Scientific Principles for Studying Sex Differences in Health and Disease

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Objectives

Outline the public health importance of studying sex difference in health and sickness, including healthcare delivery.

Discuss progress in the last 10 years, since the last IOM report “Does Sex Matter?”

Identify the scientific principles that should be considered when designing pre-clinical experiments that examine sex differences
# Sex Differences

## Incidence of Neurological / Behavioral Disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Male : Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s Disease</td>
<td>30 : 70</td>
</tr>
<tr>
<td>ALS</td>
<td>62 : 38</td>
</tr>
<tr>
<td>Anorexia Nervosa</td>
<td>7 : 93</td>
</tr>
<tr>
<td>Bulimia</td>
<td>10 : 90</td>
</tr>
<tr>
<td>Depression</td>
<td>33 : 67</td>
</tr>
<tr>
<td>Gender Identity Disorders</td>
<td>75 : 25</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>33 : 67</td>
</tr>
<tr>
<td>Sleep Apnea</td>
<td>90 : 10</td>
</tr>
</tbody>
</table>

*Adapted from Zup and Forger, 2002, Swaab et al., 2003*
Goal

• Identify factors that protect one sex from disease, and target therapies to enhance that factor (or its downstream targets) to protect both sexes from disease.

(Sex-specific factors might be particularly attractive as therapeutic agents because they occur naturally and are not generally harmful.)
What seems to be the problem?

Doctor, I have a terrible case of the sex differences.

Did anyone ever die of a sex difference?
Why compare the sexes?

• The physician never treats a sex difference. The physician treats one patient at a time, female or male.

• Why not just study what works in each sex?
Fresh perspective
Why study sex differences?

• Knowledge of the physiology of each sex gives a critically important fresh perspective on the physiology of the other sex.
Example #1 of Perspective

- 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 factor in females can be used to lower mortality?
- Without comparison of the sexes, the question would never occur.
Example #2 of Perspective

• X-inactivation, a female-specific process, can only be understood by comparing females with males.

• Females must inactivate one X chromosome to achieve a similar balance of expression of X and autosomal genes as that achieved in males – the same X and autosomal genes are carried in each sex.
What has happened in pre-clinical research since the 2001 IOM report, “Does Sex Matter”? 

1. Shift in the conceptual framework for explaining proximate signals causing sex differences

2. Increased consideration of compensation: sex-specific factors that reduce sex differences
20th Century Model

XY

↓

*Sry* (Y-linked)

↓

○○ testes

↓

testicular secretions

↓

Masculine body & brain

XX

↓

absence of *Sry*

↓

ovaries

↓

lack of testosterone

↓

Feminine body & brain
Activational Effects: REVERSIBLE
Sex differences in traits that are abolished by gonadectomy in adulthood (caused by sex differences in secretion of sex steroids at the time of measurement of the trait).

Organizational (Differentiating) Effects:
PERMANENT
Testosterone-induced irreversible commitment of a tissue to a masculine rather than feminine phenotype.
Most sex differences may be caused by Activational Effects

Example: Sexually dimorphic gene expression in mouse liver

In one microarray study;
Expression of 2597 genes sex biased in mice with gonads
After gonadectomy, 12 genes sex biased

Van Nas et al., Endocrinology 2009
Lusis Lab, UCLA
Organizational Effects
Some sex differences are permanent, established before and after birth especially by male-specific effects of testicular secretions.

Main examples:
external genitals
internal genitals
brain circuits
Spinal Nucleus of the Bulbocavernosus (SNB)

Breedlove and Arnold, *Science* 1980
XY vs. XX

At the genetic level, all sex differences result from X and Y imbalance*

*in species with heteromorphic sex chromosomes
XY vs. XX

Understanding the physiology of sex differences means identifying sex-specific factors that cause the dimorphisms.
“Unified Model”

XY vs. XX

Genetic level: All sex differences result from X and Y imbalance

Gonadal

Sry in male

Non-gonadal

Unequal expression of X and Y genes including Sry

Hormonal “Organizational Effects”

Hormonal “Activational Effects”

“Sex Chromosome Effects”

Three classes of proximate factors causing sex differences in phenotype

Arnold Horm Behav 2009
The Big Three Causes of Sex Differences

• Activational Effects of testicular and ovarian secretions
• Organizational Effects (primarily of testicular hormones)
• Direct Sex Chromosome Effects
Last decade:
New evidence that sex chromosome genes act in non-gonadal tissues to cause sex differences in brain traits and disease
“Four core genotypes” mouse model

• \( Sry \) is moved from the Y chromosome to an autosome.

• Gonadal sex is no longer determined by the Y chromosome.

• Allows measurement of the different impact of XX and XY sex chromosome in mice with the same gonadal sex
“Four core genotypes” mouse model

Gonadal type

Sex chromosome complement

Male

Female

XX

XY
Direct Sex Chromosome Effects
Example #1

- XX mice show faster response to thermal nociceptive stimuli than XY mice, irrespective of their gonadal sex.
Nociception: hotplate test in GDX adults

Latency XX << XY
gonadal males = gonadal females

Gioiosa, Chen et al.  
Horm Behav 2007
Direct Sex Chromosome Effects
Example #2

• The number of X chromosomes causes sex difference in susceptibility to neural tube closure defects in mouse model.
p53 null mutation causes neural tube defects mostly in females because of difference in number of X chromosomes

p53 (+/+) p53 (-/-)

Chen et al., *Devel Neurobiol* 2008
Direct Sex Chromosome Effects Example #3

- Sex chromosome complement, XX vs. XY, contributes to sex difference in mouse model of multiple sclerosis.
Active EAE, mouse model of MS
Smith et al., Voskuhl Lab, UCLA J Exp. Med. 2008
Direct Sex Chromosome Effects
Example #4

• Sry acts directly in the substantia nigra to influence control of movement
• male specific action of Y gene.
Sry expression in Substantia nigra

Male-specific effect of Sry

Phoebe Dewing, CWK Chiang, K Sinchak, H Sim, P-O Fernagut, M-F Chesselet, PE Micevych, KH Albrecht, VR Harley, and Eric Vilain
UCLA Laboratory of Neuroendocrinology
Current Biology 2006
Clinical Implications
hormonal vs. sex chromosomal
sex differences

- Identifying sex-specific causal factors is critical for developing therapies
- If the sex-specific protection is caused by hormones, then target therapies to hormone-driven molecular pathways
- If the sex-specific protection is caused by sex chromosome genes, then find the genes and target their mechanisms of action
What has happened in pre-clinical research since the 2001 IOM report, “Does Sex Matter”?

1. Shift in the conceptual framework for explaining proximate signals causing sex differences
2. Increased consideration of compensation: sex-specific factors that reduce sex differences:
   Similar phenotype of males and females may be achieved by sex-specific mechanisms.
The Big Three Causes of Sex Differences

• Activational Effects of testicular and ovarian secretions
• Organizational Effects (primarily of testicular hormones)
• Direct Sex Chromosome Effects

• Compensation: Each of these factors can synergize another’s sex-specific effect, or block it.

Where to focus now

- The vast majority of animal models showing sex differences in physiology or disease have been poorly studied with regard to the factors that cause sex differences
- In almost no cases have The Big Three been investigated systematically and comprehensively
Where to focus now

• Studies of humans can best investigate activational effects, not organizational or sex chromosome effects
• Need for more pre-clinical investigations to study factors that are difficult to study in human populations, to generate hypotheses that can be studied in humans.
Impediments to translation

- Need for more pre-clinical studies to understand the basic biology
- Many NIH review committees have little expertise or interest in sex differences
- Need for greater understanding among scientists that sex differences can be large and offer opportunities for developing therapies
Hormonal “Organizational Effects”

Hormonal “Activational Effects”

“Sex Chromosome Effects”

Three classes of proximate factors causing sex differences in phenotype

XY vs. XX

Genetic level: All sex differences result from X and Y imbalance

Gonadal

Sry in male

Non-gonadal

Unequal expression of X and Y genes including Sry

Unequal expression of X and Y genes including Sry
Support

- NINDS
- NIMH
- NIDCD
- Isis Fund,
  Society for Women’s Health Research
- UCLA Iris Cantor Center, CURE, Center for Neurobiology of Stress, Parkinson’s Center

Thank you!