, and in guidelines for new drug applications at the US Food and Drug Administration. Researchers are now required to include women in research studies unless there is an adequate scientific reason for doing a study in a single sex. By 1995, scientists on SWHR's Board of Directors had a clear vision of the outcome of the inclusion of women (and female animals) as research subjects: the discovery and elucidation of biological sex differences that have a significant impact on health and disease. The Society turned that vision into a proposal for a study by

the Institute of Medicine (IOM) that would address the questions, "Does sex matter?" "When does sex matter?" "How does sex matter?" Once the IOM accepted the report proposal, Society staff raised more than $650,000 in public and private funds to cover the costs of producing a report.

The Institute of Medicine (IOM) published this landmark report in 2001. The book was a product of the IOM Committee on Understanding Sex and Gender Differences, entitled *Exploring the Biological Contributions to Human Health: Does Sex Matter?* (Wizemann & Pardue, 2001) The Committee concluded that sex is a significant and often ignored biological variable, and that understanding sex differences is crucial for improving human health. They found that much of what was known about sex differences came from descriptive findings, and that hypothesis-driven research to study the mechanisms and origins of sex differences is now needed. They identified several barriers to progress in research on sex differences, including the need for more accurate use of the terms "sex" and "gender," and the need for better tools and resources for the study and analysis of sex differences.

Another barrier identified by the IOM committee was the inherently interdisciplinary nature of research on sex differences, the lack of funding for this type of research, and the lack of funding for collaborative opportunities for sex differences research. The report noted that progress in sex-based biology would require "synergy . . . between and among basic scientists, epidemiologists, social scientists, and clinical researchers." In addition, integration of findings at different levels of biological organization (genes, cells, tissues, organs, whole animals) and better "bench-to-bedside" translational research is needed.

In the six years that it took to raise the funding for and produce the IOM report, SWHR developed and launched a strategic plan for developing interest and capacity in sex differences research among basic and clinical scientists. In addition to the traditional role of SWHR as an advocacy group working with the US Congress and federal agencies, SWHR worked to expand its direct outreach to the scientific community. The Society identified two ways in which it could work to encourage research on sex differences: by providing a venue for researchers to present and discuss their work in this area, and by providing financial support for research.

From 2000–2005 SWHR produced the annual Conference on Sex and Gene Expression (SAGE), a small interdisciplinary meeting that explored all aspects of biological sex differences. The SAGE Conferences brought together researchers working at all levels of biological organization, in animal models from *C. elegans* to primates, and in various physiological systems and clinical disciplines. The SAGE Conferences were designed to allow ample time for informal discussion among the participants, and surveys of attendees found that a significant number of new collaborations and new lines of research were begun at these meetings.

In 1998 SWHR established the Isis Fund for Sex Differences Research, named for the Egyptian Goddess who was the founder of the art of medicine. The Society consulted with staff from the MacArthur Foundation, which had a program of highly successful interdisciplinary research networks to address issues in mental health. Using the MacArthur Networks as a model, funded by unrestricted grant of $1 million over four years from Ortho-McNeil Pharmaceuticals, Society staff assembled a core group of five scientists and posed to them the question "How are sex and gender differences important in the development and testing of neuropharmaceuticals?" At their first meeting in 2002, the network quickly renamed itself the Isis Fund Network on Sex, Gender, Drugs, and the Brain, and established this mission: To develop collaborations for exploratory and hypothesis-driven research on sex differences in nervous system function, and to translate the results of this research into new and/or improved therapies for advancing human health. In addition to the original goal of network members collaborating on pilot projects, the Network established the following goals in support of that mission: to promote research and education in the area of sex/gender differences in brain health and disease, and to educate and advocate among research funders, scientists, reviewers, regulators and the public. They identified three ways to accomplish those goals: through Network publications, by organizing symposia at large scientific meetings, and by seeking funding for new investigator training grants for sex differences research.

By the third meeting of the Network, which had expanded to eight members, a discussion of potential network projects brought out the need for a guideline to "best practices" for research on sex differences. The network members were concerned that the greatest barrier to the study of sex differences (or to simply including females in an experiment) was difficulty of

dealing with the ovarian cycle (estrous and menstrual cycles). Many investigators are reluctant to include females in their experiments because they are uncertain how best to account for the female cycle, or how to determine the role of hormones when they observe an effect of the estrous cycle. The Network decided to create a document that described the strategies, methods, and procedures used in sex differences research. The product that resulted was a 24-page review that was published in *Endocrinology* (Becker et al., 2005). Although the review addressed these methodologic issues in the context of central nervous system function, the basic information was widely applicable to research on sex and gender differences in other systems.

Soon after the review appeared in *Endocrinology*, the Network (which by then had 11 members) discovered that the article was only a beginning. Many researchers who read the article appreciated its value, while at the same time mentioning that there was a much wider need for this kind of information. The Network agreed that the next step was to produce an edited volume that would expand on the material presented in the review, and would include chapters on basic and clinical sex differences research in neuroscience. This book is the result of that decision.

The Society for Women's Health Research, and specifically the staff who have had a direct role in the development of the Isis Fund Network on Sex, Gender, Drugs, and the Brain,* are proud of our role in funding and supporting the work of this Network, and of the other Networks supported by the Isis Fund for Sex Differences Research: the Network on Sex Differences in Metabolism, supported by an unrestricted grant from Aventis Pharmaceuticals (now sanofi-aventis); and the Network on Sex Differences in the Musculoskeletal System, supported by an unrestricted donation from Zimmer, Inc.

The Isis Fund Networks have significantly advanced innovative interdisciplinary research on sex differences and, at the same time, have helped launch sex differences as a new field of biomedical research. Network members have organized and participated in symposia on sex differences at meetings of the Society for Neuroscience, the International Society for Psychoneuroendocrinology, and the Congress of the International Union of Physiological Societies. Network members served as guest editors for a special issue of the *American Journal of Physiology* on sex differences in pain and inflammation and a special issue of *Brain Research* featuring papers presented at a joint meeting of the Conference on Sex and Gene Expression and the Workshop on Steroid Hormones and Brain Function held in 2006.

Network members have also been instrumental in founding the Organization for the Study of Sex Differences (OSSD). The OSSD is a new scholarly scientific society for which the Society for Women's Health Research is providing fiscal sponsorship and staff support. The OSSD was founded so that the mission of the Network on Sex, Gender, Drugs, and the Brain, "to promote research and education in the area of sex/gender differences in brain health and disease, and to educate and advocate among research funders, scientists, reviewers, regulators, and the public for the study of sex differences," will continue long after the Network no longer meets.

It is our hope that this volume will prove informative and inspiring, that it will engender curiosity about the role of sex as a factor in the development and function of physiological systems, and that it will fuel the growth of a field of research that is crucial to advancing our knowledge of human biology, and our understanding of human health and disease.

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Introduction

In August 2001, the Institute of Medicine (IOM) published a report called "Exploring the Biological Contributions to Human Health: Does Sex Matter?" The IOM concluded that sex is a variable of significant importance for understanding health and disease, and for understanding human physiology more generally. The IOM report was a wake-up call to basic and clinical researchers in many disciplines. In response, the past few years have witnessed a marked growth in research on the effects of sex, as well as signs of greater awareness among professionals that scientifically and clinically important sex differences can and do exist—in susceptibility, symptom expression, response to drugs, immune responses, and many other domains. Sex-based biology has come into its own!

In this volume, we focus on the neurosciences—a set of disciplines where research on sex differences has a lengthy history. In the 1970s, pioneering studies identified sex differences in brain morphology at both the cellular and macroscopic levels, with some structural differences visible even to the naked eye (Raisman & Field, 1971, 1973; Greenough et al., 1977; Gorski et al., 1978). Outside the laboratory, neuropsychologists studying the effects of brain tumors and strokes in neurological patients noted sex differences in some of the cognitive effects of localized lesions, especially in the language domain (Kimura, 1983; Kimura & Harshman, 1984), an observation that suggested the functional organization of the brain might not be entirely the same in men and women. Now we know that even the basic neurochemistry of the brain can differ according to sex, due to developmental events and the effects of steroid hormones on neuronal and glial activity (e.g., Bazzett & Becker, 1994; Andersen et al., 1997; Auger, 2003; Walker et al., 2006). This book carries on the tradition of highlighting sex differences and illustrates the rich and varied work that is going on in the neuroscience of sex and gender today.

With this volume, we offer food for thought to both novices and experts in the field of sex differences. We open with an overview of the evolution of sex

differences (Chapter 1), and the biology of sexual differentiation of the brain (Chapter 2), emphasizing how cutting-edge ideas and discoveries are revolutionizing our concepts of what makes a male or female brain. Some expert readers might be surprised to discover a renewed emphasis on the direct actions of X and Y chromosome genes in bringing about sex differences. The endocrine model, however, is still ascendant, as many of the chapters reflect. Chapters 3 and 4 are both methodological chapters that discuss research methods and strategies for the intelligent study of sex differences. After all, discovering a sex difference is only the first step—identifying the genetic or hormonal pathways by which the sex difference is established, and understanding its significance in the context of an organism's ecology and larger behavioral context are the ultimate goals of the basic neuroscientist. The new science of pharmacogenomics is a promising tool to consider when studying central nervous system disorders, and here, too, sex differences are being discovered as discussed, in Chapter 5.

Several of the chapters in this book were written by basic scientists who study the brain and its outward product behavior, but many of these topics have exciting implications for the clinic. These include chapters on such fundamental topics as a thorough review of steroid hormone receptors and their role in sexual behavior (Chapter 7), sex differences in social bonding and affiliative behavior (Chapter 8), sex differences in the neural organization of movement (Chapter 9), as well as sex differences in motivation (Chapter 10) and sex differences in energy metabolism and eating behavior (Chapter 13). These chapters discuss information important for the understanding of the neural basis of addiction and other disorders related to the function of motivational systems.

In this volume we also discuss topics of importance for understanding the recovery from brain injury, as discussed in sex differences in neuroplasticity (Chapter 11). Three chapters deal with sex differences in cognitive function, either in rodents (Chapter 12) or in humans and other primates (Chapters 15 and 16). This has been an especially active arena for sex differences research over the past 20 years, and these chapters represent timely reviews on the topic. Newer areas of research discussed include sex differences in children's play and affiliation with same-sex and opposite-sex peers (Chapter 14).

Other chapters present sex differences in the neurobiology of disease, and illustrate how the recognition of sex differences has enlightened our understanding of a wide range of medical conditions. Chapters 17 and 18 offer insights into sex differences in infections and the activity of the immune system. Chapter 19 describes the important area of sex differences in pain, a difference with wide applicability in the medical sciences. Sex differences are a prominent feature of a number of psychiatric disorders, including major depression, and mood and anxiety-related disorders. These differences are described in Chapters 20 and 21, along with Chapter 6, where sex differences in the responsiveness to stress and in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis are discussed. As illustrated in these chapters, dysregulation of the HPA axis is a feature of many psychiatric conditions.

The book concludes with two chapters on aging and degenerative diseases of the nervous system (Chapters 22 and 23), including Alzheimer's (which shows a female predominance) and Parkinson's disease (which shows a male predominance). Understanding sex differences in aging, especially brain aging, will be an important practical issue over the next decades.

Does sex matter? To respond to the question posed by the IOM: of course sex matters! It matters to biology and medicine at every level of organization and function, from gene to behavior. The realization that there are real and identifiable differences between the sexes that can potentially have a major impact in physiology and medicine, and the potential significant applications of sex differences research, are now driving the agenda. We must have a clear understanding of the important role of sex if we are to optimize medical treatments, effectively target rehabilitation methods, and devise the most effective preventative strategies in the two sexes. Yes, sex does matter, and it matters to basic and clinical scientists in ways we can't even foresee—studying how phenomena in the brain might differ according to sex can help to illuminate the basic mechanisms and physiology that are the essential research targets of every neuroscientist.

No introduction is complete without thanking those who helped us. We thank Viviana Simon and her staff at the Society for Women's Health Research for all their assistance and support throughout the project. Without Viviana's valuable time and wonderful positive attitude, we could not have accomplished this in the short time we had. We also thank

Sherry Marts and Phyllis Greenberger, from the Society for Women's Health Research for their inspiration to create the Isis Fund Networks and for their constant efforts on behalf of sex differences research. We would not have come together without them, and we have benefited in many ways, both scientifically and personally, from our association with the Society and from our warm relationships with Sherry and Phyllis. Finally, we dedicate this book to Florence P. Hazeltine, founder of the Society for Women's Health Research, whose unstoppable energy on behalf of sex differences research is an inspiration to us all.

We hope you enjoy the book.

On behalf of the Isis Fund Network on Sex, Gender, Drugs, and the Brain
Jill B. Becker, Karen J. Berkley, Nori Geary, Elizabeth Hampson, James P. Herman, & Elizabeth A. Young
July 2007

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Part I

Strategies, Methods, and Background

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Chapter 1

Why Are There Two Sexes?

Turk Rhen and David Crews

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 (Phoenix 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. Because 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? 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 (representatives of the ancestral and the descendant species).

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.* For example, in Virago males the volume of both the POA and VMH is 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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Chapter 2

Sex Differences in the Brain: What's Old and What's New?

Margaret M. McCarthy and Arthur P. Arnold

No one will ever win the battle of the sexes; there is too much fraternizing with the enemy.
—Henry Kissinger

The study of sex differences in the brain has a long, rich history and remains a vibrant and controversial topic that is central to the field of neuroscience both for its obvious relevance and its heuristic value. The goal of this chapter is to provide a brief historical perspective, largely by directing the reader to the many excellent reviews already available, while emphasizing emerging paradigm shifts in our view of the origin and functional significance of brain sex differences. We will highlight two major new initiatives: the direct role of sex chromosome genes in determining brain sex differences, and, the novel theoretical view indicating that sometimes the sexes are striving to be the same.

We will also review 10 recent discoveries that have changed our thinking about sex differences in the brain, but emphasize that the list is not complete nor meant to place relative value on one finding over another. The study of sex differences in the brain is confounded by its biological complexity as well as the social and cultural implications of the findings.

The traditional view of a sex difference is any quantifiable endpoint with a mean value that is significantly different between males and females (Hines, 2004); however, it is becoming increasingly clear that this definition is too restrictive and does not reflect the complex and myriad ways sex differences are manifest. Males and females differ in such traits as their averages, extremes, permanence, temporal qualities, susceptibility to disease, and in their functional impact. Evolutionary processes have created sex differences that are expressed only at one life stage or maybe only at one season. Some sex differences become apparent only under unusual circumstances, such as conditions of extreme stress, or in response to drugs that humans have created but which were not available as animals evolved. Thus, a sex difference in a particular endpoint under one set of circumstances may disappear or even be reversed under a different set of circumstances.

Appreciating this complexity is not only important for a proper approach to the study of sex differences, a

topic discussed in detail in Chapter 3 of this volume, but is also important to the interpretation of the relative significance of a sex difference. Understanding the origins of a sex difference also provides insight into the potential cellular and molecular mechanisms determining the phenotype of the trait under study. In the end, all sex differences require an explanation.

It is useful to discriminate between the study of sex differences and the study of sexual differentiation. Sexual differentiation has historically meant the study of permanent, ontogenetic differentiation of tissues in males and females, and the field has focused on adaptive sex differences that produce the normal male and female phenotype required for the two different reproductive roles.

In contrast, the study of sex differences aims to explain *any* sex difference. Many sex differences are assumed to be adaptive, but because of the pleiotropic actions of genes, negative side effects of being male or female, at least in certain contexts, are unavoidable. For example, the greater susceptibility of males to X-linked mental retardation, or of females to autoimmune disease, can hardly be explained as an adaptive difference. Rather, these susceptibilities are each likely disadvantageous side effects of some adaptive sex difference that was selected for its other advantages (i.e., because of other effects on fitness).

All biological sex differences arise from the sex differences carried by the sex chromosomes. In mammals, the male sex chromosomes are XY; and the female, XX. The difference in chromosome complement leads to three genetic sex differences (Arnold & Burgoyne, 2004): male cells have Y genes absent in females (but not many, since the Y chromosome is small and gene-poor), female cells have two genomic doses of X genes (but the difference has relatively little impact at the level of gene expression because each female cell transcriptionally silences, or inactivates, one of the two X chromosomes (Itoh et al., 2006c) and, female cells receive a paternal X chromosome imprint that males lack.

These genetic sex differences cause XX and XY cells to differ. The most important difference occurs in the gonads. The Y-linked gene *Sry* is expressed in the undifferentiated gonad of males causing it to commit irreversibly to a testicular fate. The differentiation of testes in males, and ovaries in females, leads to sex differences in the secretion of gonadal sex steroid hormones. These hormones act on many tissues of the body to cause them to develop differently and function differently in adults. The sex differences caused by gonadal hormones probably represent a continuum in terms of their permanence. At one extreme are the permanent effects of gonadal steroids, the *organizational effects;* at the other extreme are reversible effects, or *activational effects*, which last only as long as the hormone is present (Phoenix et al., 1959; Arnold & Breedlove, 1985). Often activational effects are constrained by previous organizational effects. Both of these types of hormonal effects lead to sex differences in function of tissues.

THE CLASSICAL MODEL OF SEXUAL DIFFERENTIATION

The work of Lillie (1916), Jost (1947), and Phoenix et al. (1959) (Lillie, 1916; Jost, 1947; Phoenix et al., 1959) gave rise to the classic model of brain sexual differentiation, which was elaborated and confirmed by many subsequent works (McEwen, 1980; Arnold & Breedlove, 1985; Breedlove, 1994; McCarthy, 1996; Simerly, 2002; Arnold, 2004). The model states that the sex of the gonads is the primary sex difference caused directly by the presence or absence of the Y chromosome in cells of the male gonad.

The differentiation of the gonads leads to sex differences in the secretion of testosterone perinatally, which induces permanent male-specific patterns of differentiation of the genitalia and brain, and other organs. Other secretions of the testes, especially Müllerian-inhibiting hormone, cause male-specific patterns of differentiation (i.e., involution) of the Müllerian ducts. Testosterone enters the brain of the male mammalian fetus, where it is often converted to estradiol because of the presence of the catalyzing enzyme aromatase. The estradiol acts on estrogen receptors (ERs) to cause masculine differentiation of the hypothalamus and related structures, inducing the formation of circuits that are required for masculine patterns of copulation. It also acts on ERs to suppress the formation of circuits that are required for feminine receptive behaviors such as rodent lordosis and proceptive (solicitous) behaviors. These are actually two separate processes, referred to as *masculinization* and *defeminization*.

In male rodents, estradiol derived from testicular androgens permanently alters the reproductive physiology of the rodent by preventing the capacity for positive feedback effects of estradiol on luteinizing

hormone (LH) production and release in adulthood—a necessary prerequisite to ovulation. Female rodents exposed to androgen neonatally lose the capacity to ovulate and are referred to as "androgen sterilized" (Barraclough, 1961). Although the pioneers of this classical model (Lillie, Jost, & the William Young lab) focused originally on tissues and behaviors directly involved in reproduction (external and internal genitalia, copulatory behaviors), where the adaptive differences in males and females are most pronounced, the general model has been repeatedly applied in attempts to explain the many different behavioral systems in which more minor sex differences can be found. These include courtship, cognitive behaviors, the response to stress and pain, etc. A great number of experimental studies support the importance of organizational and activational effects of gonadal steroids in causing sex differences in the brain and behavior; however, in some instances, this framework applies less well, suggesting there are other principles that can guide the development and maintenance of sex differences (discussed further below).

SEX DIFFERENCES IN THE NEW MILLENNIUM: TWO PARADIGM SHIFTS

We live in the age of genetics. Not only does this mean that we have new methods for manipulating and understanding genes that control organizational and activational steroid effects on sex differences, but the exponential increase in information on genomes (including the sex chromosomes and their roles outside of the gonads) has forced us to re-evaluate the apparently complementary or opposing effects of diverse sex-specific factors that sum to produce sex differences or counteract each other to make the sexes more similar. These new ideas have led to two basic paradigm shifts in the field of sex differences.

Sex Chromosome Genes Join Hormonal Effects as Proximal Signals Inducing Sex Differences in Neural Tissues

The sex differences produced in the brain by gonadal steroids are indirect effects of sex chromosome genes—in mammals the Y chromosome gene *Sry* induces sex differences directly in the gonads. This leads to sex-specific secretions that cause sex differences in function of the brain or other tissues. The differences in sex chromosome complement also appear to act directly on the brain and other tissues to cause sex differences directly. In other words, XX and XY cells function differently, before or after they are influenced by gonadal steroids, by virtue of the direct sex-specific effects of X and Y gene expression within the cells themselves (Arnold, 2004). These effects are much less well studied than the effects of sex hormones because of the difficulty of manipulating the sex chromosome complement without also altering the levels of gonadal secretions in experimental animals.

Although the classic model of sexual differentiation has been enormously successful, and withstood many attempts to test it, a few cases do not fit this model. Several of these cases involve sex differences that occur before gonadal differentiation, before the steroid-secreting cells of the gonads have differentiated and begun to express genes leading to steroid synthesis. These include somatic differences (e.g., Renfree & Short, 1988; Burgoyne et al., 1995), but of particular interest are those observed in the nervous system. Shortly after gonadal differentiation, but before testicular secretions have been found to be sexually dimorphic, mesencephalic dopamine neurons exhibit some sexually differentiated characteristics. This appears to be due to the action of sex chromosome genes (Reisert & Pilgrim, 1995; Carruth et al., 2002). Moreover, in mice, sex differences in the expression of genes in the brain are detected prior to the differentiation of the gonads (Dewing et al., 2003), and thus cannot be the result of sex differences in gonadal secretions.

Other sex differences, which occur after the gonads are differentiated, also do not fit the classic model. In the zebra finch (*Taeniopygia guttata*), males sing a courtship song that females do not sing. The brain regions controlling song are much larger in males than females. Although treatment of females with estradiol at hatching causes about half-masculinization of the neural song circuit, the masculinization is never complete, even if different hormonal treatments are used.

The study of intersex individuals suggests a role for direct actions of sex chromosome genes on brain sexual differentiation. For example, genetic zebra finch females induced to grow testes have a feminine neural circuit and do not sing (Wade & Arnold, 1996; Wade

et al., 1996; Wade et al., 1999); whereas a genetically male zebra finch with an ovary but lacking testes (presumably a mutation in the gonad-determining pathway), had a male brain and sang. Thus, masculine differentiation of the neural song circuit appears to have occurred in the absence of testes (Itoh et al., 2006b).

Another mutant finch, a spontaneously occurring lateral gynandromorph, was genetically male on the right side of its body (containing a testis), and genetically female on the left side (containing an ovary). Although both sides of the brain would have been exposed to the same levels of gonadal steroids (and hence not differentiated by gonadal steroids), the right side of the brain was more masculine than the left (Fig. 2.1). It appears that the sex chromosome complement of brain cells contributed to differences in the two sides (Agate et al., 2003). A candidate gene encoded on the sex chromosomes, which might contribute to greater masculinization of the male, is the neurotrophin receptor trkB. The constitutively higher expression of trkB in males could facilitate greater growth of the neural circuit for song if it leads to greater action of neurotrophins (Chen et al., 2005).

Once one adopts the hypothesis that XX and XY cells are different, how does one test for such effects? Since sex chromosome complement normally is confounded by the sex-specific effects of gonadal secretions, how does one untangle the effects of hormones from the effects of sex chromosomes? The first step is

Figure 2.1. An unusual phenotype in the zebra finch, called a lateral gyandromorph, has allowed for a unique comparison of the effects of the gonads versus the genome on brain phenotype. This bird had male plumage (orange cheek patch, chest bar and strips) on one side and female plumage on the other, reflecting a genetic male on one side of the body and a genetic female on the other as demonstrated by expression of the female-specific ASW mRNA on only one side of the brain shown in this autoradiogram (lower right). The presence of testicular tissue would have provided circulating testosterone throughout the body, resulting in equal exposure to both sides of the brain. Quantification of the song nucleus, HVC, found it to be larger on the genetically male side of the brain compared to the female side, consistent with the sex dimorphism observed in the volume of this nucleus in normal males and females (based on data presented in Agate et al., 2003).

to compare animals that differ in the complement or expression of X or Y genes, to determine if this difference has an effect on phenotype. One model is to compare mice that are otherwise genetically the same, but have different strain origins (different alleles) of the Y chromosome. In some cases, for example, mice differing only in Y genes show markedly different levels of aggression (Maxson et al., 1989; Guillot et al., 1995; Monahan & Maxson, 1998), proving that allelic differences on the Y chromosome cause differences in aggression among males.

These differences probably contribute to sex differences in aggression. Is this an example of a direct effect of Y genes on the brain? Such a direct effect is possible, but it is also possible that the allelic differences on the Y chromosome led to differences among males in their levels of testosterone, which then act differently on the brain to modulate aggression. The path to answering the mechanism of Y effects on aggression is to identify the Y gene(s) responsible, and determine the sites and mechanisms of action. Another useful model for investigating sex chromosome effects is the "four core genotypes" model (De Vries et al., 2002). In the mice model, the *Sry* gene is deleted from the Y chromosome, so that the modified Y (called "Y minus," Y^-) does not induce testicular differentiation. Thus, XY^- mice have ovaries and are called females. When a *Sry* transgene is inserted onto an autosome, the mouse develops testes and is called a male (XY^-Sry). Mating XY^-Sry males with XX females produces four genotypes (XX females, XY^- females, XX*Sry* males, XY^-Sry males) in which sex chromosome complement (XX vs. XY) is varied independently of gonadal type (*Sry* present vs. absent; testes vs. ovaries).

This two-by-two comparison allows not only the unusual opportunity to measure separately the effects of testicular versus ovarian secretions on a trait, but also the effect of sex chromosome complement (XX vs. XY). To date, dozens of adult and neonatal phenotypes have been measured in these mice. Some of the classic morphological sex differences in the central nervous system (CNS) show no sex chromosome effect because gonadal males are masculine and gonadal females are feminine in these traits, regardless of chromosomal complement. That result confirms the classic model for those specific traits.

In other cases, however, sex chromosome complement has an effect. For example, XX and XY mice differ in aggression, parental behavior, and density of arginine vasopressin in the lateral septum (De Vries et al., 2002; Gatewood et al., 2006). Because the sex chromosome effects were measured in mice that had the same level of gonadal hormones in adulthood, the sex chromosome effects cannot be attributed to an indirect effect of sex chromosome complement on levels of circulating sex hormones at the time of testing (i.e., differences in activational effects of hormones). It is possible, however, that XX and XY mice of the same gonadal type experienced differences in gonadal hormone levels at earlier times of life, so an indirect organizational effect is not excluded. However, such effects seem unlikely based on the pattern of the results. For example, sometimes an XY mouse is more masculine, sometimes less masculine than an XX mouse of the same gonadal type (e.g., Carruth et al., 2002; Gatewood et al., 2006; Palaszynski et al., 2005).

Other models compare mice with different genomic imprints on the X chromosome. For example, XmO versus XpO female mice (i.e., those with a maternal vs. paternal X chromosome imprint on the single X chromosome) show differences in tests of reversal learning, suggesting that one or more X genes show different expression if the genes are inherited from the farther versus the mother. A candidate X gene has been identified which shows imprinting that causes differences in expression in the brain. Because only females receive an X chromosome with a paternal imprint, imprinting effects could contribute to sex differences in brain and behavioral traits (Davies et al., 2006).

A fourth method for studying sex chromosome effects is by observing the effects of X or Y gene-specific manipulations on traits. For example, the Y gene *Sry* is expressed in the substantia nigra of the midbrain (Dewing et al., 2006), the origin of dopamine neurons that innervate the striatum. Mice receiving unilateral injections of antisense oligonucleotides that reduce expression of *Sry* show a loss of tyrosine hydroxylase expression on the antisense side. Asymmetries in motor behavior indicate that *Sry* expression influences those behaviors. Rodents show sex differences in expression of midbrain TH. The results indicate that *Sry* has male-specific effects in the midbrain. Indeed, this is the first demonstration of a direct male-specific effect of a specific Y gene in the brain. It is not yet clear if the *Sry* effect produces sex differences in phenotypes, because its effects may be compensated by the female-specific effect of a factor operating only in females (see next section).

Sometimes the Sexes Strive to be the Same

Although it seems like almost any trait is in some way impacted by sex, in reality, there just isn't a difference in traits, in some instances, between males and females. A specific cellular or physiological process may simply be outside the sphere of influence of hormones and/or sex-specific genes. In other cases, the two sexes are similar because one sex difference is canceled by another, or because males and females reach the same end result by two different paths. Because several different factors (different hormones, different times of action, different X or Y genes) contribute to sex differences in the function of the brain and other nongonadal tissues, they can interact to modulate, enhance, or block each other. For example, testosterone has organizational and activational effects that both contribute to making the male more likely to show masculine copulatory behaviors. In other cases, however, two male-specific factors might counteract and cancel each other, reducing rather than producing sex differences (De Vries & Boyle, 1998; Voskuhl & Palaszynski, 2001; De Vries, 2004; Palaszynski et al., 2005). For example, Y genes and testosterone may work in opposition.

The compensatory effects of two sex-specific factors can be seen as adaptive if some sex-specific factor has disadvantageous side effects which are then reduced by the evolution of a compensatory process. One of the best examples of this is that the sex difference in genomic dose of X-chromosome genes (double dose in XX females, single dose in XY males) has evolved because of inevitable forces that make the X and Y chromosomes different (Charlesworth, 1991; Graves, 2006).

However, the different dose of X genes is thought to be highly maladaptive for one or both sexes, because gene dose can have a critical effect on cell function and cannot be optimal in both sexes if they have a permanent twofold difference in expression. The evolution of a female-specific mechanism of X inactivation effectively reduces the sexual disparity in X gene expression (Itoh et al., 2006a) and avoids a host of problematic sex differences in gene expression in metabolic pathways that must function equivalently in the two sexes. X inactivation is one of the best studied sex-specific mechanisms that allows the sexes to be more equal, not less.

Alternatively, the sexes may converge on the same behavioral endpoint from different origins. In mammals, the female's large investment in individual gametes, including a long gestation and period of lactation, leads to a strong maternal involvement in parental care. Maternal behavior by females is a tightly controlled hormonally-regulated process that probably evolved in the context of hormonal changes at the end of pregnancy, causing the female to become influenced by those changes. Males are less constrained in their choices regarding parenting and when the choice for parenting does appear, it must have evolved outside of the hormonal parameters that likely influenced females. Thus, parental behavior in males represents a convergence in behavior with females using divergent physiological mechanisms (De Vries, 2004) (Fig.2.2).

One system that appears to have been exploited to that end is the neurohormone, vasopressin, which is important for parental behavior and for related affiliative behaviors across a wide range of species including birds, rodents, and primates (Wang & De Vries, 1995; Lim & Young, 2006; Nair & Young, 2006). Vasopressin innervation is among the most sexually dimorphic in the brain (De Vries et al., 1994; De Vries & Panzica, 2006) and appears to have been co-opted to regulate parental behavior in males. The cellular and molecular mechanisms by which the vasopressin system is modulated developmentally to direct appropriate adult behavior in response to specific stimuli, such as neonates, is not well established.

Sometimes the sexes try to be the same, literally by using different strategies to solve the same problem. Studies of sex differences in cognition in animal models focus almost exclusively on spatial learning ability. There are a variety of experimental paradigms for assessing learning in rats, but the only model routinely employed for sex differences is the Morris Water Maze. This not because it is the best test for learning, but because it is the only test that reliably shows any sex differences in performance (Jonasson, 2005).

Performance in this instance is the amount of time in seconds (i.e. latency) for a subject animal to find a hidden platform from which to escape the aversive water. Males routinely find the platform faster than females and are thereby considered to have superior spatial learning (Jonasson, 2005). This may very well be true. Human males are also consistently better than females in some spatial tasks (Hamilton et al., 2002;

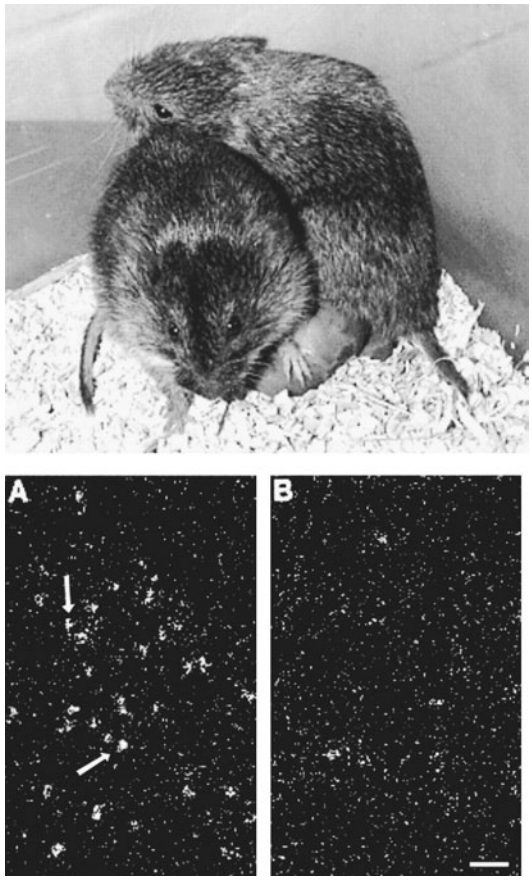

Figure 2.2. An emerging principle in sex differences research is that sometimes males and females strive to be the same. This can occur at the neuronal level in order to converge on the same behavior and is best exemplified in the parental behavior of the prairie vole. In most rodent species, including most voles, the male provides little to no parental care of his own offspring. In the prairie vole, however, the male actively takes care of and protects his young. This is correlated with an increased expression of vasopressin, a neuropetide that fosters affiliative behavior. The top panel shows a male and female prairie vole taking care of their young, and the bottom panel is a dark field image of in situ hybridization detection of mRNA for vasopressin in the bed nucleus of the stria terminalis of a male (A) and female (B). Note the much higher level of expression in males. Reprinted with permission from De Vries GJ (2004). Minireview: Sex differences in adult and developing brains: compensation, compensation, compensation. *Endocrinology*, 145:1063–1068.

Hines, 2004; Driscoll et al., 2005). However, recent studies in rodents have re-examined the Morris Water Maze and the conditions associated with the test.

Two important and related principles emerged. One is that females use a different strategy than males to solve the problem (Perrot-Sinal, 1996; Beiko et al., 2004). When the platform is raised above the water so that the animals can readily see it, males will swim the most direct path; whereas females exhibit a strong thigmotaxis, swimming close to the walls of the tank before darting out into the open water to reach the platform (Fig. 2.3). Importantly, both males and females learn the task, but the rate at which it is learned differs. Moreover, males and females differ in their

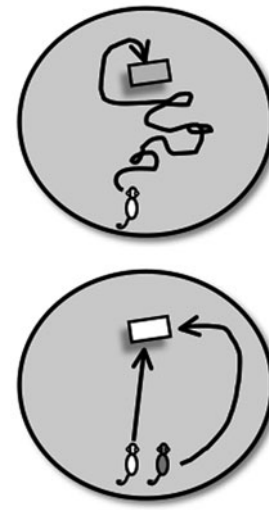

Figure 2.3. Males and females may also strive to reach the same endpoint by using different behavioral strategies. The Morris Water Maze is a well known test for spatial learning and males are consistently reported to outperform females. Performance on the task is a function of the latency to find a platform hidden beneath the surface of the water. Animals often spend considerable time searching for the platform as illustrated in the top panel. However, when the platform is raised above the surface of the water, male and female rats adopt different strategies for approaching it. Males swim directly, while females take a more circuitous, and presumably less anxiety-provoking, route that takes longer. When the stress of the task is reduced, male and female rats both swim directly to the platform. Based on studies by Beiko et al., 2004.

sensitivity to variables that impact on learning, such as stress. In general, females seem to suffer from greater "test anxiety," and are more severely impaired in their ability to learn if there is stress associated with the task (Shors et al., 2001).

Thus, with this one cognitive task we have an example of the sexes using different strategies to solve the same problem; and a situation in which the sexes perform the same unless there is an extrinsic variable, such as stress, introduced into the situation. An important point is that neither of these necessarily represents a sex difference in learning *per se*. Similar arguments have been made regarding evidence for sex differences in human cognitive ability (Spelke, 2005) and highlight the continuing gaps in our understanding of what is or is not different between males and females.

SEX DIFFERENCES IN THE NEW MILLENIUM: 10 FINDINGS THAT ARE CHANGING OUR THINKING

Despite the risks inherent in making any list, we present one here in an attempt to emphasize both major recent advances and the reemergence of decades-old problems that still lack clarification. Our goal is not to applaud some of these advances while ignoring others, but hopefully to provide a framework for determining the best avenues for future work. The topics are loosely organized along conceptual themes to highlight how they might support or contradict each other, with no intention of suggesting relative importance.

Growth Factors Mediate Effects of Gonadal Hormones

Hormonal induction of neurotrophic factors seems a fairly obvious mechanism that nature might have utilized to differentiate particular brain structures, but evidence for this mechanism is not abundant. Estradiol increases the amount of brain derived nerve growth factor (BDNF) in the developing hippocampus (Solum & Handa, 2002), midbrain (Ivanova et al., 2001), and vocal nuclei of songbirds (Dittrich et al., 1999; Fusani et al., 2003), but seems to have little effect on the primary receptor, trkB. Conversely, estradiol increases binding of nerve growth factor (NGF), and thereby, presumably, the amount of receptor in the developing telencephalon of the zebra finch (Contreras & Wade, 1999).

Estradiol and insulin have long been known to have a synergistic effect on neurite growth in fetal hippocampal explants (Toran-Allerand et al., 1991; Toran-Allerand, 1996), an effect now known to be the result of an interaction between insulin-like growth factor (IGF-1) receptors and estrogen receptors, presumably at the membrane (Toran-Allerand et al., 1999). These two receptors appear to act in tandem to promote cell survival and neurite outgrowth in a variety of brain regions, with considerable emphasis placed on a potential neuroprotective effect in the adult (Cardona-Gomez et al., 2002).

In only one sexually dimorphic system, the spinal nucleus of the bulbocavernosus (SNB), has a clear functional impact of elevated growth factor been found. In this system, ciliary neurotropic factor (CNTF) is upregulated in the bulbocavernosus muscle by androgens, and then retrogradely acts on the CNTF receptors on the motoneurons of the SNB, promoting their survival (Forger, 2006). Mutant mice lacking receptors for CNTF have no sex difference in the size of the SNB. Because the SNB motoneurons innervate muscles that attach to the penis, the functional significance of the male's greater number of neurons is evident.

When CNTF is administered to females, it rescues the motoneurons in females; and treating males with antagonists to CNTF receptor, reduces the number of motoneurons in males. Why then has it been difficult to find similar functional significance for growth factor signaling in sexual differentiation of diencephalic or telencephalic brain structures? One reason is technical. There are no receptor antagonists for BDNF, and trkB knock-out mice have only recently been developed. Moreover, BDNF signaling is so pervasively important to normal brain development, that it is difficult to interfere selectively with its putative role in hormonally induced sexual differentiation.

For instance, estradiol is a potent inducer of BDNF in the developing hippocampus (Solum & Handa, 2002), yet BDNF is fundamental to the balance of glutamatergic versus GABAergic synapses in this region (Singh et al., 2006), making it difficult to dissect out the role of estradiol-induced BDNF from BDNF in general. Nonetheless, there is good reason to suspect that growth factors are critical players in the sex differentiation process, and that this role extends beyond the spinal cord.

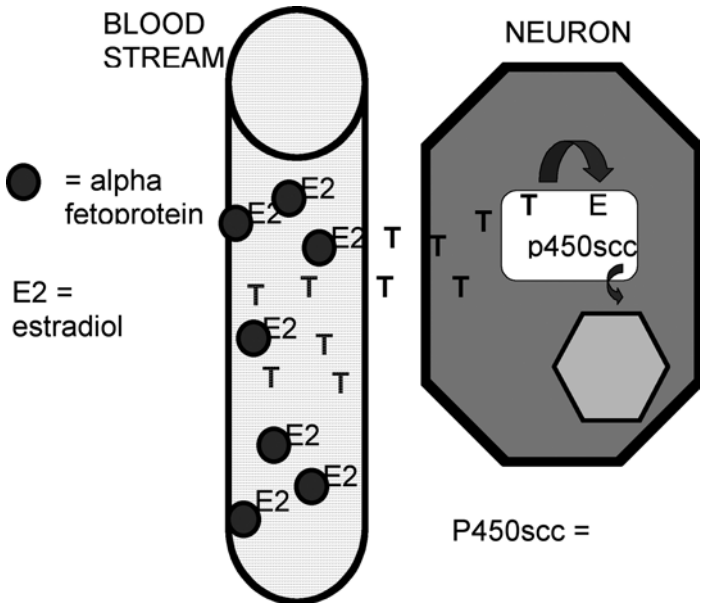

Figure 2.4. Estradiol is a major regulator of brain masculinization and defeminization. This is achieved by testicularly-derived androgen gaining access to neurons where it is locally converted to estradiol by the P450scc enzyme, aromatase. High levels of estradiol in maternal circulation also gain access to the fetal circulation, but are sequestered there by the steroid binding globulin, alpha-fetoprotein, preventing masculinization and defeminization from occurring in developing females. Testosterone is not bound to alpha-fetoprotein and so selectively gains access to the neurons, where it is aromatized to estradiol. Together, these observations form the basis of the Aromatization Hypothesis of sex differentiation of the brain.

Estradiol Induces a Target-Derived Diffusible Axonal Growth Factor

In addition to steroid-induced regulation of neurotrophins that regulate cell survival, steroids appear to alter trophic factors that control axonal outgrowth. The principle nucleus of the bed nucleus of the stria terminalis (pBNST) projects to the anteroventral periventricular (AVPV) nucleus as part of a neural circuit controlling gonadotropin secretion from the anterior pituitary.

One of the most robust morphological and functionally significant sex differences in the brain is the 10-fold larger pBNST to AVPV projection in the male. Clever use of explant cultures, in which male and female pBNST and AVPV could be mixed and matched, definitively revealed that estradiol was acting in the AVPV to produce a signal to attract the growing axons of the pBNST neurons (Ibanez et al., 2001) (Fig. 2.4).

The identity of the diffusible factor remains to be determined. The AVPV appears to be a critical node for the induction of the surge in LH release that is required for ovulation. AVPV neurons are largely glutamatergic and project to the vicinity of the LHRH neurons, which in turn project to the anterior pituitary and regulate LH release. No compelling evidence exists for sex differences in the LHRH neurons themselves. When placed in a circuit context, one can envision the inhibitory pBNST projecting to and clamping the excitatory AVPV in males, preventing the induction of an LH surge in response to elevated estradiol, one of the hallmarks of the masculinized brain.

Steroid-Mediated Sex Differences in Cell Death are Independent of Steroid-Mediated Neurochemical Phenotype

Up to this point, we have not discussed the most well-established and intensely studied sex difference in the brain, the overall size of specific brain regions. In rats, the sexually dimorphic nucleus of the POA (SDN-POA) is 5 to 7 times larger in males and the AVPV of the POA is 3 to 5 times larger in females (Simerly, 2000, 2002; Morris et al., 2004). The SNB of females has one third the number of neurons as in males (Forger, 2006) and several of the song control nuclei in birds are 5 to 6 times larger in males than females (Ball & MacDougall-Shackleton, 2001).

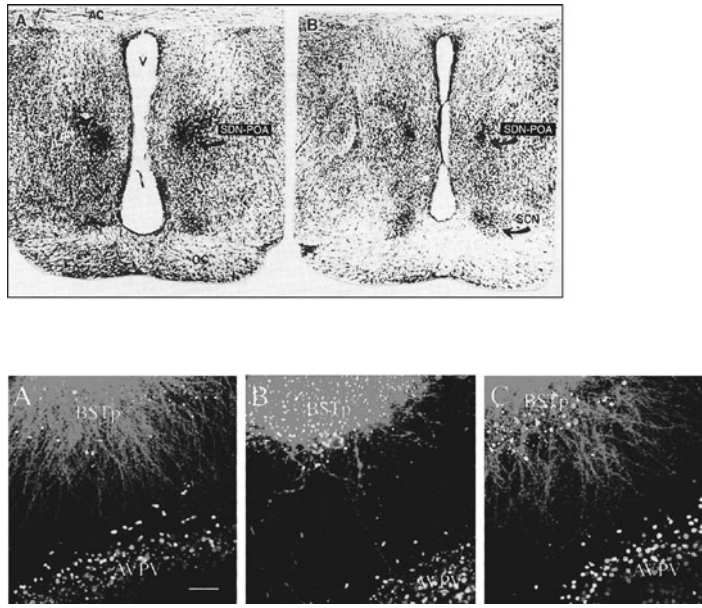

Figure 2.5. Many sex differences in the brain are characterized as the size of a structure being larger in one sex versus another. These structures include entire brain regions, major projections, and subnuclei. Work in bird brains demonstrated that differential cell death in one sex versus the other can contribute to volumetric sex differences, and this was subsequently confirmed in the mammalian brain for the sexually dimorphic nucleus (SDN) of the preoptic area, shown here in the upper panel and visualized by cresyl violet. More cells die in females than males during the perinatal sensitive period, resulting in a smaller SDN volume in females (right) compared to males (left). Alternatively, in the AVPV, more cells die in the male than in the female, resulting in the opposite volumetric difference. However, a target-derived factor from the male AVPV encourages a much larger innervation by BNST neuronal axons, resulting in a larger male projection than in females. This is illustrated in the lower panel illustrating explant cultures of the BNST (red) and the AVPV (green). More fibers grow toward the AVPV from a male BNST (left) and a female BNST treated with testosterone (right) than in an untreated female (middle). Reprinted with permission from Ibanez MA, Gu G, Simerly RB. (2001) Target-dependent sexual differentiation of a limbic-hypothalamic neural pathway. *J Neurosci,* 21:5652–5659.

The SDN-POA is arguably the most intensively studied of these, with literally hundreds of published studies since its discovery in the 1970s (Gorski et al., 1978) (Fig. 2.5). Study of various systems, including birds and mammals, demonstrate that volumetric sex differences can be established when males and females begin with the same number of neurons, but that differential hormonal exposure results in sex differences in cell death (e.g., Konishi & Akutagawa, 1985; Nordeen et al., 1985). We know very little about how steroids regulate which cells live and which cells die, however, this does not exclude other contributing variables such as differential migration or neurogenesis, in the establishment of volumetric sex differences.

Recent studies of knock-out mice provide important new information on sex differences in cell death. Studies of neuronal death during a developmental window are inherently limited by the difficulty in detecting the cell while it is dying. Dying itself occurs

quickly, in the course of a few hours (see Forger, 2006, for review). Markers for dying cells disappear with the cell and do not predict which cells might die in the future. Recognizing this limitation, Nancy Forger has exploited the benefits offered by mice that have a null mutation in the Bcl-2 gene, a potent inhibitor of cell death, or in Bax, a promoter of cell death. In the case of the latter, Forger and colleagues found that sex differences in the SNB, AVPV, and pBNST were all eliminated in Bax−/−mice (Forger et al., 2004), indicating Bax is required for sexually dimorphic cell death in the mouse forebrain and spinal cord.

Interestingly, Bax is involved in cell death that is increased by estradiol (AVPV) as well as that decreased by testosterone (SNB). One advantage of this approach is that the number of neurons observed in Bax−/−adults represents the original number generated in each sex, whereas the difference in cell number between Bax−/−and Bax +/+ adults reveals the total number of neurons lost or "integrated over the entire developmental cell death period" (Forger, 2006), further supporting the notion that sex differences in cell death contribute to volume differences in multiple brain regions.

However, there is more to the story. Within the AVPV, a heterogeneity of cell type exists, and females have 3 to 4 times more dopaminergic neurons than males (Simerly et al., 1997). In Bax−/−mice, there is no sex difference in the size of the AVPV, and AVPV is markedly larger in both sexes than in Bax +/+ mice. When one examines only the dopaminergic neurons, there is a robust sex difference and no effect of the Bax mutation (Forger et al., 2004), suggesting that estradiol directs the phenotype of a subset of neurons in the AVPV. A similar phenomenon may be occurring in regards to the vasopressin phenotype in the pBNST (Han & De Vries, 2003). Integrating these findings with the apparent role of *Sry* in differentiating midbrain dopaminergic neurons (Dewing et al., 2003; Dewing et al., 2006) will also be a fruitful area for future investigation.

A Prostaglandin Mediates Masculinization of Sex Behavior in Rats

The ability of gonadal steroids to sexually differentiate the brain during a defined sensitive period of development has been established for almost 50 years. During that time, there has been considerable effort to find the cellular mechanisms of hormone action. Early studies focused on neurotransmitters such as noradrenaline, dopamine and serotonin. These have all been proposed by various groups as important mediators of steroid-induced masculinization of the brain and important sex differences in these systems have been reported (Ani, 1978; Simerly et al., 1985; Simerly, 1998). Some differences occur very early in development and perhaps prior to the influence of gonadal steroids (Reisert & Pilgrim, 1995). Manipulation of these transmitter systems during the critical period for masculinization has deleterious effects on adult behavior. However, the converse is not true; administering serotonin, dopamine or noradrenalin analogs or antagonists to newborn females does not initiate masculinization, suggesting some important element of the story was missing.

A recent and surprising finding reveals that the missing element appears to be the prostaglandin, PGE_2. The synthesis of all the prostanoids begins with the oxygenative cyclization of arachidonic acid by cyclooxygenase. The inducible isoform of cyclooxygenase, COX-2, is an immediate early gene responsive to a variety of stimuli including fever, injury, and stimuli associated with neuronal plasticity (Hoffmann, 2000; Camu et al., 2003; Giovannini et al., 2003). COX-2 mRNA and protein are higher in the POA of newborn males than females and treating females with estradiol increases COX levels to that of males. Increased COX-2 is directly correlated with increased PGE_2 production. Treating newborn females with estradiol increases PGE_2 levels in the POA almost sevenfold.

Moreover, administration of PGE_2 to newborn females has two striking and presumably associated effects: a two- to threefold increase in dendritic spines (the primary site of excitatory glutamatergic synapses) in the POA, and a dramatic induction of masculine sexual behavior in adulthood. Conversely, blocking PGE_2 synthesis temporarily in newborn males significantly reduces POA dendritic spines, to the level seen in normal females, and severely impairs the expression of male sexual behavior in adulthood (Amateau & McCarthy, 2002b; Amateau & McCarthy, 2004). Thus, PGE_2 satisfies the criteria of being an essential mediator of steroid hormone-induced masculinization of sexual behavior in the rat in that it can both induce the masculinization process, and when blocked, disrupt the same process.

Masculinization and Defeminization are Determined by Different Cellular Mechanisms

If one takes a rodent-centric, steroid-mediated, sex-behavior-focused view of sexual differentiation of the brain (which many do, including one of the authors), sexual differentiation of sex behavior involves three independent processes: feminization, masculinization, and defeminization, but no naturally occurring demasculinization. *Feminization* is the default (yet active) pathway leading to expression of lordosis under the proper hormonal conditions in adulthood. *Masculinization* is the active developmental process initiated by testosterone during the perinatal sensitive period resulting in normal male copulatory behavior in adulthood. *Defeminization* is also an active and natural process whereby the ability to express female sexual behavior or reproductive function is lost in males. Defeminization normally occurs in tandem with masculinization in males. Thus, both masculinization and defeminization are active steroid-driven processes that can be initiated in females by exogenous treatment with steroids.

Early studies established that the two processes can be manipulated independently. There is a differential sensitivity to androgen, with masculinization being more potently induced than defeminization in females administered weak androgens. There is also a difference in the duration of the precise parameters of the critical period for each process (Whalen & Edwards, 1967) and reducing the steroid receptor co-activator, CBP, with antisense oligonucleotides, selectively impairs defeminization, however, does not effect masculinization (Auger et al., 2002). Yet all of these studies involve some sort of manipulation involving steroids. As a result, it has been difficult to clearly delineate both the anatomical region critical to defeminization of behavior (the POA is central to masculinization), and the cellular processes being regulated by estradiol or androgens that mediate each process independently of the other.

Two recent findings provide potential insight for solving this problem. The first is based on the estrogen receptors ERalpha and ERbeta. Male mice bearing a null mutation for ERbeta exhibit essentially normal male sexual behavior, but also exhibit robust female sexual behavior, suggesting normal masculinization, but impaired defeminization (Kudwa et al., 2005). Thus, the divergence in mechanisms of estradiol's effects may begin at its receptors. Still, it begs the question: What is the cellular pathway initiated to defeminize the brain?

The cellular mediator of masculinization is PGE_2, which may provide the needed tool to begin to identify the mediator of defeminization. As they exhibit normal female sex behavior as adults, females masculinized with neonatal PGE_2 are not defeminized. Likewise, males in which masculinization has been blocked by preventing PGE_2 synthesis, are still defeminized by their own gonadal steroids, reaffirming the maxim that defeminization is a hormonally driven process independent of masculinization (Todd et al., 2005). Thus, PGE_2 is both necessary and sufficient for behavioral masculinization, but plays no role in defeminization.

Any cellular process induced by estradiol (or androgen) during the sensitive period would be a logical candidate for mediating defeminization. For instance, there is a sex difference in the number of dendritic spines and the length of dendrites on neurons in the mediobasal hypothalamus, a critical brain region controlling lordosis, and therefore a logical candidate for the anatomical site of defeminization. Neonatal testosterone or estradiol treatment increases dendritic spine levels in females to that of males (Mong et al., 1999; Todd et al., 2006), but PGE_2 has no effect in this brain region. However, the actions of estradiol can be either blocked or mimicked by antagonizing or activating the NMDA glutamate receptor.

In fact, estradiol promotes the synaptic release of glutamate from immature hypothalamic neurons, leading to activation of mitogen-activated protein (MAP) kinase and the induction of dendritic spine formation (Schwarz et al., 2006). This series of cellular events has not yet been directly linked to behavioral defeminization, but highlights the utility of this approach by illustrating the general principle that the same hormone can simultaneously activate multiple cellular mechanisms to induce masculinization and defeminization to achieve a coordinated whole male brain.

Glial–Neuronal Crosstalk Is Involved in Establishment of Sex Differences

Although much attention has focused on sex differences in the shape, size, and number of neurons, there are equally robust morphological differences in the astrocytes of males versus females in several brain