Sex Differences and Implications for Translational Neuroscience Research: Workshop Summary

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Diana E. Pankevich, Theresa Wizemann, and Bruce M. Altevogt, Rapporteurs; Forum on Neuroscience and Nervous System Disorders; Institute of Medicine

SEX DIFFERENCES AND IMPLICATIONS FOR TRANSLATIONAL NEUROSCIENCE RESEARCH

WORKSHOP SUMMARY

Diana E. Pankevich, Theresa Wizemann, and Bruce M. Altevogt,
Rapporteurs

Forum on Neuroscience and Nervous System Disorders

Board on Health Sciences Policy

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The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The serpent adopted as a logotype by the Institute of Medicine is a relief carving from ancient Greece, now held by the Staatliche Museen in Berlin.

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"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe

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STEVIN H. ZORN (*Cochair*), Lundbeck USA
TIMOTHY COETZEE, National Multiple Sclerosis Society
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AMEETA PAREKH, Food and Drug Administration
VIVIAN W. PINN, National Institutes of Health

Study Staff

BRUCE M. ALTEVOGT, Project Director, IOM
SARAH L. HANSON, Associate Program Officer (until June 2010)
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EMMELINE EDWARDS, NIH Neuroscience Blueprint (since February 2010)
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KENNETH WARREN, National Institute on Alcohol Abuse and Alcoholism
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CHARLES ZORUMSKI, Washington University School of Medicine

IOM Staff

BRUCE M. ALTEVOGT, Forum Director
SARAH L. HANSON, Associate Program Officer (until June 2010)
DIANA E. PANKEVICH, Associate Program Officer (since October 2010)
LORA K. TAYLOR, Senior Project Assistant
ANDREW POPE, Director, Board on Health Sciences Policy

Reviewers

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they did not see the final draft of the report before its release. The review of this report was overseen by **Dr. William E. Bunney,** University of California, Irvine, Distinguished Professor. Appointed by the Institute of Medicine, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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1

Introduction[1]

"[S]ex . . . is an important basic human variable that should be considered when designing and analyzing studies in all areas and at all levels of biomedical and health-related research."

—Institute of Medicine, 2001

Biologically based differences between the sexes impact human development and behavior in both obvious and subtle ways. Sex differences are also apparent across the spectrum of health and disease, impacting not only individual health, but also public health, biomedical research, and healthcare delivery. Researchers have begun to elucidate these differences and their potential impact in areas such as pain and pain perception, infection, longevity, disease incidence and course, and cellular response and inflammation. Studies have shown, for example, that males and females can have markedly different responses to certain medications; in some cases these unexpected differences have led to the recall of products from the market (GAO, 2001). In the current era of translational research and personalized medicine, it is increasingly important to take sex differences into account, so that the potential effects of products and therapies can be more fully understood.

Several high-profile reports from the U.S. Food and Drug Administration (FDA), the National Institutes of Health (NIH), and the Institute of

[1] This workshop was organized by an independent planning committee whose role was limited to the identification of topics and speakers. This workshop summary was prepared by the rapporteurs as a factual summary of the presentations and discussions that took place at the workshop. Statements, recommendations, and opinions expressed are those of individual presenters and participants, and are not necessarily endorsed or verified by the forum or The National Academies, and should not be construed as reflecting any group consensus. Furthermore, although the current affiliations of speakers and panelists are noted in the report, many qualified their comments as being based on personal experience over the course of a career, and not being presented formally on behalf of their organization (unless specifically noted).

Medicine (IOM) have stressed the need to consider the biological differences between males and females in clinical research and product development (FDA, 1993; IOM, 2001; NIH, 1999). While academically it is clear that sex differences should be taken into consideration when developing research studies, in practice it is unclear when, in the course of research, the most appropriate option would be to invest resources into studying these differences. Characterizing sex differences often requires additional experimental groups or clinical protocols, adding to the overall cost and time of the research. This is particularly true for neuroscience research because of the complex nature of the nervous system and mental, neurological, and substance use disorders.

In recent years, tremendous advances have been made in the methodologies available for looking at sex differences, said Rae Silver, professor of natural and physical sciences at Columbia University and cochair of the workshop. A microarray analysis of gene transcripts from various mouse tissues, for example, was used to demonstrate sexually dimorphic gene expression in 72 and 68 percent of genes in liver and adipose tissue, respectively (Yang et al., 2006). In the nervous system, which is the subject of interest for the forum, such genome screening analysis is much more difficult as the brain is heterogeneous and likely to have localized regions or nuclei of sex differences, she said. Imaging technology, such as positron emission tomography, offers another approach that can further understanding of sex differences in the brain. As an example, Silver cited a study of sex differences in rates of serotonin synthesis, which showed that the mean rate of synthesis was about 52 percent higher in males than in females (Nishizawa et al., 1997). This may contribute to the lower incidence of unipolar depression in males.

SCOPE OF THE WORKSHOP

To explore the key principles and strategies that basic and applied researchers are using in the study of sex differences in the neurosciences and in the development of therapies for neurological disorders, the IOM Forum on Neuroscience and Nervous System Disorders convened a workshop on March 8 and 9, 2010, titled *Sex Differences and Implications for Translational Neuroscience Research*. The Forum was established in 2005 to foster partnerships among stakeholders in the scientific community and the general public; to enhance understanding of research and clinical issues associated with the brain and nervous system and associated disorders; and to advance effective clinical prevention and treatment strategies. For this workshop, participants from academia, government, the pharmaceutical industry, patient advocacy groups, medical journal publishers, and other

stakeholders assembled to consider how and when it is most appropriate to study the differences between males and females in neuroscience research, and what the implications are of sex differences for translational neuroscience research.

Specific objectives of the workshop were to

- briefly outline the public health importance of studying sex difference in the nervous system, in health and sickness, including the potential application to healthcare delivery;
- identify the scientific principles that should be considered when designing preclinical experiments that will examine sex differences, including strategies to bridge between preclinical and clinical studies;
- discuss when and how sex differences should and should not be considered;
- explore the key principles and strategies used by academic clinicians to effectively use basic research for preclinical and clinical application and study, including approaches used by researchers to decide how and when to consider the potential importance of sex differences;
- explore how and when industry considers and addresses studying sex differences, given regulatory guidelines;
- examine the advantages, constraints, and implication of performing "valid analysis" versus requiring statistical outcomes between the sexes; and
- identify the next steps that will be critical to establishing a set of principles that could be used by a variety of stakeholders in considering when and how to incorporate studying sex differences into translational research efforts.

Stevin Zorn, executive vice president for neuroscience research at Lundbeck and cochair of the workshop, charged participants to address the following questions:

- How can the pathway of sex differences research, from basic research to clinical relevance and ultimately, translation into effective medicines, be made more efficient?
- Can the efficiency of diagnostic and treatment strategies be improved by choosing diagnostic tests, drugs, and/or dosages that consider sex differences?
- Are there instances when the sex difference in effect or accuracy is large?

ORGANIZATION OF THE REPORT

The report that follows summarizes the presentations by the expert panelists, and the open panel discussions that took place during the workshop. This report is not intended to be a scholarly review but a detailed accounting of speaker presentations and commentary by panelists and workshop attendees.

An overview of the study of sex differences in biomedical research was provided by experts from four academic institutions and the NIH, and their presentations are summarized in Chapter 2. Discussion focused on the public health importance of studying sex differences in the nervous system, and the potential application of a stronger understanding of these differences to healthcare delivery. Participants discussed when sex differences should be and should not be considered, and how to design preclinical and clinical studies so that sex-based differences can be evaluated.

Chapter 3 provides highlights of four disease/condition-specific panel discussions. Experts discussed the implications of sex differences in translational research in depression, pain and pain perception, sleep medicine, and multiple sclerosis and neuroinflammation. These areas were identified by the planning committee as particularly relevant to the discussion. Issues common across these and other areas of neuroscience research were raised during an overarching discussion following the disease panels.

In Chapter 4, representatives from two key neuroscience professional journals discussed the reporting of sex differences in research publications, and current journal policies on analysis by sex in submitted manuscripts.

Chapter 5 summarizes the panel discussions of the regulatory and industry issues related to sex differences research. Morgan Sheng, vice president of neuroscience at Genentech, gave the keynote address at the workshop, offering an industry view of sex differences in translational neuroscience. Panelists from the FDA, the NIH, and industry discussed the history, guidelines, and regulations regarding the inclusion of males and females in clinical trials, and how and when industry considers and addresses studying sex differences, given current regulatory requirements.

Concluding remarks and discussion of practical next steps by participants are provided in Chapter 6, and the references, list of registered attendees, workshop agenda, and speaker biographies are available in the appendixes.

2

Studying Sex Differences in Health and Disease

In the first session of the workshop, experts from four academic institutions and the National Institutes of Health (NIH) discussed the public health importance of studying sex differences in the nervous system, particularly the potential application of a stronger understanding of these differences to healthcare delivery. Participants discussed the design of preclinical experiments and clinical studies, and the need to bridge between them. Knowing when sex differences should be considered is as important as knowing when they should not.

SCIENTIFIC PRINCIPLES FOR STUDYING SEX DIFFERENCES IN HEALTH AND DISEASE

Arthur Arnold, professor and chair of the Department of Physiological Science at the University of California–Los Angeles, stressed that basic science is the foundation for translation of knowledge about sex differences into clinical practice. Sex differences exist in the susceptibility to and progression of diseases. Identifying the sex-specific factors that protect one sex from a particular disease can guide development of therapies to protect both sexes from the disease.

Why Compare the Sexes?

A physician does not treat a sex difference, but rather treats one patient at a time, male or female. So why not simply study what works in each sex? Because knowledge of the physiology of one sex can provide fresh

perspective on the physiology of the other sex, Arnold said. One example of how studying sex differences can provide a new perspective is differential susceptibility. In humans, males die at a greater rate at every life stage than females, except at the oldest ages. This comparison leads to the question of how to lower mortality of males to match that of females. What protective factors exist in females that could be used to lower male mortality? Without comparison of the sexes, this question would not occur.

Another example is X-inactivation, a female-specific physiological process. Females inherit two copies of every gene on the X chromosome while males inherit only one copy of the X chromosome in addition to the Y chromosome. For normal female development to occur one X chromosome must be inactivated resulting in equivalent X chromosome gene product levels between males and females. This mechanism of dosage compensation (X-inactivation) can be understood through direct comparison between male and female gene product levels.

Investigators often only study sex-specific factors in one sex. Although this approach provides helpful information, it is also important to compare identical treatments in males and females to determine if responses are similar or different.

Our Evolving Understanding of Sex Differentiation

Ten years have passed since the publication of the Institute of Medicine (IOM) report on sex and gender differences in health, Arnold reminded participants (IOM, 2001). During that time, there has been a shift in the conceptual framework for explaining the proximate signals that cause sex differences, and increased consideration of the concept of compensation (the notion that some sex-specific factors make the sexes more equal, e.g., X-inactivation).

According to the traditional model for the physiologic basis of sex differences, the *Sry* gene on the Y chromosome causes testes to develop; and testicular secretions, such as testosterone, influence masculine body and brain development. In the absence of *Sry*, ovaries develop, testosterone is lacking, and a feminine body and brain develop. There are two major classes of gonadal hormone action. *Organizational (differentiating) effects* are permanent, such as the testosterone-induced irreversible commitment of a tissue to a masculine rather than a feminine phenotype. Organizational effects impact external and internal genitals, and brain circuits. *Activational effects* are reversible; the resulting sex differences in traits are caused by differences in secretion of sex steroids at the time of measurement and can be abolished by gonadectomy in adulthood.

Most sex differences, Arnold said, might actually be caused by activational effects. He cited a microarray study of sexually dimorphic gene

expression in mouse livers that found that expression of about 2,600 genes were sex biased in mice with gonads, but only 12 genes remained sex biased after gonadectomy (van Nas et al., 2009). Most genes therefore appear to be sexually differentiated or sexually dimorphic because of the action of hormones in adulthood.

All sex differences result from the imbalance of X and Y genes. These are the only genes in the fertilized egg cell and the zygote that are not sexually dimorphic. As noted above, gonadal asymmetry and gonadal hormone secretion as a result of *Sry* action in males leads to organizational and activational effects of hormones. Over the past decade, however, new evidence has emerged that sex chromosome genes act in non-gonadal tissues to cause sex differences in traits and disease. These non-hormonal actions lead to what are called *sex chromosome effects*. Arnold presented the "unified model" of sex differentiation, outlining these three classes of proximate factors causing sex differences in phenotype (Figure 2-1).

Some of these sex chromosome effects can be quite significant. To study these effects, researchers have developed a "four-core genotypes" mouse model in which the gene determining gonadal sex (*Sry*) was spontaneously deleted from the Y chromosome and through transgenic technology inserted into an autosome, so that the gonadal sex of the animal is no longer related to the chromosome complement. XX and XY no longer effect the gonads the animal develops, and four core genotypes result: XY gonadal males, XX

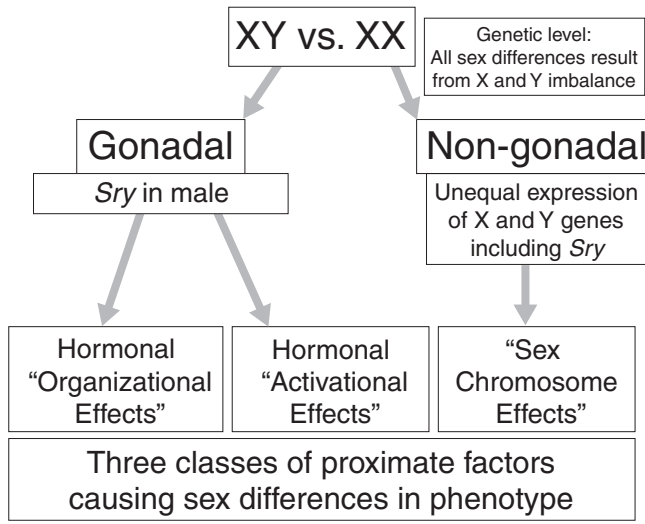

FIGURE 2-1 The unified model of sex differentiation.
SOURCE: Arnold (2009).

gonadal males, XX gonadal females, and XY gonadal females. This allows for separation of the sex chromosome and gonadal hormone effects.

One example of direct sex chromosome effects demonstrated using this model is that XX mice show a faster response to thermal nociceptive stimuli than XY mice, regardless of their gonadal sex. Another study suggests that the number of X chromosomes influences susceptibility to neural tube closure defects in a mouse model. Still other studies have shown that the sex chromosome complement contributes to sex differences in a mouse model of multiple sclerosis. The *Sry* gene itself is expressed in the brain, in the substania nigra, and influences the control of movement. As this gene is on the Y chromosome and only found in males, this effect can only be male specific.

Sometimes males and females express a similar phenotype because of different processes within the sexes. These sex-specific mechanisms cancel each other out and make the sexes more similar, such as X-inactivation. To understand the differences between males and females, we also have to understand that some of the similarities are actually based on differences that cancel out, Arnold said.

Clinical Implications of Hormonal Versus Sex Chromosomal Differences

In the search for factors in one sex that protect that sex from a disease, it is critical to understand whether the sex difference is caused by organizational, activational, or direct sex chromosome effects. Therapies directed toward genes will be different from therapies directed toward hormonal effect. If the difference is a genetic effect of X and Y genes, the gene needs to be identified and the mechanism of action targeted. If the sex-specific protection is caused by hormones, then therapies need to be targeted toward hormone-driven molecular pathways.

Next Steps

Although progress has been made, not only in the past 10 years but over the past 60 years, most animal models of disease-related phenotypes remain poorly studied with regard to sex differences. It is important to understand which of the three factors—organizational, activational, or direct chromosome effects—are important in causing observed sex differences. Animal studies are quite important because in humans, of those three factors, researchers can only ethically address activational effects by manipulation of gonadal hormones in adults. There are relatively few human models in which one can observe organizational effects of hormones, and almost no models in which one can separate the direct sex chromosome effects from endocrine effects.

To facilitate translation, more preclinical studies are needed to understand the basic biology of sex differences. For example, identify in animal models the X chromosome genes that cause sex differences, and then hypothesize studies in humans to see if those same X genes have an association with disease phenotypes.

Arnold also stressed the importance of educating both scientific grant program review staff and researchers about the importance of sex differences and how to study sex differences.

STUDYING SEX DIFFERENCES IN DRUG RESPONSE

Jeffrey Mogil, E. P. Taylor Chair in Pain Studies at McGill University, used pain and analgesia as a case example to illustrate when, how, and why sex differences in drug response should be studied. Even if one is not specifically studying sex differences, Mogil said, both sexes should be included in basic science experiments from the beginning. Adding to Arnold's review of how sex differences should be considered, Mogil referred participants to a consensus report for sex differences research specific to the domain of pain and analgesia (Greenspan et al., 2007).

Why Study Sex Differences?

One reason to study sex differences is that they are an important and known factor contributing to individual differences. Assessing pain in more than 8,000 mice using the tail-withdrawal test, researchers found an overall 0.4-second difference in tail withdrawal latency between males and females. The data could be thought of in one of two ways, Mogil said. One could be impressed by the nearly half-second difference in the overall range of about 8 seconds as explained by sex differences. A second way would be to attribute the difference to genetics and be unimpressed. Genes are certainly responsible for much of this observed difference, but which genes these are is still unknown. Instead, questions can be addressed within the context of explaining individual differences because the two variants (male and female) are known and there are methods to study them.

Another reason to study sex differences is that for many disease states, including pain, there is a sex difference in prevalence. Many common painful disorders are more prevalent in females than in males (Berkley, 1997). However, this epidemiological difference has not been fully utilized by basic scientists when experimental protocols are designed, Mogil said. As a consequence the biological underpinnings of this difference are not entirely known. Reviewing reports of rodent animal model studies published in the journal *PAIN* over a 10-year period, Mogil found that 79 percent of all papers used male subjects only (Mogil and Chanda, 2005). Another

5 percent of them used both male and female animals, but did not discuss whether there was a sex difference (presumably because no sex-based analysis was done). An additional 3 percent simply did not report the sex of the subjects (which likely means they were male). In total, 87 percent of these studies simply ignored sex differences, he said. A few pain researchers specifically study sex differences, but most in the pain field are not contributing to knowledge about sex differences at all because they only study male animals.

One reason for the lack of studies in female animals is the misconception that data from female mice are more variable than data from male mice. The variability is the same in males and females, and this is an empirical fact, Mogil said (Mogil and Chanda, 2005). Females do have an estrus cycle that adds a source of variability that males do not have. But there are male-specific sources of variability as well, such as dominance hierarchies and fighting among males.

Sex Differences in Pain Sensitivity

Sex differences in sensitivity to pain are not always reported in studies, but when they are, they almost always show that females are more sensitive to and less tolerant of pain, and better able to discriminate among different levels of pain (although the magnitude of the difference depends on the type of pain). In addition to these differences in sensitivity, there are important differences in pain processing mechanisms.

One evidence-based example is the male-specific involvement of the *N*-methyl-D-aspartate (NMDA) receptor and the apparently analogous female-specific involvement of the melanocortin-1 receptor (MC1R) in pain and analgesia. MC1R is involved in regulating skin and hair pigmentation. Mogil and colleagues (2003) compared female and male redheads to brunettes and found a female-specific genetic effect associated with kappa-opioid (pentazocine) analgesia. The same sexual dimorphism has recently been demonstrated in mice for opioid hyperalgesia (Juni et al., 2010). After chronic opioid treatments, instead of producing analgesia, morphine and other opiates start to produce hyperalgesia that can actually make chronic pain worse in both sexes, Mogil explained. However, in mice lacking a functional MC1R (essentially redhead mice), nothing changed in males while females experienced analgesia, but not hyperalgesia, suggesting that hyperalgesia in female mice was due to MC1R.

The Impact of Sex Differences in Pain Treatment

Dextromethorphan, the active ingredient in cough medicine and an NMDA receptor antagonist, potentiates morphine analgesia at low doses,

and attenuates it at high doses. To capitalize on this effect, a Phase II clinical trial of a drug called MorphiDex, a 1:1 combination of morphine and dextromethorphan, was conducted, but was an undeniable failure, Mogil said. As it turns out, the interaction between morphine and dextromethorphan cannot be demonstrated in females, at any dose of morphine or dextromethorphan. This information was unknown until this clinical trial was conducted because not one of the nearly 100 animal studies conducted over the prior 10 years had included females. In a subsequent conversation with the drug developer, Mogil was told that while women were included in the study, they did not analyze the clinical trial data by sex and did not intend to as they had become focused on other priorities. Mogil postulated that it is possible this clinical trial in humans failed because the drug worked in men, but not in women, and the results cancelled each other out. As a result, a drug that might have potential use for men will not be developed further.

As another example, the Toll-like receptor 4 (TLR4) is involved in neuropathic pain development. Male C3H/HeJ mice without functional TLR4 receptors have reduced mechanical allodynia, which is a symptom of chronic pain, but females have normal mechanical allodynia. Current studies suggest that the role of TLR4 in pain is, in fact, entirely male specific. This will be very important to elucidate as TLR4 antagonism is currently of great interest in analgesic drug development.

STUDYING SEX DIFFERENCES IN DISEASE SUSCEPTIBILITY

Kathryn Sandberg, director of the Center for Study of Sex Differences at Georgetown University Medical Center, discussed when and how sex differences in disease susceptibility should be studied (summarized in Box 2-1).

When to Study Sex Differences

An obvious situation in which sex differences should be studied is when there is a difference in anatomy, Sandberg said. Even though there are no disparities in general intelligence, when differences in brain size are taken into account, women have ten times more white matter while men have almost 7 times more gray matter as related to intellectual skill. This suggests a significant sex difference where intelligence is manifested. Because function often follows structure, these sex differences in neuroanatomy need to be understood.

Better understanding of known sex differences in nervous system physiology may improve care after injury. For example, functional magnetic resonance imaging conducted while participants listened to a book being read aloud showed that in women, both sides of the brain were active, while in men, only one side was active. Both were listening, but through different

BOX 2-1
When and How to Study Sex Differences

Study the impact of sex differences when they exist in

- Anatomy
- Physiology
- Incidence and age of disease onset
- Symptoms impacting diagnosis of disease
- Disease severity, progression, and outcome
- Response to treatment

Study sex differences across the lifespan by expanding the number of experimental models that can address

- Aging
- Disease susceptibility
- Sex chromosome dosage

NOTE: This box summarizes remarks made by workshop participants.

physiological mechanisms. Other studies show that men and women use different mechanisms to navigate; women rely more on landmarks whereas men prefer compass directions. Furthermore, while men use both the right and left hippocampi when navigating, women only use the right hippocampus. Instead of using the left, women invoke the aid of the right prefrontal cortex. The disease implications of these functional brain differences are significant. It is easy to see how a stroke in a sexually differentiated brain region could result in very different outcomes for each sex.

Differences in disease prevalence or age of disease onset are another instance when sex differences should be studied, Sandberg said. Using stroke as an example again, males have a higher incidence of stroke across much of their lifespan; however, after age 80, women have a higher incidence of stroke. A better understanding of what makes men in their 40s more susceptible to stroke while women are protected may lead to better therapies or preventive measures for both sexes.

Studying sex differences may provide an important health benefit when there sex-specific symptoms. The commonly known symptoms of stroke, for example, include sudden numbness of one side of the body (face, arm, or leg), difficulty speaking or understanding, inability to see out of one or both eyes, difficulty walking including dizziness or loss of balance, and a severe headache. But these symptoms do not present equally in males and females. Women have less loss of balance and coordination, and more

changes in mental status (confusion, unconsciousness) than men. Women also have more nausea and heart attack-like symptoms, and tend to present with more severe headaches. These symptoms are not unique to women, Sandberg clarified, but they are experienced more often by women than men. These sex differences have the potential to negatively impact diagnosis and consequently recovery if emergency room personnel are not trained to recognize sex-specific symptoms.

Sex differences in the type of stroke also occur. Men have more atherosclerotic strokes (68 percent of men versus 19 percent of women), which is perhaps related to the fact men have a higher prevalence or an earlier onset of atherosclerosis than women, whereas women have more cardioembolic type of strokes. Clearly, there are underlying mechanistic differences behind these sex differences that need to be studied that may lead to better targeted preventive therapies.

Studying sex differences may also shed light on disease severity, progression, and/or outcome. Following a stroke, women are institutionalized for longer periods of time, and have lower functional recovery. Although increases in length of institutionalization could be related to the fact that women live longer than men, when the age difference is ruled out of the analysis, there remains something inherently different between the sexes that does not explain the lower functional recovery observed in women. We must learn why this is the case.

Potential differences in responses to therapeutic intervention provides another important reason to study sex differences. Aspirin has been shown to be cardioprotective in men, but it does not reduce the incidence of myocardial infarction in women; however, aspirin does decrease the incidence of stroke in women. At a basic science level, with the majority of studies still conducted only in male animal models, drug development is inherently biased toward what works well in males, suggested Sandberg. Furthermore, because Phase I and II clinical trials do not require sufficient numbers of women to assess sex differences in safety and efficacy, sex differences in treatment responses only become obvious when large clinical trials take place. Thus research bias may, in turn, bias drug development leading to better treatments in men and obscuring potential adverse drug side effects in women.

How to Study Sex Differences in Disease Susceptibility

Sex differences must be studied across the entire lifespan, Sandberg said. Recall that women appear protected from stroke until their mid-80s, when their incidence of stroke surpasses that of men. As another example, asthma peaks early on, between ages 2 and 10 in boys, and is more prevalent in boys than girls. However, adult women have a higher incidence of

asthma than adult men. These age-specific differences suggest the need to study asthma across the lifespan instead of during a single time point to better understand the mechanisms.

Experimental models need to be expanded and improved, as most do not take into account the significant hormonal differences between males and females, and the changes over the lifespan of each. Aging can also affect the processes of disease and should be considered in animal models, as most experimental models focus only on young animals. Better models for disease susceptibility are also needed. For example, to study stroke using the Dahl salt-sensitive rat model, animals are kept on a low-salt diet for one year and then ovariectomized after which blood pressure rises and the animals start to have strokes. Gonadally intact young animals are not hypertensive and do not have strokes on the low-salt diet. Waiting a year before experiments can be done in this model is expensive. Finally, the impact of sex chromosome dosage should be studied. Sandberg referred to her recent study results using the four-core genotype model, described by Arnold (above), in which she found sex chromosome effects on blood pressure that were independent of the sex of the animal (Ji et al., 2010).

In Sandberg's conclusion, she noted that just because a sex difference is not apparent does not mean it is not there. Analysis of sex differences should always be done, and sex differences should be studied across the lifespan by developing and expanding experimental models.

OFFICE OF RESEARCH ON WOMEN'S HEALTH AT NIH

Federal attention to the issues of sex differences in health began in the late 1980s with a focus on the inclusion women in clinical studies, explained Vivian Pinn, director of the Office of Research on Women's Health (ORWH) at the NIH. Advocates raised concerns that clinical research on conditions that affect both women and men was being conducted primarily in a homogeneous white male population, but results were then applied in medical practice to both men and women of all races. This drew the attention of the Congressional Caucus for Women's Issues and led to the establishment of the ORWH, which was charged with ensuring that women were included in clinical studies in a way that the results of the research could compare the effects of the intervention on both men and women. The NIH instituted policies on inclusion that were subsequently incorporated into public law.[1] As a result, all clinical studies funded by NIH, with rare exceptions, must include women and minorities, and Phase III (see http://www.nlm.nih.gov/services/ctphases.html) clinical trials must be designed to

[1] The *NIH Revitalization Act of 1993* (Public Law 103-43), section on "Clinical Research Equity Regarding Women and Minorities."

facilitate "gender analysis," that is, to be powered so that valid analysis of potential sex differences can be accomplished.

The intent of the law and associated policies was to determine whether the outcomes studied would apply equally to both males and females. However, some of the language of the law has raised issues, Pinn noted. For example, as originally drafted, the law said that a "statistically significant difference" must be demonstrated by the research. This raised the concern that demonstration of statistically significant results pertaining to women and members of minority groups and their subpopulations would present difficult challenges in defining and enrolling study populations. Eventually the terminology that was agreed on called for a "valid analysis" to be conducted to determine whether or not there might be a significant difference, defined as a difference that is of clinical or public health importance based on substantial scientific data.

Pharmacokinetics and pharmacodynamics have known and suspected sex-specific aspects, Pinn said, that can influence absorption, metabolism, and excretion of drugs. Following the establishment of the Food and Drug Administration (FDA) *Guideline for the Study and Evaluation of Gender Differences in Clinical Evaluation of Drugs* (FDA, 1993), the Office of Women's Health at the FDA and the NIH ORWH developed an online course on the science of sex and gender in human health, which was made available at no charge.[2] The course was designed to provide a basic scientific understanding of the major physiological differences between the sexes, the influence of these differences on health, and the policy, research, and healthcare implications.

Early on, one of the major areas that ushered in the establishment of analysis of research results by sex was cardiovascular research. Despite the fact that heart disease is the leading cause of death for U.S. women, a 2003 report from the Agency for Healthcare Research and Quality (AHRQ), supported by the NIH ORWH, found that only about 20 percent of evidence-based articles reporting coronary heart disease studies that included women actually provided separate findings for women (AHRQ, 2003). The report recommended that, in addition to requiring the inclusion of women and minorities in research, the results of that research should be published or made easily available. A subsequent review of the literature for publication of sex-specific results in 2007 recommended that journal editors require authors to provide sex-specific data; the review found that 51 percent of NIH-funded trials, and only 22 percent of non-NIH trials, reported outcomes analyses by sex (Blauwet et al., 2007). Although NIH could require analysis by sex in final progress reports of the studies it funded, it had no role in journal editorial policies. The *Journal of the National Cancer Insti-*

[2] See http://sexandgendercourse.od.nih.gov/.

tute, however, has incorporated into its editorial policy a recommendation that clinical and epidemiologic studies should be analyzed to see if sex has an effect, and if not, that should be stated in the results. Furthermore, as discussed in Chapter 4, some neuroscience journals are considering similar changes to their editorial policies.

Basic Research: Sex at the Cellular Level

A significant barrier to the progress of research on sex difference in health, Pinn said, is that many in the scientific and policy communities and funding agencies still think of sex-specific research as being exclusive to clinical research. In fact, research on sex differences at the molecular and the cellular levels is very much needed. A 2001 IOM consensus study called *Exploring the Biological Contributions to Human Health: Does Sex Matter?* helped draw attention to this issue (IOM, 2001). This report discussed the need for new knowledge about biological differences or similarities between the sexes, and the translation of information on sex differences into preventive diagnostic and therapeutic practices to improve healthcare and patient outcomes.

Before addressing sex differences in basic research, the IOM committee defined the use of the terms *sex* and *gender*. These terms are often used interchangeably, but to be scientifically correct, Pinn said, *sex* should be used to refer to those biological functions assigned by one's chromosomes. In contrast, *gender* is a social construct—how people represent themselves, influenced by biology and shaped by their environment and experience (IOM, 2001).

The report concluded that being male or female is an important, basic human variable, and that sound medical research and treatment must account for sex and gender differences and similarities. Not all sex differences are due to differences in the hormonal milieu. Every cell has a sex and sexual genotype (i.e., XX for females and XY for males), which can effect the pathophysiology and prevalence of some diseases. Sex also affects behavior and perception. Without question, sex clearly affects health. Thus, expanding the understanding of sex differences at the cellular level will offer key insights into underlying biological mechanisms of health and disease.

Challenges

Pinn highlighted some of the challenges to progress in sex differences research that she has observed across NIH programs and working groups (Box 2-2). She noted that even though the NIH has an inclusion policy in place, the ORWH sees a need for continued emphasis on the importance of conducting clinical analyses by sex. She also pointed out that the law man-

BOX 2-2
Challenges to Future Progress in Sex Differences Research

- **Clinical trials:** Getting sex differences research accomplished *and* results reported.
- **Basic research:**
  - Many researchers are not aware of the pervasiveness of sex differences.
  - There are concerns that trying to control for all variables (e.g., hormonal cycles) may result in impractically large experiments.
  - Experimental standards and phenotypes historically have been defined using male animals.
- **Translational research:** Facilitating collaboration and awareness among basic, clinical, and industry researchers about the needs of each and improving understanding about the relevance of sex differences research.
- **Interdisciplinary research:** Bridging basic and clinical science, interdisciplinary work is translational by nature, but is complex, may be expensive, and does not fit into typical academic silos:
  - New models of collaboration, institutional support, and metrics are needed.
- **Education, communication, and public engagement:** Making the case for the importance of sex differences research to health without stigma.
- **Research resources and emerging technologies:** How best to collect, analyze, and apply knowledge about sex differences.

dating inclusion is applicable only to studies funded by the NIH, and not to those funded by industry, foundations, or patient advocacy organizations.

NIH Funding Initiatives

The NIH has numerous funding initiatives, and one in particular that has helped to make a difference in the area of women's health research is the funding of Specialized Centers of Research (SCOR) on sex and gender factors affecting women's health. These interdisciplinary centers must cover the span of basic, translational, and clinical projects. Pinn highlighted several examples of SCOR research areas, such as the role of sex differences on stress responses and addiction (Goldstein et al., 2010; Quinn et al., 2007).

Another recently implemented funding mechanism is Advancing Novel Science in Women's Health Research, which is designed to fund new areas of sex and gender research (e.g., sex differences in complications in diabetic

neuropathy; acute pain and analgesic responses; myocardial ischemia; and expression and function of regulatory T-cells in lupus). As important as these initiatives are, Pinn stressed the need for more investigator-initiated research and engagement by the private sector to also integrate sex differences into their research and development portfolios. Pinn also highlighted findings from a technologies bioengineering and imaging working group, which noted that many technologies are not applicable to women because the technologies were developed and standardized based on studies conducted primarily in male subjects, including male animals.

MOVING INTO THE FUTURE: NEW DIMENSIONS AND STRATEGIES FOR WOMEN'S HEALTH RESEARCH: NEUROSCIENCE WORKING GROUP

In planning its research agenda for the next decade, the ORWH held a series of national conferences entitled Moving into the Future: New Dimensions and Strategies for Women's Health Research. In October 2009 Jon Levine of the Department of Neurobiology and Physiology at Northwestern University cochaired a working group on neurosciences, which focused on the need to better understand sex differences in brain development, structure, and function.[3]

Levine provided an overview of the working group's findings on sex differences in the brain, and translational research in neuroscience. The working group focused on two core issues: (1) translation of findings in basic neuroscience research to clinical research in practice, and (2) absence of focus on sex differences in brain function and dysfunction in basic and clinical neuroscience research.

Barriers to Progress

The working group identified three basic areas that are fundamental to translation—the scientific process, administration of science, and social and cultural aspects of the enterprise—and sought to understand the barriers to successful translation.

Many scientists entering graduate and medical school programs often do not have a basic understanding about the role sex has in the biology of disease states. In the neurosciences this is often perpetuated due to the absence of sex differences in brain function as an integral topic in neuroscience graduate programs and medical school neuroscience courses. There is also a lack of recognition of the importance of sex differences in brain func-

[3] Discussed further by Levine, below.

tion and disease within the scientific community at large, and as it relates to grant reviews and funding decisions.

Furthermore, basic scientists, clinical researchers, and industry find themselves working in parallel universes. Although progress is being made on understanding sex differences in all three spheres, cross-talk is limited. In addition, often some basic science researchers focus on the study of sex differences in general, and others study diseases that are sexually dimorphic in either presentation or responsiveness to drug therapies. Ultimately, better communication and collaboration is needed between those who are specifically investigating sex differences in brain function and those who are not.

The limitations of current animal models present another challenge. Great progress has been made using animal models, for example, in understanding how genes and hormones direct sexually differentiated function in adulthood. But, Levine said, there has been limited feedback from clinical and basic science studies in humans to validate the current animal models of human brain diseases. It is not at all clear if some of the same basic sexual dimorphisms studied in animal models parallel those in humans.

Neuroscience Working Group Recommendations

Levine reviewed the set of six recommendations that the neuroscience working group provided the NIH ORWH following the October 2009 session (Box 2-3).

PUBLIC HEALTH IMPORTANCE OF STUDYING SEX DIFFERENCES

This session emphasized the significant impact that increasing basic scientific knowledge and examination of sex differences would have on public health initiatives. Critical to these efforts is the need for closer evaluation of underlying causes of sex differences in disease prevalence, age at onset, severity of progression and symptom presentation. Participants highlighted several potential outcomes that would have a direct influence on public health including identification of better drug targets. In addition, greater awareness of sex-specific symptoms would decrease incidents of emergency room misdiagnosis and improve standards of care. Overall, targeted inclusion of both sexes in current research programs will directly improve public health.

BOX 2-3
Office of Research on Women's Health
Neuroscience Working Group Recommendations

1. Promote recognition and understanding of sex differences in brain function and brain disorders in neuroscience graduate and medical school training curriculums.
2. Convene a panel of experts to make recommendations to the National Institutes of Health Peer Review administration on the inclusion of female subjects and/or focus on sexually differentiated brain function and disease in basic neuroscience research.
3. Develop new paradigms to study the epigenetic influences impacting development of neurological and psychiatric disorders. What determines sex-specific or sex-biased brain disease vulnerability, course, and/or response to therapeutics?

    - Develop experimental paradigms that model sex-specific or sex-biased experiential, hormonal, and psychosocial effects on gene expression, and intra- and intercellular signaling properties in the brain.
    - Develop and use high-throughput epigenomic approaches to characterize the large-scale epigenetic alterations associated with experience and related to sexually differentiated brain function and disease.

4. Develop new paradigms and molecular genetic approaches to study the impact of experience, hormones, developmental stage, and aging on sex differences in steroid hormone signaling in vivo; these could include new generations of transgenic and gene targeting approaches.
5. Develop and support new approaches to define similarities and differences in sexually differentiated brain function and disease in human and animal models, for example, through the use of comparative imaging of sexually differentiated brain function.
6. Develop new methodologies for targeted imaging and application of pharmacological agents to sexually differentiated cell populations in the brain.

3

Studying Sex Differences in Translational Research: Examples from Four Major Disease Areas

Following the introductory presentations on the challenges and opportunities for studying sex differences in neuroscience research, four specific disease areas within neuroscience were discussed in greater detail: depression, pain and pain perception, sleep medicine, and multiple sclerosis and neuroinflammation. These areas were identified by the planning committee as particularly relevant to the discussion with known sex differences and therefore areas with potential for critical advances. In addition, these diseases have very different etiologies and thus allowed a broad overview of many different mechanisms. (Key points of the presentations in each disease area are provided in boxes at the end of each set of panel presentations, Boxes 3-1 through 3-4.)

DEPRESSION

Characterization of Sex Differences in Depression

In science, we seek to define variables on which populations are similar to and differ from one another, said Katherine Wisner, director of Women's Behavioral HealthCARE at the University of Pittsburgh Medical Center. Two sexes provide a source of "variable partitioning" that creates a natural opportunity for comparative investigation. Disease states have been studied for a long time, considering a variety of different impacting factors (e.g., environment). The task at hand is to look at disease states by sex or gender across the lifecycle, and harness that information for treatment. The

ultimate question is whether treatments will be optimized by incorporating sex and gender principles into interventions.

Women are 1.5 to 2.5 times more likely to experience major depression than men, from puberty onward. Women have the highest prevalence of depression during the childbearing years and are also at increased risk during the perimenopausal period (the 5-year period before the cessation of menses). According to a World Health Organization study, depression is the leading cause of days lost to disability for women worldwide. Depression is often discussed as if it was a homogeneous illness, but further study is needed into the different subtypes of depression and how they vary by sex and gender.

Wisner cited one recent study that included sex-specific analyses is the STAR*D (Sequenced Treatment Alternatives to Relieve Depression) study, a large-scale clinical trial of multiple depression treatments (Marcus et al., 2005). The investigators found differences in symptoms between the two sexes. Depressed women experienced more anxiety, physical somatoform symptoms, and bulimia. For men, the symptoms concurrent with depression tended to be obsessive-compulsive symptoms and substance use. Depressive episodes were longer in women than men, and suicide attempts occurred more frequently. Interestingly, women were more likely than men to have remission (loss of all symptoms) in response to the drugs under investigation (30 percent of women compared to 24 percent of men), and half of women responded (had symptom reduction) compared to 44 percent of men. The question facing clinicians now is how to apply this information.

Individualized, personalized treatment for depression and other psychiatric illnesses is a primary goal of translational research. In addition to sex and gender, individuals vary with regard to symptoms, comorbidities, clinical factors, personal history, family features, social background, genetic polymorphisms, developmental stage, and characteristics identified from brain imaging or other technologies. Differences in the longitudinal development of males and females also naturally provide a variety of hormonal conditions under which to study sex differences as well as the hormonal changes that are unique to females: in utero differentiation, menarche, the premenstruum, pregnancy, postpartum, and menopause.

When considering a disease state or a process, there is a broad biological-to-societal spectrum of distal health determinants that fluctuate throughout an individual's lifetime; from basic genetics, to gene–environment interactions, to the physical and social environments (e.g., which pollutants or other stressors an individual is subjected to often vary by gender) (Misra et al., 2003). Proximal determinants, including biomedical responses (e.g., nutritional status, inflammatory response) and behavioral responses (e.g., alcohol use, actively practicing a religion) impact the disease process acutely. Health outcomes are influenced by these distal and proximal determinants, as well as by inputs and processes such as health care.

Wisner closed noting that the so-called "valleys of death" in clinical and translational research are, in fact, valleys of opportunity. Mechanisms such as the Specialized Centers of Research and Building Interdisciplinary Research Careers in Women's Health programs are bringing people together to eliminate these valleys. Questions to be addressed when translating neuroscience research are whether there is enough of a sex difference to merit changing the way medicine is practiced to accommodate those differences, and if so, how to train individual practitioners to consider these differences in practice.

Fetal Antecedents to Sex Differences in Depression

Jill Goldstein, director of research at the Connors Center for Women's Health and Gender Biology at Brigham and Women's Hospital and professor of psychiatry and medicine at Harvard Medical School, discussed fetal hormonal programming of sex differences in the brain, and its role in understanding sex differences in depression.

The incidence of major depressive disorder has an approximately 2:1, female-to-male ratio. Furthermore, depression is comorbid with several chronic diseases, including the fact that the comorbidity of depression and cardiovascular disease is the fourth leading cause of morbidity and mortality worldwide. Goldstein and colleagues are currently testing the hypothesis that there are shared etiologies associated with understanding sex differences in depression and cardiovascular disease; that they are initiated during the sexual differentiation of the brain; and that they involve disruption of the fetal hormonal programming of the brain, which leads to endocrine disruptions throughout life, and sex differences in adulthood in these chronic diseases.

Throughout life windows of opportunity are available for studying sex differences in these disorders, Goldstein said. These occur when the brain and the body are flooded differentially with hormones: fetal development, puberty, pregnancy, perimenopause, and menopause. Although depression and cardiovascular disease are, for the most part, adult-onset disorders, they have developmental precursors, and considering this lifespan perspective is important.

Some risk factors for depression that have been identified from population-level studies include small for gestational age; low birthweight; obstetric complications (e.g., preeclampsia, oxygen deprivation); second trimester influenza; and second to third trimester famine. Population-level studies in the field of cardiovascular risk and hypertension have identified some of the same fetal risk factors for cardiovascular disease: small for gestational age; low birthweight; preeclampsia; and maternal prenatal famine.

Studies on the fetal programming of cardiovascular disease have focused on prenatal and early life stress and the disruption of the hypothalamic pi-

tuitary adrenal (HPA) axis in development. The HPA axis is also the focus of studies on the fetal programming of sex differences in depression.

Timing is critical for understanding sex effects, Goldstein said. Population-level studies that have looked at first trimester factors have found fewer sex differences in the incidence of these disorders than those that looked at second and third trimester factors. This may, in part, reflect the fact that hormonal regulation of the sexual differentiation of the brain starts at the beginning of second trimester, when testes begin to secrete testosterone, which has direct and indirect effects (through aromatization into estradiol) on brain sexual differentiation. In addition, as described by Arnold (see Chapter 2), prior to gonad differentiation, genetics play a critical role in sex differentiation.

Estrogen and testosterone have major effects on neuronal growth and development. These effects are not all over the brain, but are region specific, in areas such as the hypothalamic and amygdala nuclei, the hippocampus, medial dorsal thalamus, and areas of the cortex. Although much of the previous work on the sexual differentiation of the brain has been conducted in animals, magnetic resonance imaging (MRI) of healthy human brains shows that brain regions affected by sex hormones during development are highly sexually dimorphic (i.e., exhibit sex differences in brain volumes relative to the size of the cerebrum) (Goldstein et al., 2001).

Brain imaging studies of depression show crossover between those brain regions that are normally highly sexually dimorphic and those that are implicated as abnormal in depression, including the paraventricular nucleus, lateral hypothalamic area, hippocampus, and areas of the amygdala. Imaging studies of central nervous system (CNS) control of the autonomic nervous system show that some of those same brain regions are also important for regulation of the heart. This, Goldstein said, is the basis for her studies on shared etiologies for depression and heart disease.

Studying the stress response circuitry is a model system for the study of hormonal regulation of the brain and of the impact on major depressive disorder, Goldstein explained. Stress response circuitry crosses over with mood regulation, control of the HPA axis, and brain regions that regulate heart and blood pressure through autonomic nervous system function, such as the hypothalamic paraventricular nucleus and hippocampus. To characterize the hormonal phenotype in response to stress using this model, blood was collected and heart rate was monitored while an individual was lying in the MRI scanner, viewing a visual stress response challenge. The responses of the brain to low- and high-arousal pictures (e.g., a cow in a green field versus a serious car crash) were compared. Results showed that the stress response circuitry in the healthy brain activates differently at different points in the menstrual cycle, and those hormonal differences contribute to explaining sex differences in stress response circuitry activation (Goldstein

et al., 2005, 2010). The physiology of male and female healthy brains is different, and findings show that male and female brains act differently to maintain homeostasis with regard to one's response to stress.

Stress response circuitry function is abnormal in women with recurrent depression. Furthermore, in depression, there is a lower parasympathetic control, which can be operationalized as the high-frequency component of heart rate variability, and which has been found to be significantly associated with estradiol in women. Thus, brain activity deficits, hormonal deficits, heart rate and autonomic nervous system function, and sex differences in the brain are all highly related to each other and increased understanding will contribute important new knowledge regarding depression and its comorbidity with major general medical diseases, such as cardiovascular disease.

Goldstein is now looking at the shared fetal antecedents to sex differences in depression and risk for cardiovascular disease using the National Collaborative Perinatal Cohort developed in the 1960s. This study initially followed a New England cohort of 17,000 women in Boston and Providence through their pregnancies, and their children for 7 years after birth, until study funding ran out. Over the past 20 years, study participants (who are now adults) have been re-recruited and interviewed, and a subsample have been brought to the brain imaging center, facilitating human studies of fetal antecedents to brain, hormone, and heart regulation phenomenology and risk for different psychiatric and general medical diseases.

In a separate study, Goldstein and colleagues are following 300 discordant sibling pairs, one of whom has been exposed to fetal growth restriction or to preeclampsia, and the other as the unaffected control. As an example, one initial finding shows that healthy adult males who were exposed to fetal growth restriction or preeclampsia have significantly less parasympathetic control of the heart than females.

In conclusion, Goldstein stressed that understanding sex differences in depression and its comorbid conditions is absolutely critical for sex-specific drug discovery and development. One must take a life-course perspective for understanding the medical implications of sex differences for both the healthy brain and for models of disease. Taking a brain–body approach for understanding the impact of sex differences in the brain will be fruitful for new sex-specific drug discovery and other treatment modalities. Finally, clinical and population-level research is critical for informing the development of basic animal models and vice versa.

Sex Differences in Translational Studies of Major Depression

Etienne Sibille, associate professor in the Translational Neuroscience Program at the University of Pittsburgh, explained that major depression

is a heterogeneous syndrome that is characterized by chronic low mood. Mechanistically, depression is a chronic, recurrent disease known to be influenced by genes and environment. The higher prevalence of depression in women is cross-cultural, and is probably one of the most robust findings in all of psychiatric epidemiology, Sibille said, and yet this finding often is not considered in basic studies.

Perspectives differ on the origin of sexual dimorphism in depression. A *societal perspective* focuses on the interaction between increased victimization and female character traits. In the *Darwinian perspective* on the adaptive role of mood, mood is defined as emotion over time that is less dependent on immediate triggers. Low or high mood is a source of information about goal achievement and serves as a regulator of effort and energy allocation. For example, behavior inhibition associated with low mood is an adaptive response that saves resources in the face of unachievable goals or potential negative outcomes. Low mood, under normal conditions, is critical in strategy reassessment. Under this definition, based on sexual selection theory, women allocate more effort and energy in long-term reproductive goals and are more sensitive than men to negative outcomes about lifetime strategies in the context of normal mood regulation. In the Darwinian perspective, depression is a chronic maladaptive state of mood dysregulation. For reasons as yet unknown, the female system is evolutionarily more at risk of a maladaptive state. The *biological perspective* seeks to determine if increased female vulnerability to develop depression is due to sex hormones in early development (organizational) or in adulthood (activational).

For translational studies, mood states (e.g., anxiety-like and antidepressant-like behaviors) can be modeled in animals, including rodents. Mood regulation neural networks are conserved across mammalian species. Still, the primary pathology of depression is poorly characterized because there are numerous limits with current animal models. The models are often oversimplified; there is poor conceptualization of baseline traits versus induced depressive-like states; conceptualization of syndrome versus single behavior is poor; and little consideration is given to sex differences. Specific concerns include differences between behavioral tests, genetic models designed to characterize a trait, and animal models that induce depressive states. The forced swim test as a behavioral animal model of depression, for example, is not a really a model at all, but rather a single behavioral response. Its only value is predictive validity for short-term response to antidepressants. Genetic models are generally very good, but we must recognize that often, what is reported is the impact of the lack of a specific gene on traits. These are not multisystem models, but a single entry into complex disease.

Sibille described her work with the unpredictable chronic mild stress (UCMS) model, which induces a depressive-like state in mice that mimics, in a naturalistic way, both the role of stress in precipitating depressive

pathology and the time frame of therapeutic response to antidepressive treatment. Mice are subjected to an unpredictable regimen of mild psychosocial stressors such as forced bath, predator's song or smell, tilted cage, or social stress. Over 4 to 6 weeks, they develop a syndrome, or a collection of symptoms, including measurable outcome of behaviors that relate to emotions (e.g., increased anxiety, increased depressive-like behavior), increased anhedonia-like behavior). Physiological changes also occur, such as decreased weight gain, reduced quality of coat, and neuroendocrine changes. One study using this model has also shown cardiovascular changes. After onset, this syndrome can be blocked by chronic application of antidepressants. Using the UCMS model, Sibille and colleagues have shown that emotionality (expressed as Z score) is much higher after stress in female subjects than male. In another test, female mice genetically altered to express low levels of serotonin transporters are more vulnerable to chronic stress than male counterparts (Joeyen-Waldorf et al., 2009).

This model has also been used to test the translational hypothesis that the molecular pathology of altered mood regulation will manifest as conserved gene changes across species. Using large-scale gene expression data from human postmortem brain analysis, researchers have shown that changes in the amygdala of depressed human subjects actually predict what is observed in the amygdala of chronically stressed mice, and vice versa (Sibille et al., 2009). A set of 32 core genes has been identified that form a tight gene network, which is structurally conserved across species. This, Sibille said, suggests that in the context of depression or chronic stress, existing cellular pathways are abnormally recruited.

In summary, Sibille said, in the evolutionary context of mood regulation, these findings suggest that sexual dimorphism in biological mechanisms of depression should be expected. Animal models are associated with considerable limitations, at the levels of both concept and interpretation. UCMS could serve as an appropriate model of the human syndrome, and rodent findings parallel sex differences of human depression, setting the basis for development of realistic studies of sexual dimorphism. Ultimately, evidence shows sexual dimorphism in the primary pathology of depression in humans.

Industry Perspective on the Implications of Sex Differences for Translational Research

Carla Canuso, senior director of external innovation, Neuroscience Therapeutic Area at Johnson & Johnson, provided an overview of how and when industry considers sex differences, particularly in antidepressant drug development, during each phase of development, from preclinical through postmarketing.

Industry is necessarily concerned with regulations and guidance from the Food and Drug Administration (FDA) and regulatory bodies around the world. The FDA issued guidance in 1993 about the inclusion of women in the clinical evaluation of drugs, lifting the restriction on the participation of women of childbearing potential in Phase I and early Phase II trials (see http://www.nlm.nih.gov/services/ctphases.html), even before the completion of all animal toxicology studies. This placed greater onus on investigators to employ strict inclusion criteria regarding the use of birth control or abstinence, as well as strict guidance for pregnancy monitoring, and put more responsibility on institutional review boards to monitor clinical protocols. The intent was to have fair balance and representation of both sexes in the study so the data could be analyzed to detect any clinically significant differences. The guidance also addressed the assessment of demographic differences in pharmacodynamics in Phase I and II studies. Interestingly, Canuso said, the guidance specifically noted that the effects of menstrual cycle on pharmacokinetics should be evaluated when feasible, but this is not routinely done.

The European Medicines Agency (EMEA) does not have specific guidance on the inclusion of women, but EMEA did conduct a recent review of International Conference on Harmonisation (ICH) guidelines to determine whether special guidance for inclusion of women was needed. EMEA concluded that the current ICH guidance is sufficient to address the special needs of women and, in a review of recent clinical trials, found that women were adequately represented.

Canuso concurred with the previous speakers regarding the limitations of animal models, which are necessary for drug development. Animal models show sex differences in depression and stress, and that these differences in stress response are related to differences in corticotropin-releasing factor and serotonin neurotransmission. These are core regulators of mood and the coping response. The vast majority of preclinical studies done in the pharmaceutical industry are done in males, partly because of variation across the estrous cycle in laboratory animals. Nonetheless, studies are rarely replicated in females of the species, Canuso said. Preclinical studies have poor predictability of sex differences with respect to clinical response and toxicology, including reproductive toxicology, teratogenicity, and carcinogenicity.

Despite the 1993 FDA guidance, the vast number of participants in Phase I clinical trials are male, Canuso said. Reasons include the logistical challenges of birth control for women participants (e.g., double-barrier methods, the need to be on oral contraceptives for 3 months prior to entry into the study) and the lengthy informed consent process for early phase studies of drugs in women. The pharmacokinetics of drugs differ between women and men, not just because of body weight or volume of distribution, but also hormonal interplay. Also, drug–drug interaction studies must be

done for coadministration of antidepressant drugs, which are substrates or inhibitors of the cytochrome P-450 system, and drugs women commonly take that are metabolized by the P-450 system (e.g., oral contraceptives, tamoxifen).

Throughout all phases of clinical development, the consideration of sex differences should include designing studies to be appropriately enriched for women; requiring birth control or abstinence; pregnancy reporting; data analysis using sex-specific laboratory ranges; and studying sex-specific pharmacodynamic responses and adverse drug reactions.

Other sex-specific factors are considered for proof of concept and pivotal trials conducted for product registration. Products may have sex-specific indications (e.g., premenstrual dysphoric disorder; vasomotor symptoms associated with menopause; postpartum depression), or have been developed for use in only one sex (e.g., a safety concern in the opposite sex). As a result of the 1993 FDA guidance, inclusion of women in Phase II and III studies is generally adequate, and subgroup analyses by sex is included in labeling.

Finally, sex differences also come into play in Phase IV and postmarketing studies. Populations of interest are studied further, such as those with comorbidities. Investigator-initiated studies are conducted by academic researchers. Epidemiological studies are used to revise labels as new information comes to light following widespread use. Pregnancy registries are also used more often.

In closing, Canuso offered several ways industry can foster translational research in neuroscience, as follows:

- Partner with academia to advance the science of personalized medicine, while considering sex and gender in every phase of drug development so that differential responses in dosing, efficacy, and safety can be fully appreciated.
- Partner with academia to develop and validate better preclinical animal models that are truly predictive of the diseases, and then study both sexes of the species in those models.
- Identify and evaluate sex-specific endophenotypes and other biomarkers, such as increased stress sensitivity.
- Identify moderators and predictors of disease, specifically those that may confer resilience.
- Establish multisector collaborations across industry, academia, and the National Institutes of Health (NIH) and create data-sharing mechanisms (e.g., the Psychiatric Genome-wide Association Study [GWAS] Consortium and the North American Antiepileptic Pregnancy Registry) so that once viable drug targets are identified, there will be large datasets that can be used to assess and validate them.

BOX 3-1
Key Points: Depression

- Major depressive disorder is a leading cause of disability worldwide.
- Depression is a significant contributor to other systemic and organ diseases.
- While there has been some progress in treatment options, current approaches are inadequate.
- Primary pathological mechanisms of depression are poorly characterized.
- Women are 1.5 to 2.5 times more likely to experience major depression than men, from puberty onward. Symptomatology is also different between the sexes.
- Sex differences must be studied across the lifespan:
  - Natural variation of hormone levels across the lifespan provides opportunities for the study of sex differences in psychiatric and neurological disorders.
  - Adult-onset disorders have developmental precursors.
  - Consideration of comorbid conditions is important.
- Current animal models of depressive disorders have significant limitations at the levels of both concept and interpretation.
- Sex and gender should be taken into account in every phase of drug development (Phases I through III and postmarketing studies, as well as preclinical studies in animals).

PAIN AND PAIN PERCEPTION

Sex Differences in Pain and Pain Perception

Studies in humans have shown that females generally experience more clinical pain and often show greater experimental pain responses (i.e., have lower thresholds and less tolerance for pain) than males, said Karen J. Berkley, professor of psychology and neuroscience at Florida State University. That difference, however, can be manipulated by a variety of experimental factors (e.g., stimulus type, pain scale used, testing paradigms, endpoints selected) and impacted by individual factors (e.g., age, reproductive status, general health, blood pressure, food intake, odors, social and cultural factors).

Although individuals show significant variability when it comes to alleviating pain, some generally accepted sex differences in pain are worth considering. First, more painful conditions have a higher prevalence in females than males. In other words, women are more likely to have painful chronic conditions than men. The underlying basis for this disparity is not known, but probably has multiple causes, Berkley said, and is an opportunity for further research. Second, hundreds of therapies are available to

alleviate pain, and women use more of them (e.g., drugs, herbal products, complementary alternative medicine) than men. Yet little attention has been paid to how this usage difference affects the efficacy and side effects of various treatments.

One of the key questions considered in a 2007 consensus report on studying sex differences in pain and analgesia was "is there enough evidence to warrant sex-specific pain interventions?" The authors concluded that "the findings are mixed" and that "the evidence does not appear strong enough to warrant sex-specific pain interventions in most situations" and noted that more studies are required, including clinical trials that should take sex into consideration and report any differences in outcomes (Greenspan et al., 2007, p. 14).

The consensus group also expressed concern about the "translation hindering" effects of the "disconnects" among specialties, and between basic and clinical researchers. Berkley also noted that translational research is not unidirectional from animal research to clinical practice, but is really circular, and evidence from human and clinical research should inform animal models.

In conclusion, Berkley said that knowledge of statistical sex differences is already beginning to save lives and improve the health of both females and males, but dissemination of this knowledge is key. Better understanding of the interplay between social roles and health is needed. These issues are complex, but seemingly small increments in knowledge can have large lifetime impacts.

Dissecting Pain and Pain Perception into Sex-Related Endophenotypes

Emeran Mayer, director of the University of California–Los Angeles (UCLA) Center for Neurobiology of Stress, studies persistent pain syndromes, with a focus on visceral pain from the gastrointestinal and urinary tracts. Based on reported spontaneous symptoms, persistent pain syndromes are more common in women (including irritable bowel syndrome [IBS] and interstitial cystitis). Awareness is growing that persistent pain syndromes (e.g., fibromyalgia, temporomandibular joint disorder, vulvodynia, interstitial cystitis, IBS) are not distinct diseases, but rather, they significantly overlap with each other, and with disorders of affect and mood, particularly anxiety, depression, and somatization. Most of these disorders are studied using experimental pain assays to determine if an individual has a high or a normal pain threshold or pain sensitivity. Such measurement would be simple if the relationship between pain perception and a stimulus was linear. But pain perception is a highly complex, modulated system (Figure 3-1). These networks prepare the system and modulate perception of a stimulus

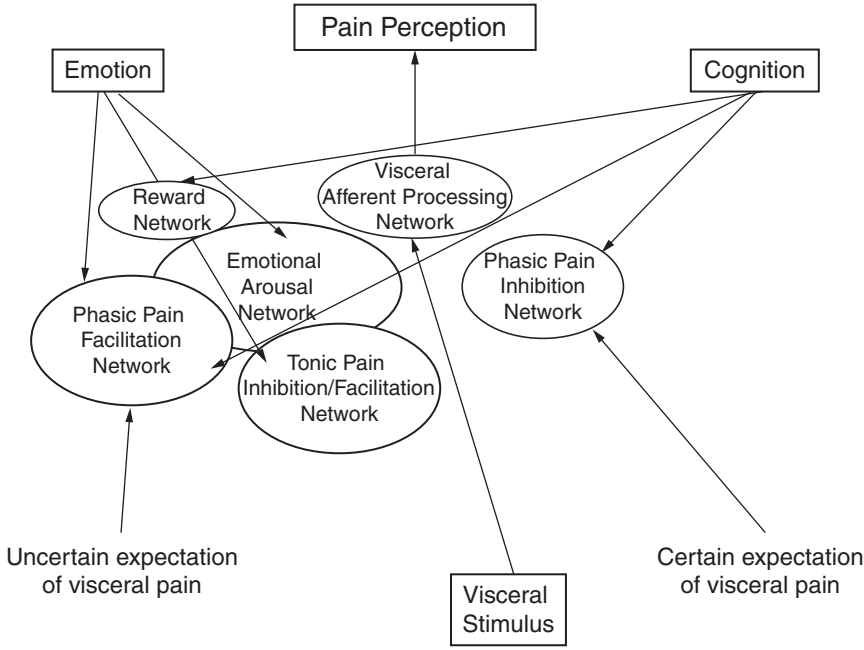

FIGURE 3-1 Pain perception is not a linear pathway from stimulus to pain but is a highly complex, modulated system, and each component potentially could be sex specific.

whether it is painful or not. In the case of a distending gastric stimulus, there are sex differences in the expectation of pain (certain and uncertain) much more than physical responses to the actual stimulus. Therefore, differences in one of these modulated systems may result in a sex-specific bias for pain perception and spontaneous pain.

Mayer proposed a reverse translational approach that takes into account sex-related differences in the endophenotypes. An endophenotype is a physiological or biological abnormality that cuts across categorical disease definitions, and may be shared by several disorders. In deconstructing complex symptom-based syndromes into biological endophenotypes, the goal is to work backward from the syndrome through the symptoms; to the cognitive phenotype; to the underlying neural networks, cellular systems, and component cells; and ultimately, to the genes or network of genes that correlate with endophenotypes.

Sometimes sex and gender differences can be studied only in humans, as in the clinical phenotype of IBS, a common persistent pain disorder affecting the gastrointestinal tract. Because no animal models exist that

can report the painful symptoms, investigators have relied on measuring reflexive and behavioral responses to noxious stimuli. However, there are neurobiological endophenotypes that can be studied in animals, such as visceral sensitivity, descending pain inhibition, emotional arousal, and associated brain responses.

Mayer provided several examples of how endophenotypes have been used to deconstruct complex syndromes in pain conditions. In closing, Mayer said that studying endophenotypes in humans and in animals can be productive in understanding sex differences in pain.

Sex and Gender Differences in Pain Across the Lifespan

Although many painful conditions are more prevalent in females than males overall, the prevalence of different painful conditions varies across the lifespan, resulting in variations in sex ratios across the lifespan, explained Linda LeResche, professor at the University of Washington School of Dentistry. Neck and shoulder pain as well as joint pain, which occur more frequently in females, tend to increase with age in both sexes (Hasvold and Johnsen, 1993). Abdominal pain (excluding menstrual pain) decreases with age in both sexes (Agréus et al., 1994). Migraine exhibits a large sex difference after puberty, but the curve is more bell shaped and the prevalence disparity between females and males lessens with age (Stewart et al., 1992).

LeResche described her work on temporomandibular joint and muscle disorders (TMD), which cause musculoskeletal pain in the region of the jaw joint and associated facial muscles. TMD is more common in women than in men, and peak prevalence occurs during the reproductive years. Her initial studies suggested that women taking hormone replacement therapy (HRT) were at increased risk of being treated for pain from TMD (LeResche et al., 1997). As a follow-up, subsequent studies are focusing on the association of endogenous hormone patterns and pain.

Whether sex differences in pain begin in adolescence and are associated with pubertal development was a question LeResche and colleagues explored (LeResche et al., 2005). Looking at TMD pain, headache, stomach pain, and back pain, she found that prevalence of pain in boys varied by painful condition (some increasing, some decreasing with pubertal development). For girls, pain increased across pubertal development for all four conditions. Another study assessed whether cyclic changes in levels of reproductive hormones (i.e., the menstrual cycle and pregnancy) are related to pain in female TMD patients. The results indicate that when estrogen levels are predicted to be high, pain is actually lowest, and correspondingly, when estrogen and progesterone levels are low, pain is the highest. In other words, TMD pain was highest for all

women during the menstrual period. Similar patterns in clinical facial pain were observed across pregnancy (pain was lower during the later months of pregnancy, when estradiol and progesterone levels are high). Note that this pain is not in the reproductive system, but is potentially influenced by hormone levels.

In summary, the presence and intensity of pain in women are related to hormone (especially estradiol) levels. Questions remain as to whether these relationships are strong enough to be taken into account in research and treatment. Large individual differences exist in the correlation between pain and hormone levels, suggesting avenues for further research, including further lifecycle studies (e.g., menopause); relationship of gender-related factors (e.g., social role expectations, coping) to pain in non-Western cultures; common mechanisms underlying negative affective states (pain, depression, somatic symptoms) in women; and differential pain mechanisms by sex (even if pain outcomes are the same).

Open Discussion: Pain and Pain Perception

During the open discussion, panelists and participants delved further into the research aspect of sex differences in pain, including animal models and endophenotypes. Mayer reemphasized that more focus is needed to understand the mechanisms underlying the generation of chronic spontaneous pain. Current thinking is that the main stimulus produces a pain, but another perspective is that these disorders may have a developmental aspect. At an early stage, there may have been a nociceptive input to the brain, and later in life a pain memory may be recalled based on mood states, affective states, or other stressors. Berkley concurred with the need to understand basic mechanisms, but noted that studying stimulus-induced changes also has a role.

A participant agreed that spontaneous pain is a significant clinical problem. He noted that animal models are studying allodynia, which is a real symptom of chronic pain, but is not a particularly important symptom or one that patients often complain about. He also expressed concern about studying endophenotypes, suggesting that perhaps it was more of a step backward than forward. Panelists responded that proxies are useful when the painful experience cannot be quantified. Current research suggests that male and female brains issue the subjective report of chronic pain by different mechanisms. A rational approach to drug development would be to target these sex-specific mechanisms that together generate the same symptom. In terms of drug development, the participant responded, the critical factor is predictability; do the existing models in the field have predictability or not? If a disorder has a high sex prevalence, and that sex prevalence cannot be shown in the animal model, it does not have predictability.

BOX 3-2
Key Points: Pain and Pain Perception

- Persistent pain syndromes are more common in women, but sex ratios vary by painful condition and life stage.
- The presence and intensity of pain in women are related to hormone levels. Large individual differences exist in the correlation between pain and hormone levels.
- Questions remain as to whether these relationships are strong enough to be taken into account in research and treatment.
- Development of drugs for pain is largely based on evoked reflexive measures in male rodents, which is a poor model for spontaneous pain in humans.
- A reverse translational approach is proposed, which considers sex-related differences in endophenotypes.
- Better understanding of the interplay between social roles and health is needed.
- These issues of sex differences are complex, but seemingly small increments in knowledge can have large lifetime impacts.
- A disconnect remains among specialties, and between clinical and basic research. Dissemination of knowledge about sex differences in pain is critical.

SLEEP MEDICINE

Sleep Regulation

Although sleep medicine has become a huge field, our understanding of basic sleep regulatory processes and their consequences for disease are still lacking, said Roseanne Armitage, director of the Sleep and Chronophysiology Laboratory at the University of Michigan. Understanding sex differences in any clinical disorder requires knowing about those differences in healthy individuals, and the developmental time course of when those differences emerge. During sensitive periods in development, the magnitude of the sex differences is more likely to increase or decrease, depending on what modifying conditions are in play.

The time course of slow-wave activity is a proxy for sleep homeostasis, the recovery function that occurs during sleep. In healthy adults (women and men, ages 18 to 40), a mild challenge to sleep (e.g., extending wakefulness by a couple of hours) results in only subtle sex differences in the amount of slow-wave activity across the night. However, as the magnitude of the sleep challenge increases, the sex differences become larger with women displaying significantly greater accumulation and dissipation of slow-wave activity. After 40 hours of sleep deprivation, the magnitude of the sex difference is nearly doubled. There is an extraordinarily large response in the initial accumulation of slow-wave activity in women and a

very rapid decay across the night in women compared to men (Armitage, 2007). This suggests a greater adaptive response in homeostasis in females than in males, Armitage said, but it takes a challenge to the regulatory system to elicit that difference.

Important factors to understand are the conditions under which sex differences increase in magnitude, what creates a greater adaptive response in females than in males, and what other factors interact with those. Although baseline sleep studies of the organization of sleep are important, they do not provide information on the sleep regulatory mechanism the way evoking a response in brain regulation through challenge does. Challenge studies are necessary for understanding risk factors for certain diseases that are sex specific, either in their prevalence or in a variety of conditions within the disorder.

The magnitude of the sex difference in slow-wave activity in depressed individuals of the same age range is nearly three times greater than it is in healthy control individuals with no personal or family history of psychopathology. Following a mild challenge to sleep regulation, there is a significant difference between depressed women and depressed men, suggesting that depression itself is a challenge to sleep and brain regulation. There appears to be a sex-dependent propensity to move outside normal homeostasis—an overresponse in depressed women and an underresponse in depressed men.

Age-related sex differences in slow-wave activity occur within healthy control groups. Most studies are not powered sufficiently to consider age subgroups within sex subgroups, and such studies can become very expensive if they include longitudinal designs. But this area needs additional focus, Armitage said, to truly appreciate sex differences in sleep regulation and their consequences for disease. Depression is just one model of conditions under which larger sex differences in sleep regulation are observed than in healthy individuals.

Interaction of Sleep and Circadian Rhythmicity

Prominent sex differences have been observed in slow-wave sleep and slow-wave activity, concurred Jeanne Duffy of the Division of Sleep Medicine at Harvard Medical School and Brigham and Women's Hospital. As the amount of slow-wave sleep declines with age, sex differences become more pronounced. When an individual is deprived of sleep, he or she responds with increased slow-wave sleep. Individuals differ in the amount of slow-wave sleep they have, and what those differences imply for actual sleep need or sleep deprivation.

Profound sex differences are observed when comparing objective and subjective measures of sleep quality. In one study of adults, for example,

the number of awakenings that were (objectively) recorded on a polysomnogram was greater in women than in men, but (subjective) self-reported awakening from the same individuals on the same nights was greater from men (O'Donnell et al., 2009). The basis of this disconnect is not understood.

The ability to sleep depends, in part, on sleep/wake homeostasis, which depends not only on how long a person has been awake, but also on the time of day they are attempting to sleep which is influenced by the circadian timing system. This system also influences most aspects of human physiology, producing rhythmic daily variations to time events optimally (e.g., body temperature is lower at night and higher during the day).

Growth hormone release is an example of a sex-specific rhythmic variation where the hormone is release in large amounts at the beginning of the night in men, while women tend to have more pulses of release during the day. Early studies exploring the role of sleep on hormonal changes and circadian rhythms were largely done in men resulting in some misleading generalizations for both sexes.

The effect of sleep on circadian rhythms is not well understood, nor whether there are sex differences, but data suggest there may be differential impacts on sleep deprivation. The body's response to attempts to sleep at the wrong time of day may have a sex difference as well. As noted earlier, sleep changes profoundly with aging, a change also associated with sex differences.

Males and females differ in their tolerance for staying awake for extended periods. During the usual nighttime hours, women unintentionally fall asleep more frequently than males when they are asked to stay awake for a 36-hour period (Buysse et al., 1993). However, women have faster reaction times after being awake for extended periods (Duffy et al., 2009).

Coregulation by the circadian timing system and the "sleep–wake homeostat" allows humans to keep a stable level of alertness and performance across a normal 16-hour waking episode. This is different from most animal species, which have polyphasic sleep–wake patterns. In addition, most current model systems involve nocturnal animals.

Misalignment between the timing of sleep and the underlying circadian rhythm timing has both health and safety consequences. These are exacerbated by the associated reduction in sleep. For example, there are large differences in the way hormones related to metabolic and cardiovascular functions are regulated under circadian misalignment versus alignment. Studies suggest important differences in how circadian and sleep misalignment may affect the two sexes, but data are currently limited.

Duffy closed by describing some of the challenges to understanding sex differences in sleep regulation. Similar to other areas of research, many basic studies on sleep and circadian rhythms are done in male animals. Con-

ducting sleep studies is technically challenging because sleep feeds back on circadian rhythms. Although women are now included in clinical studies, many studies are not powered to test whether sex differences exist.

Sex differences occur in sleep, both in slow-wave activity and self-reported sleep need and sleep duration. Reductions in sleep have important metabolic consequences, and influences depression and subjective reports of pain. A better understanding of sex differences in sleep has implications for many of the other sex-related health disorders.

Sex Differences in Subjective and Objective Measures of Sleep

Rachel Manber, director of the Stanford Sleep Medicine Clinic at Stanford University, expanded on the discussion of sex differences in the objective and subjective measures of sleep. Objectively, compared to men, women seem to have shorter sleep-onset latency, spend less time awake in bed, have fewer awakenings, and ultimately get more total sleep time and have higher sleep efficiency. Sleep efficiency is the ratio between time spent asleep and time spent in bed, Manber explained. Women also have less slow-wave sleep during the second half of the night. Subjectively, however, across all ages, women report more disturbed sleep than men. A National Sleep Foundation poll found that women report that they need more sleep, or their sleep is insufficient and more disturbed, compared to men. These differences persist even after controlling for psychiatric conditions. Given less than 7 hours of sleep, men are more likely than women to report better functioning during the day. However, on objective vigilance tests or performance tests, not much difference is apparent.

Subjective/objective differences in sleep exist across the board. There are subjective, but not objective, differences in the consequence of menstrual phase and menopausal status on sleep. Menstrual phase effects also cause large individual differences in sleep. About 15 percent of women experience a clinically meaningful disturbance in sleep when they are premenstrual compared to when they are in their follicular phase (Manber and Bootzin, 1997).

Manber stressed the need to better understand subjective–objective sleep discrepancies. Subjective sleep is extremely relevant because the perceived lack of sleep causes people to seek help. Subjective sleep is also easier to study, she noted.

The sexes show differences in sleep disorders in terms of both prevalence and presentation. Insomnia, for example, is more prevalent in women, at a ratio of 2:1. Restless leg syndrome is also more prevalent in women, but a related disorder, periodic limb movement disorder, shows no sex difference. Narcolepsy, rapid eye movement (REM) behavioral sleep disorder,

and obstructive sleep apnea are all more prevalent in men (Krishnan and Collop, 2006).

Differences in presentation can be important for diagnosis and treatment. Obstructive sleep apnea, for example, presents differently in men and women (Valipour et al., 2007; Wahner-Roedler et al., 2007). Women with obstructive sleep apnea tend to be more obese and report more fatigue and less energy. However, they are less likely to report witnessed apnea or heavy snoring, considered the most important clinical presentations warranting a sleep study to confirm or rule out sleep apnea. Women are less likely to respond to sleep apnea treatment and their apnea is less likely to abate or decrease in severity following weight loss than men.

Sleep is very sensitive to perturbations in stress and emotional and physical well-being. Sleep, or the lack of it, also influences a person's ability to regulate emotions. Therefore, Manber said, sex differences in sleep in psychiatric disorders are not surprising. Not much is known, but as Armitage discussed, studies have shown that men are more likely to experience slow-wave sleep deficiencies in depression, which may indicate impaired homeostatic regulation of sleep. Another area in which little is known about sex differences is sleep and posttraumatic stress disorder (PTSD). A meta-analysis found that by restricting the sample to studies of only men, PTSD-specific sleep disturbances had a very strong effect compared to controls, but looking at studies that included both sexes, or female-only samples, the effect was not as strong (Kobayashi et al., 2007).

Manber described her current work on moderators of treatment response in depressed individuals with comorbid insomnia as part of the TRIAD (Treatment of Insomnia And Depression) study. Both sexes are represented, she said, and the sample is large enough to facilitate analysis of the interaction between moderators of treatment response and sex.

In summary, Manber reiterated the need to better understand sex differences in the discrepancy between objective and subjective sleep; the need to understand sex differences in the presentation, course, and treatment of specific sleep disorders; and the need to have sufficiently large samples to allow examination of the interaction between moderators and sex in treatment outcomes research.

Health Consequences of Sex Differences in Sleep and Circadian Rhythms

Although one can argue that sleep is of, by, and for the brain, the brain sits in a corporeal body, and the body is essential for the brain's continued adventure throughout life, said Martica Hall, associate professor of psychiatry, psychology, and clinical and translational studies at the University of Pittsburgh School of Medicine. Every dimension of health

and functioning is effected by circadian rhythms, and vice versa. As already discussed, normative sleep and normative circadian rhythms have marked sex differences, and those differences affect mental health. But do these sex differences matter to the sleep–health relationship with regard to physical health and corporeal health disorders?

Compelling evidence shows that sex differences in sleep bear on both resilience and vulnerability to health and disease. In studies of sleep and health, the three current areas of emphasis are obesity, cardiovascular disease, and metabolic disease. The common focus in these studies is sleep apnea, and the usual subjects are males, including male rats or mice. However, until menopause, men and women have different susceptibility and vulnerability to sleep apnea syndrome.

One example is sex differences in the cardiovascular response to hypoxia during the sleep period. Differences in mean arterial pressure have been demonstrated between male and female rodents in an induced hypoxic condition. Mean arterial pressure of ovariectomized females was similar to that of males.

In addition to apnea, other factors to consider include subjective sleep quality, sleep duration, fragmented sleep, sleep depth, and cortical arousal during sleep. Hall cited a recent study showing that short sleep was a risk factor for incident hypertension in women, but men showed no relationship between sleep duration and incident hypertension.

Women who have sleep disorders also have more hyperaroused brains during sleep. Fast frequency electroencephalography (EEG) activity was shown to be increased during non-REM sleep in women with insomnia compared to controls, but men with insomnia showed no difference from controls. Women with increased fast frequency EEG activity during sleep also have been shown to be at greater risk for metabolic syndrome.

In conclusion, Hall said she and others are now looking at real-time evaluation of sleep and physiology (the pathways through which sleep may affect health outcomes, such as hearing rate variability, autonomic imbalance, or inflammation), and studying people in their habitual environments (i.e., in participants' homes rather than in sleep laboratories). Interdisciplinary collaborations are also important, and Hall noted the need for emphasis on sleep and accelerated aging, both at the cellular and molecular levels.

Open Discussion: Sleep Medicine

Animal models were once again a topic of much interest during the open discussion. Panelists and participants also discussed sleep patterns in adolescents, and how subjective and objective measures factor into the drug development process.

Animal Models

A fundamental issue with animal models is that rodents have polyphasic sleep, while human adults are monophasic. Big differences occur in sleep regulation across the lifecycle, so animal models should be developmentally appropriate, Armitage said. Although animal neurophysiologists have worked on sleep studies for years, few have included both sexes of rodents in their studies.

A participant raised the issue of the disconnect between objective and subjective measures of sleep in humans and pointed out that there are no animal models that can take this into consideration. Animal research has been useful, he said, in telling us what we should look for in humans. For example, early studies of rodents established a sex difference in circadian rhythms by demonstrating that the estrous cycle can influence the rhythm.

Subjective Versus Objective Measures in Drug Development

The subjective versus objective sleep measure conundrum is particularly relevant to the development of new drugs for sleep, a participant observed. Is it more important that the subject feels subjectively better? Or is more important that the subject is objectively better? Which measure should be used in developing a new drug?

Hall responded that going forward, researchers are using more quantitative and sophisticated techniques to measure what is happening in the brain during sleep. This is one area where animal models are helpful and directive, she added.

A methodological issue is whether the objective measures being used are just not good enough to capture the subjective experience, Manber said. Which method to use really depends what disorder is being studied. In insomnia, for example, subjective measures may be more important.

A participant from a pharmaceutical company concurred that in insomnia research, subjective measures are important to patients. But objective measures are important in drug development, he said, and can be useful in translation. In early drug development, researchers are looking for outcome measures that can facilitate smaller studies. Polysomnography, for example, is much preferred to the subjective measures, which have a smaller effect size and larger variability.

BOX 3-3
Key Points: Sleep Medicine

- Understanding of basic sleep regulatory processes and their consequences for disease is still lacking.
- Sex differences are not often considered when studying the relationship of sleep and physical health status.
- Comprehending sex differences in any clinical disorder requires an understanding of sex differences in healthy individuals, and the developmental time course of when those sex differences emerge.
- Baseline sleep studies of the organization of sleep are important, but challenge studies are necessary for understanding risk factors for certain diseases that are sex specific, either in their prevalence or in a variety of conditions within the disorder.
- Sex differences occur in sleep in both objective and subjective sleep measures. Reductions in sleep have important metabolic consequences, and influences on depression and subjective reports of pain.
- Conducting sleep studies is technically difficult because sleep feeds back on circadian rhythms.
- Studies must be sufficiently powered to allow examination of the interaction between moderators and sex in treatment outcomes research.
- Promising sleep research directions include
  - real-time evaluation of sleep and physiology;
  - studies conducted in participants' habitual environments;
  - interdisciplinary collaborations (e.g., sleep and accelerated aging, at both the cellular and molecular levels).

MULTIPLE SCLEROSIS AND NEUROINFLAMMATION

Sex Differences in Multiple Sclerosis: Clinical, Imaging, and Pathology

Multiple sclerosis (MS) is a multifocal inflammatory disorder that affects the CNS, including the brain, spinal cord, and optic nerve, explained Robert Fox, associate staff and medical director of the Mellen Center for Multiple Sclerosis at the Cleveland Clinic. MS is typically diagnosed between ages 25 and 45, and is the most common non-traumatic cause of disability among young adults, affecting one in 1,000 persons in the United States. By 15 years from onset, about 75 percent of individuals have a progressive course of disease, and moderate to severe disability. Multiple sclerosis lesions can be seen on axial brain and spinal cord MRI, and are apparent pathologically postmortem.

Disease Course

About 85 percent of MS patients start with a relapsing–remitting form of disease, with episodes of neurological dysfunction that go into remis-

sion, but may leave them with some residual disability. Of those, about 75 to 80 percent will eventually, over about 15 years or more, transition into a gradually progressive course of disease with few, if any relapses. This is the secondary progressive phase of MS, when there is gradual progression of disability.

About 10 percent of new patients will present initially with a primary progressive course, a gradual worsening over time. This is similar to secondary progressive MS, just without the preceding relapsing–remitting phase. This subset is probably a mixture of both secondary progressive patients who had non-clinical events or subclinical relapses, as well as patients with a true primary neurodegenerative disorder without much active inflammation.

A smaller group of patients, about 5 percent, have what is called progressive relapsing MS. They appear to have primary progressive MS, and then they have a remission.

Finally, a rare variant of MS called neuromyelitis optica is sometimes considered a completely different disease. Inflammation targets primarily the optic nerve and the spinal cord. When it affects the brain, it causes large atypical brain lesions that do not look like typical MS.

Two categories are used for measuring disease: active inflammation and accumulated injury. To assess active inflammation, clinicians consider the frequency of relapses and measure gadolinium-enhancing lesions. Accumulated injury assessments factor in disability progression, MRI lesion burden, brain atrophy, and information obtained from advanced imaging technologies.

Sex Differences in Multiple Sclerosis

Fox reviewed the current knowledge about sex differences in MS based on clinical observations, imaging, histopathology, and clinical trials. Overall, MS is two to three times more common in women than in men (Alonso and Hernán, 2008; Orton et al., 2006). This ratio decreases with age (Marrie et al., 2010). Note that the sex ratio among primary progressive MS patients, who have a worse prognosis because they are progressing from the beginning, is equal. Also of note, neuromyelitis optica is at least 3 times more common in women than in men (and some surveys suggest up to 10 or 20 times). Peak incidence of MS is earlier in females, occurring around ages 35 to 39 in women, and 45 to 49 in men. Peak prevalence is also earlier in women compared to men (ages 45 to 49 for women and 55 to 69 for men). This difference is important to keep in mind when interpreting studies on prognosis, Fox said, as many studies have not properly controlled for differential age, and differential age at diagnosis.

Over time, the prevalence and peak age of MS have been increasing. The incidence has remained fairly stable, and the ratio between men and

women in terms of the incidence is roughly the same. This may be due to less diagnostic delay in more recent times (which will increase the prevalence without changing the incidence) and greater survival (which does not necessarily mean greater quality of life).

In terms of prognosis relative to relapses and progressive disability, observations vary. A large number of earlier studies suggest that females have a favorable prognosis. Quite a few other studies have shown no sex difference in the prognosis of MS. Although a sex difference is prominent in the incidence in neuromyelitis optica, no sex differences have been observed in progression.

With regard to the role of sex hormones in MS, from the clinical point of view it is clear and widely accepted that relapse rate decreases during pregnancy, particularly the third trimester. Then, in the months after delivery, a rebound brings the relapse rate back to the prepregnancy rate or perhaps even higher. The effect of oral contraceptives is less clear. A lower incidence of MS is seen among women using oral contraceptives, although some studies have found no effect. One study, interestingly, found an increased risk in long-term users of oral contraceptives, suggesting the need for further research in this area.

Earlier and smaller MRI studies (n = 50 to 413) of active inflammation found gadolinium-enhancing lesions were more common in women, but they did not control for the differential age and the disease course of patients. Later studies that did control for some of these covariates, enrolling 700 and 1,300 patients, found no sex difference in gadolinium-enhancing lesions.

MRI studies of cumulative injury found no sex difference in T2 lesions after covariate adjustment. Studies of T1 lesions observed no sex difference in relapsing–remitting MS, and greater T1 lesion volume in male primary progressive MS. No sex differences in atrophy have been demonstrated in cross-sectional or longitudinal studies. Results are conflicting about gray-matter atrophy; some studies suggest it is greater in men, and others indicate it is equal in both sexes. Studies using advanced imaging metrics of MS tissue injury, including diffusion-weighted imaging and magnetization transfer ratio, have not demonstrated any sex differences.

Only a few histopathologic studies of MS have evaluated sex differences. No differences have been observed in studies of active inflammation (equal number of microglial cells and T-cells in MS lesions and normal-appearing white matter in men versus women), tissue damage (equal number of amyloid-precursor, protein-positive spheroids; equal reduction in axonal density; no difference in number, type, or distribution of cortical lesions), or repair (no difference in the pathologic evidence of repair, oligodendrocyte precursor cells, and other related lineages; no difference in remyelinating lesions between men and women). The only pathologic sex

difference available in the literature, Fox said, was a low fiber density in spinal pyramidal tracts in men. All together, evidence showing a sex difference is limited in the histopathology of MS.

The vast majority of clinical trials have found no effect of sex on outcome. Many of these trials had a preplanned covariate analysis of sex, and while some reported no effect of sex, others did not report any sex-based results. However, notable exceptions include two studies of interferon in secondary progressive MS, which found that only women had slowed progression of disability. A third interferon study did not find any effect of sex. Whether these studies adjusted for covariates such as age of patient and age at diagnosis was unclear, Fox said.

A post-hoc analysis of data from a study of glatiramer acetate and primary progressive MS showed that only men had a slowed progression of disability. This difference persisted after covariate adjustment. Several other glatiramer acetate studies (in relapsing–remitting MS and primary progressive MS) did not identify a sex difference. No clinical trial has shown a differential effect of sex on any MRI measure.

In summary, Fox said, MS is two to three times more common in women. Onset is later in men and is more likely to be primary progressive MS, making interpretation of studies challenging. Women may have a better prognosis, but the differences decrease after adjusting for age at diagnosis and disease course. Pregnancy decreases disease activity, but whether oral contraceptives have an impact is unclear.

Despite the observations of smaller studies, the larger MRI studies fail to suggest an effect of sex on MRI measures of either inflammation or tissue injury. Histopathology shows virtually no effect of sex, nor did most clinical trials. A few post-hoc analyses suggest some sex-based effect, but how to interpret these findings is unclear in light of all the other studies that found no impact.

Altogether, while a sex difference is clear in the incidence of MS, little evidence is available on sex differences in the clinical course of MS. That does not mean, however, that consideration of sex differences should not be included in planning future studies.

MULTIPLE SCLEROSIS AND NEUROINFLAMMATION: CONSIDERING SEX DIFFERENCES IN DESIGNING THERAPEUTIC AGENTS

Multiple sclerosis is just one of a list of autoimmune diseases that show sex differences. Halina Offner, professor of neurology and anesthesiology and perioperative medicine at Oregon Health and Science University (see ohsu.edu), said 78 percent of people affected with autoimmune diseases are women.

Experimental autoimmune encephalomyelitis (EAE) is the prevailing animal model for MS. Disease can be induced in genetically susceptible rodents by immunization with spinal cord homogenates, myelin, or specific myelin peptides in combination with adjuvant (active EAE), and by adoptive transfer of encephalitogenic cells (passive EAE). Typically, EAE is thought to be a Th1/Th17-mediated disease. However, other cellular players such as antigen-presenting cells also play a role in disease progression. Increased numbers of antigen-presenting cells in the CNS increase susceptibility to and severity of EAE in female mice. T-cell response to foreign antigen increases the numbers of activated T-cells in female versus male CNS. This could be influenced by sex steroids, Offner said. CNS T-cells recruit higher numbers of antigen-presenting cells to the CNS, which enhance the encephalitogenic activity of myelin-specific T-cells, thus producing the "high susceptibility" phenotype found in female mice.

MS does have sex-based immune differences. Females mount a stronger immune response, with higher levels of antigen-presenting cells, CD4+ Th2 cells, and immunoglobulin responses. Males have severe inflammation and enhanced CD4+ Th1 and CD8+ T-cell activity. The immune functions of males and females have fundamental differences, which may require different immunomodulatory strategies in MS. The regulatory balance between the detrimental and beneficial effects of immune cells in MS is very complex, Offner explained.

In MS, the initiating factors are susceptibility genes and environmental factors. Sex hormones and neuroendocrine factors are very important modulatory factors in the immune and autoimmune responses, Offner said.

Some data on MS are rather controversial. Offner cited a report on the recent increase in incidence rates of MS in females compared to males (Debouverie, 2009) and several studies that showed increased ratios of females to males (Eikelenboom et al., 2009; Maghzi et al., 2010; Sadovnick, 2009). One study connected the increase in the sex differences to vitamin D, suggesting that higher levels of vitamin D are associated with a lower incidence of multiple sclerosis only in women (Kragt et al., 2009).

MS cases are well known for increasing with geographic distance from the equator, and vitamin D levels decrease with distance from the equator. Decreasing MS cases with increasing ultraviolet (UV) light also has been documented in several countries. UV light catalyzes the first step of vitamin D_3 synthesis (the inactive form of the vitamin), and serum D_3 levels correlate with exposure to the UV light.

To evaluate this further, researchers looked at the effects of vitamin D_3 in the EAE model. The severity of disease was much lower in females who were fed a vitamin diet, but there was no difference in males in clinical disease (Spach and Hayes, 2005). Further studies demonstrated that estrogen was needed for vitamin D_3 to inhibit EAE in female mice. Ovari-

ectomy eliminated the vitamin D_3-mediated inhibition of EAE, and estrogen replacement in these animals restored vitamin D_3-mediated inhibition of EAE.

If humans have similar gender differences in vitamin D metabolism, Offner said, then sunlight deprivation would increase the MS risk more significantly in women than in men, which could explain higher incidence of MS in females.

The current hypothesis is that there may be a female bias in the protective effects of vitamin D_3 in MS, and that insufficiency in vitamin D_3 may contribute to the higher female/male sex ratio. Lifestyle changes could be linked to insufficient sunlight exposure in recent years (women in the workforce, indoor lifestyle, sunscreen use). Also, declining ovarian function and limited vitamin D_3 supplies may be driving the transition of relapsing–remitting to chronic progressive MS.

In contrast, Offner described a study in which male and female animals with EAE were treated with a recombinant T-cell receptor ligand (RTL) construct at the onset of disease. The results showed a significant reduction in disease activity in mice treated with RTL, which was comparable in both sexes. Spinal cord imaging of mice at the onset of disease and at 3 days posttreatment with RTL showed reversal of T-cell infiltration, again with no difference between females and males. Based on these preclinical studies with RTL in the EAE model, a Phase I safety study in MS patients was designed. Thirty-four adult subjects (male and female) who received varying doses of RTL injections or placebo were followed for more than 90 days. The primary outcome measure was whether the RTL construct increased MS disease activity, and the conclusion was that the RTL did not increase MS disease activity by any measure in either females or males.

In conclusion, Offner said that sex differences matter in many clinical diseases and animal disease models. Disease models such as EAE often have used males, with the assumption that this decreases experimental variability caused by female hormone cycling. There are also misconceptions that disease mechanisms or treatment effects will be the same for both sexes. This is not the case, as demonstrated in the two examples provided, showing that response to vitamin D is sex dependent, while response to RTL is not. Therefore, preclinical studies should always include both sexes.

Studying Sex Differences from Bedside to Bench to Bedside

When discussing sex-based disparities in health, separating incidence from progression is important. Incidence is akin to susceptibility, or the immune system's overactivation in autoimmune diseases like MS and lupus, whereas progression or disability accumulation can be a reflection of not only the immune system, but the overlay of the CNS reaction to that im-

mune attack. These are very different situations, said Rhonda Voskuhl, director of the Multiple Sclerosis Research and Treatment Program at UCLA. Although sex differences in the progression of MS generally have not been observed, there is a clear difference in incidence.

Sex differences in MS could be from the basic differences between males and females, including sex hormones (estrogen and testosterone) or sex chromosomes (XX or XY gene effects). Conditions that occur in one sex, but not in another, such as pregnancy, could also be major factors. In the case of MS, pregnancy reduces relapses by 80 percent, significantly more than most of the currently available drug therapies, which reduce relapses by about 33 percent. (Some reduce relapses by half to two thirds, but these carry with them risks of significant adverse events.) Understanding this effect of pregnancy on MS pathogenesis could aid the discovery and development of better therapies for MS.

Voskuhl described a "bedside-to-bench-to-bedside" approach to considering MS. The classic bench-to-bedside approach is somewhat risky, she said, starting with a molecule or a pathway, studying it in vitro and in vivo and then in human trials, often finding that the treatment does not work as predicted in people. Sex differences are known to be clinically important as major disease modifiers. A better approach in the case of MS is to go from what is known clinically, to characterize the cellular and molecular mechanisms, then to go back to the patients with a clinical trial.

One example of this approach is the bedside observation of reduced relapses during pregnancy. As discussed, this could be related to any number of things, including hormones or vitamin D. Several laboratories have now shown that estrogens, particularly estriol, are protective in the animal model of MS. Based on this clinical observation, and the subsequent results from animal models, estriol was then administered in pill form to people with MS in a Phase I trial, and reduction in the number and size of lesions was observed. A multicenter Phase II trial is under way.

A second example stems from the clinical observation of the sex difference in incidence of MS, and the fact that men are older than women at disease onset. Based on this decreased susceptibility of younger men (when testosterone levels are high), studies of the potentially protective role of testosterone in MS were conducted in the animal model. Results supported the protective role of testosterone, and a pilot study of men with MS found that treatment with testosterone gel slowed brain atrophy and caused immune shifts that are biomarkers for improvement. A Phase II trial of testosterone in men with MS is being planned.

As discussed by Arnold (Chapter 2), sex differences are not all about adult hormones. Developmental hormones and sex chromosomes also have effects. Using the four core genotype mouse model describe by Arnold to study EAE and lupus, Voskuhl found sex chromosome clearly had an effect,

with the XX genotype promoting disease development in both EAE and lupus (Smith-Bouvier et al., 2008). Voskuhl is also studying developmental hormone effects using the same system.

In summary, Voskuhl stressed that clinical observations can lead to promising potential treatments for MS. Research on sex hormones has reached clinical trials, and research on sex chromosome effects are still in the early stages, but may have potential as well. Whether the sex chromosome effect is an X dosage effect or a Y gene effect—and what that gene is—remains to be seen.

Animal studies to elucidate mechanisms, and pilot trials of potential products in humans, are possible, but funding is a major obstacle, Voskuhl said. The small pilot trials described by Voskuhl were funded by the MS Society and the NIH. But the challenge is funding a potentially $30 million Phase III trial, especially when the drug under study is not a patent-protected product (e.g., estriol). Although estriol is already broadly used and well characterized with regard to safety, the fact that it is not patent protected means that a pharmaceutical company that might otherwise invest in developing a new product may not be able to recoup the significant product development costs through future product sales.

Open Discussion: Multiple Sclerosis and Neuroinflammation

In the open discussion, panelists further considered the implications of sex differences in MS, and how MS is different from the other diseases discussed so far. Participants were interested in further information on the studies discussed, and raised issues about approaches to research.

Fox noted that for the other diseases discussed at the workshop, sex differences are apparent in the disease course. However, for MS, although the incidence is different for men and women, the disease course appears similar. Voskuhl noted that although progression may not be different, the immune response is more aggressive in women. The question is why, if immune response is different, is progression not different? A difference in the brain must be counter to the difference in the immune system.

A question was raised as to whether estriol had been tested only in women, and if there are any obstacles to trying it in both sexes. Voskuhl responded that estriol has been shown to have an effect in male mice with EAE as well. This has not been done in clinical trials. The adverse events could be different, she noted; there would be no concern about endometrial or uterine cancers in men. Precedents have been set for giving estrogens to men with other diseases.

A participant suggested that another way to study sex differences is to look at the factors at play when, for example, a male develops a disorder that is common in women. Another participant cautioned that care must

be taken when looking at clinic populations versus the larger community. People with the most severe conditions are the ones who come to tertiary care.

BOX 3-4
Key Points: Multiple Sclerosis (MS) and Neuroinflammation

- Clear sex differences are evident in the incidence of MS:
  - MS is two to three times more common in women.
  - Onset occurs later in men, and is more likely to be primary progressive MS.
  - Women appear to have a better prognosis, but differences are less apparent after adjusting for age at diagnosis and disease course.
  - Pregnancy decreases disease activity (but the effect of oral contraceptives is unclear).
- The clinical course of sex differences shows little evidence of sex differences:
  - Despite observations in smaller studies, large magnetic resonance imaging (MRI) studies suggest no effect of sex on MRI measures of either inflammation or tissue injury.
  - Histopathology shows virtually no effect of sex differences.
  - Most clinical trials show no effect of sex differences.
- There are sex-based immune differences in MS:
  - Females mount a stronger immune response.
  - Males have more severe inflammation.
- Studies suggest a role for ultraviolet light and vitamin D in the pathogenesis of MS.
- Clinical observations can lead to potential treatments for MS:
  - Animal studies to elucidate mechanisms of the observed clinical phenotypes and pilot trials of potential products in humans are possible, but funding can be a major obstacle.
  - Preclinical studies should include both sexes.

In the community, there are large sex differences in pain, but within the tertiary care setting there are not many differences between males and females.

OVERARCHING DISCUSSION

Following the disease-specific panel discussions of issues related to sex differences in translational research, the four panel moderators and the workshop cochairs assembled to consider overarching issues across major disease areas.

Making the Decision to Study Sex Differences

The panel first considered when, during development of products to treat disorders of the nervous system, consideration of sex differences would be most appropriate, taking into account financial, time, and material resource constraints.

Richard Nakamura, director of the Division of Intramural Research Programs at the National Institute of Mental Health,[1] said that ideally, information should be collected on any sex differences that may emerge, but sex differences are unlikely to be identified in relatively small proof-of-concept trials. The larger studies, such as STAR*D, are the ones that need to focus on sex differences. Investigators should publish any supplementary data on sex differences that emerge from trials, even when the data are not statistically significant.

He also noted that large databases, such as the NIH's GWAS databases, could provide a valuable resource in trying to understand sex differences. In addition, the Veterans Administration and other large healthcare entities that have large electronic health records systems should be encouraged to develop common features and nomenclature so that these systems could be analyzed for information on sex differences. Progress toward a system of universal medical records in the United States would aid this effort as well.

From an industrial perspective, Chi-Ming Lee, executive director of Translational Science at AstraZeneca Pharmaceuticals, noted that unexpected failures in later phases of product development (Phase II or III) are extremely costly. A company usually has a very strong rationale, and has met certain criteria, before moving a drug from early phase into the later phases of development. Data from animal models are considered in these decisions, but experience has shown that some animal models that were relied on failed, in the sense that they did not predict the outcomes later observed in humans.

Theoretically, sex differences should be addressed early, Lee said, but there are many considerations. Should every animal model study include both male and female? Simply including females in studies will not necessarily provide the answer, and can provide a false sense of security. If either preclinical or clinical evidence suggests that sex makes a difference, then further research on these differences should be conducted. Many factors must be considered, including, but not limited to, hormonal effects, sex chromosome effects, lifespan, psychosocial factors, or species differences.

Paul Hoffman, associate chief of staff for Research and Program De-

[1] Dr. Nakamura's comments were based on personal experience over the course of a career, and do not necessarily represent official NIH policy or official NIH endorsement of potential policies.

velopment at North Florida/South Georgia Veterans Health System, concurred that evidence of an increased prevalence in one sex versus another merits further attention. The question is where the funding is best applied. In clinical trials, sex should be treated as a variable, and studies should be designed with enough power to allow for analyses by sex (rather than sex being a post-hoc analysis).

Zorn emphasized the need for a business case before a pharmaceutical company expends significant resources. The current costs of bringing a drug from the bench to the patient are in the range of $1.8 billion. To include sex as a variable in preclinical studies and clinical trial design, a hypothesis based on data is needed. While much of the data presented at the workshop have been very good and could be used to generate hypotheses about sex differences, some of those data are not yet clear enough to justify the significant investment required. A stronger investment is needed in basic research, he said, looking at differences in the sexes all the way down to the molecular level, so that drug developers have a solid base on which to test these differences in humans. The responsibility for obtaining this evidence falls to basic, preclinical, and clinical researchers of all kinds, communicating and working together.

A participant pointed out that beyond the costs of development, a company must also bear the costs of educating physicians and providers about the use of the product.

Nakamura added that over the past few years, there has been discussion that the business model for CNS disorder medication development is basically failing, and that many pharmaceutical firms are leaving this therapeutic area. Studying sex differences, to the extent that they are a complicating factor, adds costs when developing a profitable drug. On the other hand, there is a limited business case for developing reasonable molecules that are inexpensive. Perhaps the NIH could help share the risk and leverage the strengths of federally funded research and industry by performing proof-of-concept studies while providing industry with the opportunity to develop distributable drugs.

A participant said that in recent years, charities such as the Bill and Melinda Gates Foundation have funded the development of compounds that are either not patentable, or are for a rare disease in countries where people cannot pay for products. Perhaps this alternative funding approach should be considered for sex differences.

Lee noted that many companies are now involved in various consortiums, such as the Foundation for NIH's Biomarker Consortium, where money is pooled and risk is shared. Lee also said that the blockbuster business model is really in the past. Moving forward, there is definitely consideration of personalized medicine, but the investment has to be justified.

Another industry participant concurred that industry is moving away

from big blockbuster drugs and is much more comfortable with smaller markets. In personalized healthcare, it is implicit that not only is the target population restricted, but the efficacy of the drug is potentially increased and thus has a higher chance. A product may be approved for the 20 percent of the people who have a particular mutation or amplification of a gene, with payers willing to reimburse the manufacturer for it because it works.

Another participant wondered if a sex difference is something that a drug developer would simply rather not know because that information prevents them from marketing the product to everyone, potentially cutting the marketing in half.

An industry participant responded that he was not aware of many drugs in development that were so much more efficacious for one sex than the other that they merited pursuing a separate, sex-based efficacy claim. However, safety problems detected in animals can often be restricted to one sex, or the therapeutic index may be different. The company is then faced with the difficult decision of whether to continue development of the drug for one sex, or stop and try to switch to another molecule in the family (assuming it is not a mechanism-based toxicology or adverse event). Risk/benefit also needs to be considered. If toxicology in one sex is bad, should the drug still be developed for the opposite sex? If the other sex takes it inadvertently or doctors do not read the label or prescribe it off-label for the other sex anyway, that is a significant risk.

Encouraging Basic Research into Sex Differences

Many participants asserted that if basic researchers were somehow required to study both males and females, the amount of information available about sex differences could be rapidly expanded, but such a mandate would be too expensive, and there would be a huge pushback from the research community. Other, more practical options could be to require study sections to rate grants on their comparison of sexes, or to put greater power in the hands of program officers, asking them to commit a certain amount of their funding to basic research grants that consider sex differences. There could be a study section, or similar alternative mechanism, for vetting which sex differences in human conditions and diseases should be funded.

A participant added that, as a previous recipient of NIH funding, he believed that forcing researchers to include both sexes in animal research was a bit unpalatable, but that it would be reasonable to ask them to justify why they use one sex or another, or both.

Interdisciplinary Collaboration

As the panel presentations demonstrated, there are commonalities and shared factors across different clinical conditions with respect to sex differences. Given limited funds, participants suggested that one way to have a greater impact is to target funding toward those commonalities. One way is to encourage interdisciplinary cooperation through Requests for Applications. This would not need to be a large multicenter effort, but could be two researchers from different disciplines who are working in collaboration. A participant noted that the NIH interdisciplinary programs are intended to do this.

4

Reporting Sex Differences in Research Publications

Journal policies lay out what is required of manuscripts that are submitted for publication, Workshop Cochair Rae Silver noted as she opened the session on reporting sex differences in research publications. If journal editors believe that knowing the sex of origin of the cell type discussed in the report is important, or likewise the sex of the animals or human participants in a study, then investigators will have to include that information when describing strains, species, and participants in manuscripts. In this session, two panelists representing peer-reviewed, professional neuroscience journals provided perspectives on the status of reporting sex differences in neurological health and disease.

JOURNAL OF NEUROCHEMISTRY

Sean Murphy, professor in the Department of Neurological Surgery at the University of Washington Medical School, is midway through an 8-year appointment as editor of the *Journal of Neurochemistry* (JNC). Established 50 years ago, JNC was the first neuroscience journal. JNC receives about 2,000 manuscripts each year and publishes about 30 percent of those, in a variety of areas from molecular biology through animal studies (JNC does not publish clinical studies).

In conducting his own informal survey, Murphy found that out of 30 journals that focus on or report neuroscience studies, only one, the *European Journal of Neuroscience*, stated directly in the author instructions a requirement for reporting the species, strain, sex, age, supplier, and

numbers of experimental animals used. He was surprised to find that his own publication, JNC, did not require the sex of animals to be reported.

A recently published survey of 271 randomly selected articles that reported the results of animal studies found less than 60 percent stated clearly the hypothesis, and the number and characteristics of the animals used, including sex (Kilkenny et al., 2009). More than 85 percent did not report any attempt at randomization or blinding to reduce bias in assessing outcomes, and 30 percent did not report statistical methods. This lack of information sharing may well contribute to why animal models are not optimized.

In response to this, guidelines are currently being developed that will ask editors of journals to require authors to address a checklist of 20 items that are the minimum information that should be included in all scientific publications reporting research using animals (e.g., number and specific characteristics of animals used, including species, strain, sex, genetic background; details of housing and husbandry; and experimental, statistical, and analytical methods, including methods to reduce bias).

Murphy noted that although these guidelines will call for the reporting of the sex of animals used, it does not ask authors submitting an article to give the rationale for studying either males or females, or to describe what the potential implications are for not studying the other sex. Including these requirements would be an educational step, Murphy said, and as editors are currently sensitive to these issues, the time could be right to implement such requirements.

EXPERIMENTAL NEUROLOGY

Marie-Francoise Chesselet, chair of the Department of Neurobiology at the University of California–Los Angeles and associate editor of *Experimental Neurology*, is also treasurer of the Society for Neuroscience (SFN) and an ex-officio member of the publications committee for the SFN publication, the *Journal of Neuroscience*. Money is often a primary reason why many studies do not use both sexes, she said. But a valid scientific explanation and justification should be given for studying only one sex. Chesselet supported Murphy's suggestion that authors be required to state the potential implications of studying only one sex.

In many papers, Chesselet observed, authors indicate that they balance experimental groups by sex, but do not disclose the exact balance. In some cases that "balance" turns out to be, for example, two males and seven females in one group, and one female and eight males in the other group. This information should be explicitly disclosed, she said.

Proper analysis of data by sex should also be required because, as mentioned in the earlier discussions, even National Institutes of Health–

sponsored studies that do include both male and female animals do not always analyze the two sexes separately.

Journals have an educational role to play and can lead the way by providing, for example, checklists for authors and reviewers to consult when preparing and reviewing manuscripts. Reviewers should increase their awareness of the potential importance of sex-related effects, and they should not consider a finding that applies to only one sex as a shortcoming. Journal editors can also write editorials to increase awareness about the need for reporting sex differences. Researchers who serve on editorial boards should raise these points with their colleagues.

Silver suggested that for journals to be effective in changing the way researchers report sex and sex differences, a concerted and coordinated effort is needed to revise and enforce policies; otherwise authors may simply choose to submit their manuscripts to journals with less stringent requirements.

OPEN DISCUSSION

Many participants agreed that having journal editors require specification of sex in publications would not create an undue burden on scientists. One participant cited a 1994 statement by the New York Academy of Sciences that recommended journal editors and reviewers require specification of the numbers and proportions of males and females studied, and that generalization from single-sex studies should be restricted to the sex investigated. In 1992, the University of California–Berkeley similarly encouraged reviewers to ensure that authors state the sex and reproductive state of the species studied, and that editors adopt sex specification as part of journal policy. These recommendations had no way to be enforced, and fell on deaf ears, he said.

Decisions to invest in studying sex differences during drug development are based, in part, on the presence of symptomatic or epidemiologic differences in disease characteristics between males and females. On the other hand, it was also noted that studies of the underlying pathophysiology of diseases between sexes suggest large differences that may, in essence, cancel each other out, and lead to a common symptomatic or epidemiologic presentation. Therefore, dissemination of all information obtained regarding sex differences is very important.

5

Sex Differences in Drug Development: Policy and Practice

The innovative pharmaceutical and biotechnology industries are critical stakeholders in the long process of bringing new therapies from bench to bedside. Morgan Sheng, vice president of neuroscience at Genentech, gave the keynote address at the workshop. He offered an industry view of sex differences in translational neuroscience. Panelists from the Food and Drug Administration (FDA), the National Institutes of Health, and industry discussed the history, guidelines, and regulations regarding the inclusion of males and females in clinical trials, and how and when industry considers and addresses the study of sex differences, given current regulatory requirements.

SEX DIFFERENCES IN TRANSLATIONAL NEUROSCIENCE: A VIEW FROM INDUSTRY

Those in the neuroscience field would generally agree that there are significant sex-based differences in how the brain works, and in diseases of the nervous system, Sheng said. They concur that it is important, even ethically imperative, to consider sex differences in all levels of research, but especially in research that involves humans and drugs given to humans. But we live in a resource-limited world, and a challenge is determining the right time to invest these limited resources so that data on sex differences can be translated into actions.

Drug Discovery

Quite simply, the business of the pharmaceutical industry is to make drugs that can help people—and to sell those drugs. Genentech focuses on

development of novel drugs and targeted therapies, always with the best interests of patients in mind, Sheng said. Targeted therapies focus on treating the underlying mechanism of the disease to modify the disease course. Treatment or palliation of symptoms is also important, Sheng said, but Genentech's philosophy is to develop disease-modifying medications. Even patients with the same basic indication for treatment can have different mechanisms of disease. To define the most appropriate population for treatment, and work toward personalized health care, more diagnostic tests that differentiate these subsets are also needed.

Although some are pessimistic about the "broken business model" of the modern pharmaceutical industry, Sheng said Genentech remains optimistic about the future of the industry. Tremendous technological advances have been made in the understanding of basic biological mechanisms. Much is known about disease pathways, which are beginning to be linked into a systems biology. Sheng anticipated that in another few decades, thousands of plausible or rational disease targets would be identified for possible drug treatment. To truly personalize health care, thousands of targets and thousands of drugs are needed.

The standard flow of drug discovery starts with a basic understanding of the mechanisms of the disease. Then drug targets are identified based on rational understanding. Those targets are "druggable," meaning they can be attacked by small molecules, proteins, antibodies, or maybe in the future, gene therapy with small interfering RNAs. Differentiated molecular medicines are then developed to attack these targets, tailored to the groups for which they are most effective.

Sex difference is one obvious way to describe subgroups of a patient population. Another is genetic groups, which are independent of sex. Race and age also have significant effects on disease. In general, Sheng said, drug development in neuroscience is at a disadvantage because the basic workings of the brain and its disease mechanisms are still poorly understood.

Considering Sex Differences in Drug Development

Sex differences can provide information about disease mechanisms and clues about why people contract a disease. Sex differences also affect the quality of animal disease models, and should guide choices of animal models. Most importantly, from a treatment point of view, sex can affect how a drug is metabolized and how well the patient responds to the drug.

How should sex differences be considered during drug development? One could analyze sex differences in a "blanket fashion." This means covering sex differences at a purely descriptive level, with no hypothesis, regardless of the amount of labor or time expended and without thinking about the cost. This would entail including equal numbers of males and females

in all genetic studies, pathological examinations, translational experiments, and efficacy studies, and analyzing them together and separately. This approach does not consider race (or strain, for mice), age, environment, or other important factors. For certain neurodegenerative illnesses, these other factors may be more important to consider than sex.

Another issue is that of experimental animals raised in cages. Disease phenotypes in mice that have been genetically engineered to have defects can be reversed simply by changing their environment: giving them more space and several other mice with whom they can interact. Environmental enrichment is costly in an animal care facility, but it may be more or just as important as sex differences.

A better approach to determine when to consider sex differences is when doing so provides the most *value*, or the most benefit for the expenditure. The first step is to analyze sex differences in general, unbiased descriptive studies of humans. A great deal of existing data can be mined for sex differences, Sheng said. One approach would be to look at the human genetics of nervous system diseases, particularly through genome-wide association studies (GWAS). Other approaches include, for example, prospective clinical studies of the natural history of disease, and biomarkers of disease and its progression.

One example from the recent literature involves a genetic polymorphism in the gene for brain-derived neurotrophic factor (BDNF). BDNF is involved in many processes in the brain, including plasticity of synapses, neuronal development survival, and neurogenesis. Reduction in BDNF has been implicated in stress and depression, and antidepressant drugs increase BDNF. The Val66Met polymorphism in BDNF is associated with impaired secretion of BDNF and is linked to several neuropsychiatric illnesses. Researchers looked at the genome-wide association between the Val66Met polymorphism and major depressive illness, and found no association overall. But when they segregated males and females, they found a significant association in males (Verhagen et al., 2010). The implications of this are not clear, but it is an example of interesting studies to come, where sex differences are parsed out through large-scale, genome-wide association studies.

A more difficult question than when should sex differences be *studied* is when they should be *investigated*, moving from descriptive studies to experimental hypothesis-driven studies. This question needs to be addressed in an indication-specific fashion. One factor that might compel one to move from descriptive to experimental studies is if there are large sex differences in the human clinical disease (epidemiology, features, outcomes, etc). Experimental studies are also possible if the basic pathogenesis of that disease is understood, and if there is a reasonable animal model in which to study the potential sex difference. If the sex difference is largely due to environmental

or social factors, studying that in an animal is difficult. If there is not a large sex difference, if basic pathogenesis is poorly elucidated, or if there is no appropriate animal model, then it is very difficult to *scientifically* study the sex differences in preclinical translational studies, Sheng said.

Studying Sex Differences in Neurological Diseases

Neurodegenerative Diseases

Neurodegenerative diseases are a scourge of modern civilization, Sheng explained. Alzheimer's disease (AD) affects about 5 million people in the United States. It results in impaired memory, progressing to profound dementia. The disease mechanism is not yet established, and even though textbooks focus on beta-amyloid plaques, that is still a hypothesis, Sheng said. No disease-modifying treatment is available yet, although several are in various phases of clinical development.

Alzheimer's disease is about twice as common in women than in men. This could be partly due to age, because women live longer, or it could be that men get other diseases before they get AD. Whether or how this observed sex difference may be important for the disease is unclear, Sheng noted. Although the sex difference is not huge, it would be worth studying if the right kind of animal model were available. But current animal models for AD are not ideal.

With this in mind, when should sex differences in AD be studied? Given a finite pool of money to try to find a cure or a new target for AD drugs, Sheng said he would not spend it studying the sex difference. Rather, studying age factors would be a better use of resources because of the hundredfold increase in its incidence between the ages of 60 and 90. Another area to focus on would be the apolipoprotein E gene variant, *ApoE4*. People who carry a single allele of *ApoE4* are 3.5 times more likely to get AD, and those with two copies of the gene are 10 times more likely.

Given the lack of good animal models, the difficulty in accounting for sex differences, and the fact that not enough is known about the basic mechanism of Alzheimer's disease, drug discovery is more likely to be successful by focusing on some of these other factors.

Another neurodegenerative disease being studied is amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease), a relatively uncommon illness that is more prevalent in men. A mouse model does exist for these studies. Although the underlying mechanisms are not fully understood, mutations in the superoxide dismutase 1 (SOD1) gene are associated with a small percentage of ALS cases. Transgenic expression of the human SOD1 mutation in mice recapitulates the human disease. Animals survive between 21 and 25 weeks, with females surviving longer by several days to a week.

In addition, Sheng noted, an investigational therapy also shows a sex difference. In this case a sex difference in humans can be translated to a sex difference in the animal model and in a therapy response.

Neuropsychiatric Disorders

Sex differences have also been observed in neuropsychiatric illnesses such as depression (twice as common in women) and schizophrenia (1.4 times as common in men, with earlier onset). Sex differences in neuropsychiatric disorders are important, but may be due in part to environmental and social factors. As a result, studying relevant neuropsychiatric sex differences is difficult in preclinical experiments. Another primary hurdle in studying sex differences in these diseases is the lack of good animal models.

When so little is known about the basic physiologic and disease mechanisms of the brain, Sheng asked, how high a priority should studying sex differences in preclinical research be given? In many diseases where the pathophysiology is relatively unknown (and where there are no large sex differences), is it more reasonable to start by trying to understand the basic mechanism with the assumption that it might apply to both sexes before we attempt to analyze the differences between the sexes?

Neurodevelopmental Disorders

Autism is a common neurodevelopmental disorder with a significant sex difference; males are four times more likely to be affected than females. One theory for this disparity is the "extreme male brain theory of autism" by Simon Baron-Cohen. This theory posits that males generally have less empathy than females, and autism is an extreme form of that sex distribution that occurs in the general population. More recently, autism has been shown to have a significant genetic component. Very good mouse models are available for the specific genetic causes of subsets of autism spectrum disorder, including Fragile X syndrome, tuberous sclerosis, and Rett syndrome (which affects only females).

This area should be fertile ground for hypothesis-driven translational neuroscience research that investigates sex differences, Sheng said. However, opportunities are often missed because experimental expediency can corrupt the study and weaken conclusions. Behavior analysis in translational research is labor intensive and can take a very long time. The significant variability requires a very large sample size, and multiple assays are involved. Although Genentech typically includes both males and females in all behavior experiments, in general, males are often the preferred sex for behavior studies because they are considered to be less variable, and because using only one sex halves the numbers needed for analysis.

Sometimes males are selected because they make the research easier. Rett syndrome, an autism spectrum disorder, is a severe form of X-linked mental retardation affecting only females (because affected males do not survive). Rett syndrome is caused by mutations in a DNA binding protein, MECP2. There is an excellent animal model in which MECP2 knockout mice display a range of physiological and neurological abnormalities that mimic the human syndrome. The phenotype can be rescued by activating the gene again weeks after birth.

Sheng described a study showing that insulin-like growth factor 1 (IGF-1) administered to MECP2 mutant mice reverses much of the Rett syndrome-like symptoms in the animals (e.g., it prolongs life by about 50 percent, improves locomotor and autonomic functions, enhances brain plasticity). A look at the methods, however, reveals that this study was conducted on males even though this is an almost exclusively female disease in humans. The reason is that males express a far more severe phenotype. In humans, males are stillborn or die early, but in mice, they do not die immediately and express a variety of defects. As a result, measuring the effects of rescue treatments is easier in males. The researchers really should have used both sexes of mice, Sheng said, but added that the study would have taken much longer, and the female results could have been ambiguous because the phenotype is less severe.

Conclusions

Sex differences are significant in normal brain structure and function as well as in behavior, Sheng said. These differences are critical to understanding and treating human diseases of the nervous system. Translational research in neuroscience is particularly complex and arduous, but sex differences should be explored in translational experiments if at all feasible, and investigated when appropriate.

However, basic mechanisms of normal brain function and pathogenesis of most central nervous system disorders are still poorly understood. Therefore, for many indications, studying sex differences may not be completely appropriate yet. In some cases, the most valuable investment of resources may be in basic neuroscience, to gain improved basic knowledge (and hence improved animal models) that is essential to the investigation and understanding of sex differences in physiology and disease.

WOMEN IN CLINICAL TRIALS: FDA POLICIES

Beginning around 70 years ago, and into the early 1970s, women were prescribed diethylstilbestrol to prevent miscarriages and premature deliveries, said Ameeta Parekh, director of research and development in the

Office of Women's Health at the FDA. Years later, as daughters of these women became adults, the daughters faced miscarriages, infertility, and a higher prevalence of vaginal and cervical cancers. Another drug, Thalidomide, taken by pregnant women in the 1950s to prevent nausea related to pregnancy, was later discovered to result in phocomelia (flipper-like limbs) and stunted limb growth in children. These examples represent the most extensive outbreaks of drug-induced birth defects in medical history, and were the basis behind the regulatory history that starts with 1977 FDA guideline, *General Consideration for Clinical Evaluation of Drugs*. With a strong tone of protectionism, the guideline said that women of childbearing potential should be excluded from the earliest dose-ranging studies. However, the guideline was broadly taken to mean that women should be excluded from all phases of clinical trials rather than just the earliest phases. As a result, women were excluded or underrepresented in clinical trials, which in hindsight was more detrimental than beneficial. Critics of the guideline said it precluded a woman's ability to decide for herself whether to participate, and violated the principle of informed consent. Advocacy groups contended that females were being denied access to important and innovative therapies.

In response to these concerns, several new FDA guidelines followed: the 1988 guideline, *Format and Content of the Clinical and Statistical Section of an Application*, and the 1989 guideline, *Study of Drugs Likely to Be Used in the Elderly*, both of which recommended analysis by age, race, and sex.

But a guidance is not a regulation or a mandate, and there was concern as to whether these guidelines were enough. Advocacy groups lobbied Congress, and in 1992, Congress requested a Government Accountability Office (GAO) survey of the representation of women in clinical trials. GAO concluded that women were not adequately included (GAO, 1992). For 60 percent of the drugs GAO reviewed, the representation of women in the trial was less than the prevalence of the disease. Even when women were included in the studies, the data were not analyzed for sex differences. Overall, GAO concluded that there was a lack of understanding of sex and gender differences.

In 1993, following the GAO report, a new guideline was issued that reversed the 1977 policy of exclusion of women of childbearing potential from early trials. The guideline, *Study and Evaluation of Gender Differences in the Clinical Evaluation of Drugs*, recommended collection and analysis of data on sex differences for effectiveness, adverse events, and pharmacokinetics. The guideline also addressed reducing the risk of fetal exposure through protocol design.

In 1998, the FDA issued regulations addressing investigational new drug applications (INDs, generally submitted to the FDA before an investigational

product can be shipped for clinical trials) and new drug applications (NDAs, submitted to obtain marketing approval for a new product).[1] The regulations require that NDA submissions and IND annual reports include information on trial participation, safety, and efficacy, and with data presented by age, race, and sex.

IND regulations required that the data be *tabulated* by age, race, and sex, and NDA regulations required that safety and efficacy data be *presented* by age, race, and sex. However, there is no requirement for any specific number or percentage within any subgroup or subpopulations, and as a result, the studies may or may not be powered sufficiently to look at differences between subgroups.

In 2000, the regulation was amended to allow the FDA to put a clinical hold on IND studies of treatments for life-threatening diseases if women were excluded due to reproductive potential.

In 2001, nearly 10 years after the 1992 GAO report, Congress requested that GAO again report on the status of women in clinical trials. This time GAO found that appropriate numbers of women were included in studies submitted as part of NDAs, and that participation of women was similar to that of men, except for the earliest phase clinical trials and in select therapeutic areas, particularly cardiovascular disease (GAO, 2001). However, analysis by sex was not consistently present. More recent data, Parekh concluded, show that women's participation in early phase trials has continued to increased since the 2001 GAO report (Pinnow et al., 2009).

A STRATEGY FOR TRANSLATIONAL PSYCHOPHARMACOLOGY IN MOOD DISORDERS

Carlos Zarate, Jr., chief of experimental therapeutics in the Mood and Anxiety Disorders Program at the National Institute of Mental Health (NIMH), described a strategy for translational psychopharmacology in mood disorders involving multimodal imaging, complex math modeling, and psychiatric stress testing.

As discussed in Chapter 3, depression and other mental disorders are complex behavioral disorders with clear biological differences between men and women. However, little has been found in terms of sex differences in treatment response in depression (e.g., dosing, pharmacokinetics, adverse effects, drug interactions, or the roles of sex-linked genetic traits, menopause, perimenopause, and the menstrual cycle).

The association between sex differences in depression and treatment response remains unclear. One area of focus is genetics, and there has been

[1] 21 CFR 314.50 and 21 CFR 312.33.

considerable interest in certain single nucleotide polymorphisms within the serotonin transporter gene that may be associated with observed differences in clinical response to selective serotonin reuptake inhibitors (SSRIs). But whether polymorphisms are relevant in clinical practice is questionable.

The largest study conducted to date on sex and treatment response in depression is the STAR*D (Sequenced Treatment Alternatives to Relieve Depression) trial funded by NIMH, in which 3,000 outpatients received the SSRI citalopram for 10 to 14 weeks. The remission rate (absence of depressive symptoms) was about 24 percent in men and 29 percent in women, or about a 5 percent difference in favor of women over men. The time to response and remission was not different between males and females. In the end, Zarate explained, the sex differences in remission are small, with low overall remission rates and an extended time to achieve remission.

Much work is currently being done on how endophenotypes might relate to genes associated with depression. In a similar approach, Zarate is looking at treatment response first, and then which genetic underpinnings might be responsible for that treatment response. His approach employs multimodal imaging, objective data, and psychiatric stressors or challenges.

Ketamine, a non-barbiturate anesthetic used worldwide for anesthesia, is useful as a tool for translational psychopharmacology in mood disorders. Ketamine acts by blocking the N-methyl- D-aspartic acid receptor, and has been studied in many conditions throughout the years (e.g., schizophrenia, cognition, alcoholism, chronic pain syndrome). In patients with treatment-resistant major depressive disorder, treatment with ketamine resulted in a robust, rapid, and sustained antidepressant response within 2 hours of a single infusion, compared to the weeks to months that other therapies take to achieve remission (Zarate et al., 2006). Zarate is now working to identify biomarkers that will predict response to ketamine.

In preclinical models, ketamine's mechanism of action appears to be enhanced α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate throughput. On a clinical level, Zarate has found that synaptic plasticity or potentiation is associated with the antidepressant effects of ketamine (using slow-wave activity as a putative marker of synaptic plasticity). Using ketamine as a model, within about 4 to 6 hours responders and non-responders can be identified using multimodal imaging technologies. One can then apply psychiatric stress testing and complex math models to see what moderators might be relevant.

In conclusion, Zarate noted that sex difference matters, but the interaction with other variables is the important issue. To date little is known about the impact of sex difference in response to depression treatment. Neurobiological parameters may be valuable in predicting treatment response, and may better explain variance in response than common subdiagnostic

categories. One approach to looking at sex differences in treatment response is to identify biomarkers of response and determine which combination of factors, including sex difference, explains the greatest variance of response. This is a step toward personalized health care.

PRACTICAL ISSUES OF ADDRESSING SEX DIFFERENCES DURING TRANSLATIONAL RESEARCH AND CLINICAL DRUG DEVELOPMENT

Douglas Feltner, vice president of Global Translational Medicine and Neuroscience at Pfizer Inc, reviewed some of the practical issues in drug development. First, it is important to remember that the molecules being studied in translational research are drug candidates, not drugs, and little is generally known about them. Safety and efficacy must be characterized, but cost and time are critical constraints. Ultimately, a candidate does not become a drug until it receives marketing approval and has an approved product label.

In animal toxicology studies, rodent and non-rodent animal species are exposed to drug concentrations that are far in excess of the projected efficacious concentration so that safety issues that might be of concern in humans can be identified. In the IND toxicology studies (conducted before filing an IND to support moving the product into humans), both sexes of animals are used. Animal toxicology data are used to set a human exposure limit. The key toxicology questions are, is there a therapeutic index (a dose that is effective, but not toxic); are any toxic findings in animals reversible; and can they be monitored in humans? For example, tissue necrosis or cellular hyperproliferation are generally not reversible and usually not monitorable. Changes in heart rate, blood pressure, or QT intervals are generally reversible and monitorable.

Sex differences do occur in toxicology findings, Feltner said, and fall into three main types: (1) the same finding may occur in both male and female animals, but at different exposures; (2) a unique safety finding may occur only in one sex, sometimes in a reproductive organ, but other times in other organ systems; and (3) safety findings may occur at the same exposure in male and female animals, but the associated doses may be quite different due to variations in bioavailability, distribution, metabolism, or elimination.

The translatability of irreversible and non-monitorable animal safety findings with no projected therapeutic index are really not known because in nearly all of these cases, these drug candidates are dropped from development. If there is a backup molecule, and it is suspected that the toxicology findings are not related to the mechanism of action of the drug candidate, but rather some structural effect, that backup candidate might be investigated further.

On the other hand, for reversible and monitorable safety findings in animals, something is often known about translatability because those problems may have been addressed before. Sometimes sex differences in animal safety findings are reversible and monitorable, and have an acceptable therapeutic index, Feltner said. If, for example, the safety finding occurs in males at 100 times the efficacious concentration, and is not observed in females until 1,000 times the efficacious concentration, that is not really a concern because the dose would not be set that high in humans.

With regard to efficacy, the point was made throughout the workshop that most animal efficacy studies are done in males to reduce variability. Feltner noted that regardless of whether male or female rats would be more or less variable, having just one sex of rodent is likely to produce less variability than having both. Male/female efficacy differences are generally not explored in animals. The bigger issue for neuroscience discovery is successfully matching novel targets to the right patients. Too often the efficacy observed in animal models does not translate to efficacy in a patient population. Would studying both male and female animals help with this problem?

IND toxicology is completed prior to starting reproductive toxicology studies, so results are not generally available at the start of Phase I trials. Key data are necessary before exposing women of childbearing potential to an investigational product, including one that has potential maternal and fetal toxicity, which is derived from embryo-fetal development studies in rats and rabbits. Female and male fertility studies are completed prior to initiation of Phase III trials, and pre- and postnatal development studies are completed prior to submission of the NDA. Embryo-fetal development studies are usually completed shortly before Phase IIa in order to allow for adequate patient recruitment, including a more representative population, but it depends on indication, Feltner said.

This means that, for practical reasons, males predominate in Phase I studies because embryo-fetal toxicology is not yet done. Women of nonchildbearing potential can also participate in Phase I studies. After embryo-fetal developmental toxicology is done, the male-to-female ratio for recruitment depends on the disease being studied (although for reasons unknown, the actual recruitment may have slightly more males than the epidemiology of the disease would predict).

Differences in tolerability related to male/female exposure differences may be found in Phase I studies. Careful pharmacokinetic characterization allows for understanding of these sex differences in exposure by dose, but with the small sample size of Phase I studies, only limited information on differences in tolerability can be obtained, and none on efficacy.

As product development approaches the NDA submission, sex difference effects in exposure are examined in a population pharmacokinetic

analysis across all of the studies that will be part of the submission. A pharmacokinetic/pharmacodynamic model is built as data accumulate; efficacy and common adverse events are related to either exposure or dose, by sex; and findings are then used to support dosing recommendations.

Ultimately, moving a drug candidate through development involves responding to the new data that are always emerging, Feltner said. In some circumstances more research is necessary to understand sex differences in efficacy or safety in the clinic, and in other circumstances, nothing more needs to be done than is currently being done. The next steps depend on the cumulative data up to that point.

OPEN DISCUSSION

Animal Models

Participants made a variety of additional points regarding preclinical research, including the value of animal models, reporting results of animal studies, and the need for clinical and basic researchers to work together on animal model development.

One participant expressed the opinion that psychiatric disorders are essentially human, and it is unlikely that, for example, an animal model will ever be depressed from a human point of view. However, understanding traits in animal models and looking at endophenotypes does provide relevant information about the disorders. Understanding the mechanisms behind the disruption of the reward circuitry around food motivation, for example, is extremely important for understanding the nature of eating disorders in humans.

A participant suggested that it is not necessarily the animal models that are at fault, but the quality of the science that is done around those models. A participant urged industry to make preclinical animal data available as soon as possible so that those in the public sector could review outcomes. The data do not have to be published in the traditional sense, he said, but simply made available, especially for systematic reviews.

Stevin Zorn, workshop cochair, noted that many animal models used in drug discovery research were developed more than 50 years ago, when clinicians and basic animal researchers worked closely together to model human diseases. Given what is now known about the complexities of diseases, and the impacts of not just single, but multiple, genetic defects, it is time to get basic and clinical researchers back together to reevaluate what the animal models are, what information the models can provide, and what can be modeled.

Diagnostic Criteria

Current diagnostic criteria were raised as a barrier to progress in research. Symptom-based disorders are qualitatively different from neurodegenerative

or neuroinflammatory disorders, a participant said. In the ongoing large-scale GWAS studies, the biggest bottleneck is going to be the phenotyping of patients. The symptom-based diagnostic categories used in pain and psychiatry are consensus-based criteria that subclassify further into artificial categories that do not consider the full syndromes. Much better phenotyping is needed, and sex-based differences should be included automatically in these phenotypes. Otherwise they are not complete, accurate, and descriptive phenotypes.

Zarate concurred with concerns about diagnostic categories. Statistics show that an individual with two comorbid anxiety disorders has a 50 percent chance of having a third. With three or four comorbid anxiety disorder diagnoses, why not just have an anxiety disorder across all of the comorbidities and focus on that? As time passes, hopefully more biomarkers will be recognized in psychiatry, and diagnostic groups will be needed less.

6

Needs, Opportunities, and Next Steps

Sex differences in neurological disorders have been observed in disease susceptibility, disease incidence, time to onset of symptoms, manifestation of illness, prognosis, and drug response, including pharmacokinetics, phamacodynamics, efficacy, adverse events, and treatment outcomes. The differences may be a result of sex hormone activation of genes and circuits, developmental pathways, sex chromosome effects, or a combination of these factors. Breakthroughs in molecular biology and noninvasive brain imaging have redefined the goals and promise of neuroscience research, and knowledge about sex differences must now be incorporated into basic and translational research strategies.

Over the course of the workshop, participants provided numerous examples of when and how researchers are studying these differences across the spectrum of neurological diseases. We now know that some tissues have huge sex differences in gene expression. The challenge is how to utilize our current knowledge to inform the practice of the variety of stakeholders involved in central nervous system (CNS) drug development enterprises (e.g., preclinical researchers, funding agencies, Food and Drug Administration [FDA], journals, industry, patient advocacy groups).

In the keynote address, Morgan Sheng highlighted the challenge of determining when limited resources should be invested in studying sex differences. For some diseases, investments in basic science research of nervous system disorders may be more appropriate even if there are known sex differences.

In the closing session, Workshop Chairs Stevin Zorn and Rae Silver reviewed the barriers impeding sex differences research that were identified

during the panel discussions. They challenged participants to identify the next steps necessary to overcome these challenges, other factors that could ease the successful translation of sex differences from preclinical to clinical studies, and additional priority areas for research.

Much of the workshop discussion focused on females because they often bear the greater burden across many parameters in the neurological disorders discussed. However, sex difference research offers the opportunity to determine why one sex may be more predisposed to certain diseases, or have worse outcomes, while the other sex is protected. Therefore, the results from sex differences research will have a significant impact on the public health of *both* sexes.

BARRIERS

Over the course of the workshop, a variety of barriers to conducting more appropriate, extensive, or prioritized sex-based research in diseases of the nervous system were identified (Box 6-1). Participants broadly acknowledged that sex differences are important in neurological disorders, but that current knowledge of underlying biology is insufficient, both in health and disease. Participants were very concerned that almost no attention is paid to the importance of personalized medicine (age, race, ethnicity, sex, genetics) in health science education curriculums.

Participants identified a lack of expertise by study section reviewers in recognizing the importance of sex differences research in funding applications. Another difficulty is that no study section or special emphasis panel exists that specifically funds research on sex differences. For example, locating funding to develop a mouse model for estrogen receptor action in the brain would be difficult. The study of sex differences transcends tissues, diseases, and the Institutes at the National Institutes of Health (NIH). The traditional way that the NIH has been organized does not allow for a concentrated interest in sex differences; they must be studied in the context of something else.

The absence of animal models of human neurological conditions was also highlighted by participants as a barrier, as was the fact that many studies predominantly use male animals. The absence of reliable animal models is a tremendous barrier to CNS drug development. As animal models are developed, strategies will need to be established that take into account potential sex differences.

Dissemination of information on sex differences was also a topic of interest. Even when studies include male and female animals, results are often not reported by sex. Journals generally have no standard policy about including information on the sex of subjects studied or cell lines used, or the

BOX 6-1
Barriers to Sex-Based Research in Diseases of the Nervous System

- General lack of recognition of the importance of sex differences in health and disease.
- Lack of implementation of policies by large influential organizations, such as neuroscience professional societies, to require attention to sex differences.
- Inadequate focus on sex differences in graduate and medical education curriculums.
- Financial, resource, and time constraints associated with studying both sexes.
- Lack of expertise in sex differences in study sections and program review staff.
- Difficulty of securing funding to study sex differences in general (as grants generally fund studies only in the context of a particular disease).
- Limitations of animal models of nervous system disorders.
- Basic studies are largely conducted in male animals; concerns and confusion about variability in female animals due to estrous cycles.
- Institutional review boards face an ethical dilemma in approving the conduct of studies in humans in which the researchers do not have a strong basis in animal or preclinical work.
- Clinical studies may include both male and female participants, but are often not designed to allow for analysis of sex differences.
- Sex-specific data are often not reported in the medical literature, especially if no difference was observed.
- Difficulty of engaging commercial sponsors in investing in late-phase development to repurpose drugs that are already available generically (e.g., estriol).

reporting and analyzing of data by sex. Private-sector sharing of preclinical data was also discussed. Industry participants pointed out that little animal research done in industry goes unpublished. In some cases, however, the information may not published until compounds involved are patented.

Some participants acknowledged that communication between basic and clinical researchers is lacking, and facilitating collaborations among the NIH, the FDA, and industry can be a challenge. They also pointed out that workshops, studies, and reports over the past 30 years have drawn attention to the need to address sex differences in health, but few recommendations have been implemented.

Contributing to this general disregard for sex differences is the fact the large and influential bodies, such as neuroscience professional societies and journal publishers, have acknowledged sex differences, but have not implemented policies that require researchers to address them.

ADDITIONAL PRIORITY AREAS FOR SEX DIFFERENCES RESEARCH

Four specific neurological disease areas were reviewed during the workshop, including depression, pain and pain perception, sleep medicine, and multiple sclerosis and neuroinflammation. Other conditions were raised as examples during the discussions. At Zorn's request, participants offered additional suggestions of priority areas for sex differences research.

Researchers should be looking for possible sex differences during the time in life at which vulnerability to adult problems arise, a participant said. For example, studies suggest that vulnerability to depression and drug abuse in adults may be associated with prenatal stress in males, but early childhood stress in females. Differences in when early stressors produce vulnerabilities may also impact cardiovascular disease and pain. Multiple-syndrome liability and the times during the lifespan when these vulnerabilities are established may be a worthwhile commitment of resources and effort in studying sex differences.

Stuttering was suggested as another example of a dramatic sex difference in which there is also a differential sex recovery. Stuttering occurs in 1 percent of the general population, with a 4–5:1 male-to-female ratio. Interestingly, at the onset of symptom occurrence in early childhood, the sex ratio is closer to 2:1, female to male, but there is a differential recovery among females. Many more girls recover within years of stuttering onset, leaving many more boys who stutter by adolescence and adulthood. Research in this area is still in the early stages, but preliminary data show exaggerated deficits in structural and functional connectivity among left hemisphere speech-related regions of the brain in persistently stuttering females compared to persistent males.

One function that is clearly sexually differentiated is reproduction, a participant pointed out. Yet there is still little understanding of the brain mechanisms associated with reproduction, especially at the cellular and molecular levels. This would seem to be an obvious priority when looking at cellular mechanisms of sexually differentiated function.

NEXT STEPS

In introducing the workshop, Silver said the mission was to "look backward at what we already know and race forward to do something about it." Over the course of the workshop, participants discussed practical next steps that could be taken to facilitate a better understanding of sex differences in neurological health and disease, and translation of that knowledge into effective treatments for both sexes (summarized in Box 6-2). Those who fund, regulate, perform, or report research all have important roles to play,

BOX 6-2
Possible Next Steps Recommended by Individual Workshop Participants

Government

Funders

- Fund basic and clinical integrative, interdisciplinary research to
  - establish the scientific basis for sex differences in health and neurological disease,
  - build predictive and translatable models of human neurological disease and identify potential biomarkers.
- Establish mechanisms to fund sex difference (i.e., not necessarily in the context of a specific disease).
- Set standards for reporting of sex-related clinical data.

Regulators

- Require that studies be sufficiently designed to allow for analysis by sex.

Industry

- Establish precompetitive, public–private partnerships with academia and government to understand the biological basis for sex differences as they relate to disorders of the nervous system.
- Apply this knowledge to generate hypothesis testing studies, with the goal of establishing evidence-based therapeutic approaches for personalized medicine.

Academia

- Incorporate information about sex differences into the curriculums of graduate and medical education programs to
  - raise awareness of the pervasiveness of sex differences,
  - highlight the opportunities for medical advances related to them.
- Design clinical trials around evidentiary needs.
- Develop action steps and set disease priorities for mining of existing data sets for sex differences.
- Conduct studies or mine existing data to answer questions regarding female variability due to estrous cycle.

Journal Publishers

- Develop and institute guidelines for the inclusion of sex-related subject information in all publications, including sex of origin of tissues, cell lines, etc.
- Establish guidelines to encourage authors to analyze data by sex and to report sex differences, or the lack thereof:
  - requiring reporting of means and standard deviations by sex would help facilitate systematic reviews.
- Develop checklists for authors and reviewers to consult when preparing and reviewing manuscripts to ensure inclusion of sex-related information.

from the bench to the bedside, in acquiring and applying knowledge about sex differences in neurological disorders.

Facilitating Sex Differences Research: Funding and Collaboration

Many workshop participants recognized that mandating research on sex differences was not appropriate. But rather, and especially given the limited resources, beginning to establish a comprehensive plan is important. The plan should review available data sources and develop a process to prioritize sex differences research. Existing databases provide a rich source of available data that could be analyzed. Analysis of available databases and development of new databases for analysis of materials that have been overlooked could serve as a valuable starting point.

A variety of funding models were suggested, from government grants specifically for the study of sex differences to public–private partnerships. One opportunity that resonated with the workshop participants was the need to establish multisector partnerships that can share risk and stimulate research and development for CNS drugs, a participant said. An example is the Alzheimer's Disease Neuroimaging Initiative, a $60 million, multisector, public–private partnership among the NIH, the FDA, and 12 industry and other partners. They are conducting longitudinal studies at sites across the United States to attempt to map the cause of Alzheimer's disease and identify biomarkers that could be used in clinical trials. No single company, university, or NIH Institute could fund a study this large, but in partnership it is possible, and high-quality data are already emerging.

With regard to government funding through grant programs, efforts should be made to increase the expertise of reviewers on study sections so they recognize the importance of sex differences. Another suggestion by some workshop participants was to issue Requests for Applications for general neuroscience research, outside the context of a particular disease, where a significant sex difference has been identified. Within the NIH the "Blueprint for Neuroscience Research" is a cooperative effort across NIH Institutes and Centers that supports neuroscience research and is aimed at pooling resources and expertise to advance basic and clinical research. Another approach to studying sex differences would be as an initiative of the NIH Roadmap.

Education

Some participants stressed the need to incorporate the importance of sex differences into health science education curriculums at all levels. In the developing age of personalized medicine, graduate and medical students should be learning about the impacts of not just sex, but also age, race,

ethnicity, and genetics. Not enough is known about sex differences in health and disease, or how to apply what is known to healthcare practice. Building this area of study into the core medical education curriculum will better prepare the next generation of scientists and clinicians to advance understanding of sex differences and better translate that knowledge to improve patient lives. These scientists would then be better equipped to consider sex differences if they serve on study sections.

With regard to raising general awareness of sex differences, a participant suggested mining the power of the Internet. Websites such as Google and Wikipedia are very popular, yet information on sex differences is lacking on these sites. These public information venues present another opportunity to increase understanding of sex differences as they relate to health care.

Disseminating Information About Sex Differences: The Roles of Journal Publishers and Professional Societies

Suggestions for how journals could foster dissemination of data on sex differences included developing checklists for authors and reviewers to consult when preparing and reviewing manuscripts, and requiring authors to state the potential implications of studying only one sex.

A participant suggested that manuscripts that include both sexes should be required to report means and standard deviations by sex because this would facilitate systematic reviews. Authors often simply report that there were, for example, five males and five females and no statistically significant was difference observed, and do not include numerical data. But there may be a direction or a trend that could be found if the data could be included in a meta-analysis.

Sex Is Always There, So Always Look at Sex

Sex differences can be studied in multiple, one participant said. Studies of sex differences that are hypothesis driven should be sufficiently powered to test the hypothesis and analyze the results by sex. But in studies in which a sex-based hypothesis is not being tested, researchers should be encouraged to explore sex differences in whatever phenomenon they are studying, even though it might not be sufficiently powered, and convey those results in publications.

A

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B

Registered Attendees

Roseanne Armitage
University of Michigan

Arthur Arnold
UCLA

Patrick Bader
Stanford University

Samuel Barondes
UCSF

Simret Beraki
Stanford University

Karen Berkley
Florida State University

David Bredt
Lilly Research Laboratories

Louann Brizendine
UCSF

Carla Canuso
Johnson & Johnson

Soo-Eun Chang
Michigan State University

Marie Chesselet
Experimental Neurology /UCLA

Sophia Colamarino
Autism Speaks

Dena Dubal
UCSF Neurology and Gladstone Institutes

Chris Duffield
Sci Tech Biomed Consultant

Jeanne Duffy
Brigham and Women's Hospital

Douglas Feltner
Pfizer Inc.

Robert Fox
Mellen Center for Multiple Sclerosis at Cleveland Clinic

Stephen Glickman
UC–Berkeley

Peter Goadsby
UCSF Headache Group

Nirupa Goel
University of British Columbia

Jill Goldstein
Brigham and Women's Hospital

Melvin Grumbach
UCSF

Martica Hall
University of Pittsburgh School of Medicine

Nancy Hills
UCSF

Paul Hoffman
North Florida/South Georgia Veterans Health System

Emily Jacobs
UC Berkeley

Lisa Kilpatrick

Sofie Kleppner
Stanford Cardiovascular Institute

Chi-Ming Lee
AstraZeneca Pharmaceuticals

Linda LeResche
University of Washington

Jon Levine
Northwestern University

Lynne Love

Rachel Manber
Stanford University

Emeran Mayer
UCLA

Sonia Mayoral
Stanford University School of Medicine

Robert Meisel
University of Minnesota

Dawn Meyer
UCLA

Jeff Mogil
McGill University

Sean Murphy
Journal of Neurochemistry/ University of Washington

Stephanie Murphy
Oregon Health and Science University

Richard Nakamura
NIH/National Institute of Mental Health

Halina Offner
Oregon Health and Science University

Kathleen O'Leary
NIH/National Insitute on Mental Health

Ameeta Parekh
FDA/Office of Women's Health

Vivian Pinn
NIH/Office of Research on Women's Health

Linda Porter
NIH/National Institute of Neurological Disorders and Stroke

Nancy Raymond
University of Minnesota

Kathryn Sandberg
Georgetown University Medical Center

Nirao Shah
UCSF

Morgan Sheng
Genentech

Etienne Sibille
University of Pittsburgh

Rae Silver
Columbia University

Viviana Simon
Society for Women's Health Research

Offie Soldin
Georgetown University Medical Center

Rhonda Voskuhl
UCLA

Lauren Weiss
UCSF

Cora Lee Wetherington
NIH/National Institute on Drug Abuse

Katherine Wisner
University of Pittsburgh Medical Center

Ruth Wood
Keck School of Medicine of USC

Corinna Wu
Freelance Science Writer & Editor

Carlos Zarate
NIH/National Institute of Mental Health

Stevin Zorn
Lundbeck USA

Irving Zucker
UC–Berkeley

C

Workshop Agenda

SEX DIFFERENCES AND IMPLICATIONS FOR TRANSLATIONAL NEUROSCIENCE RESEARCH: A WORKSHOP

Background: Basic research that involves delineating meaningful drug effects and behavioral and physiological responses that differ between the sexes can be costly and time consuming because the research requires additional experiment groups and protocols. However, epidemiological and clinical studies indicate substantial sex differences in response to drugs. The sex differences cut across other parameters such as socioeconomic factors, race, age, etc. In the current era of translational research and personalized medicine, taking sex differences into account is important so that these drug effects can be more accurately understood. This is particularly important in the neurosciences because of the complex nature of many disorders of the nervous system, including mental, neurological, and substance use disorders. Consequently, the Institute of Medicine's Forum on Neuroscience and Nervous System Disorders is hosting a workshop to explore the key principles and strategies used by basic translational researchers and industry in studying sex differences in the neurosciences for the therapy development pathway.

Meeting Objectives: The objectives of this workshop are to

- briefly outline the public health importance of studying sex difference in the nervous system, in health and sickness, including the potential application to healthcare delivery;

- identify the scientific principles that should be considered when designing preclinical experiments that will examine sex differences, including strategies to bridge between preclinical and clinical studies;
  - discuss when and how sex differences should and should not be considered;
- explore the key principles and strategies used by academic clinicians to effectively use basic research for preclinical and clinical application and study (i.e., Phases 0–IV), including approaches used by researchers to decide how and when to consider the potential importance of sex differences;
- explore how and when industry considers and addresses studying sex differences, given regulatory guidelines;
- examine the advantages, constraints, and implication of performing "valid analysis" versus requiring statistical outcomes between the sexes;
- identify the next steps that will be critical to establishing a set of principles that could be used by a variety of stakeholders in considering when and how to incorporate studying sex differences into translational research efforts.

March 8, 2010

Franciscan Ballroom
Sir Francis Drake Hotel
450 Powell Street, San Francisco, CA

8:30 a.m. Welcome, Introductions, and Workshop Objectives

Rae Silver, *Cochair*
Professor, Natural and Physical Sciences
Columbia University

Stevin Zorn, *Cochair*
Executive Vice President
Neuroscience Research
Lundbeck

SESSION I: SEX DIFFERENCES IN RESEARCH: NEED, DESIGN, STUDY

Session Objectives:

- Briefly outline the public health importance of studying sex difference in the nervous system, in health and sickness, including the potential application to healthcare delivery.

- Identify the scientific principles that should be considered when designing preclinical experiments that will examine sex differences, including strategies to bridge between preclinical and clinical studies.
  - Discuss when and how sex differences should and should not be considered.
- Explore the key principles and strategies used by academic clinicians and industry to effectively use basic research for preclinical and clinical application and study (i.e., Phases 0–IV), including approaches used by researchers to decide how and when to consider the potential importance of sex differences.

Opening Remarks

8:40 a.m. What Are Some of the Challenges for Sex Differences Research and How Can They Be Overcome?

Vivian Pinn
Director
Office of Research on Women's Health
National Institutes of Health

9:00 a.m. What Are the Scientific Principles for Studying Sex Differences in Health and Disease?

Arthur Arnold
Professor and Chair
Department of Physiological Science
University of California–Los Angeles

9:20 a.m. When and How Should Sex Differences in Drug Response Be Studied?

Jeff Mogil
Chair, Pain Studies
Department of Psychology
McGill University

9:40 a.m. What Factors Will Affect the Successful Translation of Sex Differences from Preclinical to Clinical Studies?

Jon Levine
Professor
Department of Neurobiology and Physiology
Northwestern University

10:00 a.m. When and How Should Sex Differences in Disease Susceptibility Be Studied?

Kathryn Sandberg
Professor, Medicine and Physiology
Director, Center for Study of Sex Differences
Georgetown University Medical Center

10:20 a.m. BREAK

10:35 a.m. Panel Presentations: Depression

Katherine Wisner
Professor, Psychiatry, Obstetrics, and Gynecology
University of Pittsburgh School of Medicine
Director, Women's Behavioral HealthCARE
University of Pittsburgh Medical Center

Jill Goldstein
Professor, Psychiatry and Medicine
Departments of Psychiatry and Medicine at Harvard Medical School
Director of Research, Connors Center for Women's Health and Gender Biology
Brigham and Women's Hospital

Etienne Sibille
Associate Professor
Department of Psychiatry
Center for Neuroscience
Translational Neuroscience Program
University of Pittsburgh

Carla Canuso
Senior Director, External Innovation
Neuroscience Therapeutic Area
Johnson & Johnson Pharmaceutical Research and Development, LLC

11:25 a.m. Discussion with Panelists and Attendees

Richard Nakamura, *Moderator*
Director, Division of Intramural Research Programs
National Institute of Mental Health

11:55 a.m.	LUNCH
12:50 p.m.	Panel Presentations: Pain and Pain Perception

Karen Berkley
Professor, Psychology and Neuroscience
Department of Psychology
Florida State University

Emeran Mayer
Professor
Departments of Medicine, Physiology, Psychiatry, and Biobehavioral Sciences
Director, UCLA Center for Neurovisceral Sciences and Women's Health
University of California–Los Angeles

Linda LeResche
Professor
Department of Oral Medicine
School of Dentistry
University of Washington

1:20 p.m.	Discussion with Panelists and Attendees

Chi-Ming Lee, *Moderator*
Executive Director, Translational Science
AstraZeneca Pharmaceuticals

1:50 p.m.	Panel Presentations: Sleep Medicine

Roseanne Armitage
Professor, Department of Psychiatry
Adjunct Professor, Department of Psychology
Director, Sleep and Chronophysiology Laboratory
University of Michigan

Jeanne Duffy
Assistant Professor of Medicine
Division of Sleep Medicine
Harvard Medical School
Director, Chronobiology Core
Division of Sleep Medicine
Department of Medicine
Brigham and Women's Hospital

Rachel Manber
Professor, Psychiatry and Behavioral Science
Director, Stanford Sleep Medicine Clinic
Stanford University

Martica Hall
Associate Professor
Psychiatry, Psychology, and Clinical and Translational Sciences
University of Pittsburgh School of Medicine

2:30 p.m. Discussion with Panelists and Attendees

Rae Silver, *Moderator*
Professor, Natural and Physical Sciences
Columbia University

3:00 p.m. BREAK

3:15 p.m. Panel Presentations: Multiple Sclerosis and Neuroinflammation

Robert Fox
Staff Neurologist and Medical Director
Mellen Center for Multiple Sclerosis at Cleveland Clinic

Halina Offner
Professor, Neurology and Anesthesiology and Perioperative Medicine
Oregon Health and Science University

Rhonda Voskuhl
Professor, Neurology
Director, Multiple Sclerosis Research and Treatment Program
University of California–Los Angeles

3:45 p.m. Discussion with Panelists and Attendees

Paul Hoffman, *Moderator*
Associate Chief of Staff for Research and Program Development
North Florida/South Georgia Veterans Health System

SESSION II: REVIEW

Session Objectives: Based on today's presentations and discussions, a panel will synthesize and discuss key points and ideas that examined

- the principles that should be considered when designing preclinical experiments that will examine sex differences, including strategies to bridge between preclinical and clinical studies;
  - when and how sex differences should and should not be considered;
- the key principles and strategies used by academic clinicians and industry to effectively use basic research for preclinical and clinical application and study (i.e., Phase 0–IV), including approaches used by researchers to decide how and when to consider the potential importance of sex differences.

4:15 p.m.	Panel Review and Discussion
	Richard Nakamura Director, Division of Intramural Research Programs National Institute of Mental Health
	Chi-Ming Lee Executive Director, Translational Science AstraZeneca Pharmaceuticals
	Rae Silver Professor, Natural and Physical Sciences Columbia University
	Paul Hoffman Associate Chief of Staff for Research and Program Development North Florida/South Georgia Veterans Health System
4:45 p.m.	Closing Discussion with Attendees
5:15 p.m.	ADJOURN

March 9, 2010

9:00 a.m. Welcome and Review of Day One

Rae Silver, *Cochair*
Professor, Natural and Physical Sciences
Columbia University

Stevin Zorn, *Cochair*
Executive Vice President
Neuroscience Research
Lundbeck

9:20 a.m. Keynote Talk

Morgan Sheng
Vice President, Neuroscience
Genentech

9:50 a.m. Panel Discussion: Reporting Sex Differences in Research in Publications

Sean Murphy (*Journal of Neurochemistry*)
Professor, Department of Neurological Surgery
University of Washington School of Medicine

Marie-Francoise Chesselet (*Experimental Neurology*)
Professor, Neurology
Chair, Department of Neurobiology Reed Neurological Research Center
University of California–Los Angeles

SESSION III: FDA REGULATIONS AND PERSPECTIVES FROM INDUSTRY

Session Objectives:

- Discuss regulatory practices regarding the inclusion of males and females in clinical trials.
- Explore how and when industry considers and addresses studying sex differences, given regulatory guidelines.

- Identify industry's constraints on assessing sex differences in all phases of clinical trials.
- Examine the advantages, constraints, and implications of performing "valid analysis" versus requiring statistical outcomes between the sexes.

Stevin Zorn, *Session Chair*
Executive Vice President
Neuroscience Research
Lundbeck

10:10 a.m. Panel Presentations

Ameeta Parekh
Director, Research and Development
Office of Women's Health
Food and Drug Administration

Carlos Zarate
Clinical Professor, Psychiatry and Behavioral Sciences
George Washington University
Chief, Experimental Therapeutics
Mood and Anxiety Disorders Program
National Institute of Mental Health

Douglas Feltner
Vice President, Global Translational Medicine and Neuroscience
Pfizer

10:50 a.m. Discussion with Panelists and Attendees

Stevin Zorn, *Session Chair*
Executive Vice President
Neuroscience Research
Lundbeck

SESSION IV: NEXT STEPS

Session Objectives: Identify the next steps that will be critical to establishing a set of principles that could be used by a variety of stakeholders in

considering when and how to incorporate the study of sex differences into research.

11:20 a.m. Moderated Discussion with Attendees

Rae Silver, *Cochair*
Professor, Natural and Physical Sciences
Columbia University

Stevin Zorn, *Cochair*
Executive Vice President
Neuroscience Research
Lundbeck

Wrap-Up Discussion Questions:

a. What are the key opportunities where understanding sex differences will have the greatest healthcare impact?
b. What are some of the critical factors (e.g., biological, epidemiological, health economics, sociological, ethical) and how would they guide the consideration of studying sex differences to improve health care?
c. When and how should sex differences in disease susceptibility be studied?
d. When and how should sex differences in drug response be studied?
e. What are some of the barriers that impede sex differences research and how can they be overcome?
f. How can academic clinicians and basic researchers help improve translational neuroscience efforts in the area of sex differences research?
g. What factors will affect the successful translation of sex differences from preclinical to clinical studies?

12:00 p.m. ADJOURN