

Integrating the Dimensions of Sex and Gender into Basic Life Sciences Research: Methodologic and Ethical Issues

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ABSTRACT

Background: The research process—from study design and selecting a species and its husbandry, through the experiment, analysis, peer review, and publication—is rarely subject to questions about sex or gender differences in mainstream life sciences research. However, the impact of sex and gender on these processes is important in explaining biological variations and presentation of symptoms and diseases.

Objective: This review aims to challenge assumptions and to develop opportunities to mainstream sex and gender in basic scientific research.

Methods: Questions about the mechanisms of sex and gender effects were reviewed in relation to biological, environmental, social, and psychological interactions. Gender variations, in respect to aging, socializing, and reproduction, that are present in human populations but are rarely featured in laboratory research were considered to more effectively translate animal research into clinical health care.

Results: Methodologic approaches to address the present lack of a gender dimension in research include actively reducing variations through attention to physical factors, biological rhythms, and experimental design. In addition, through genomic and acute nongenomic activity, hormones may compound effects through multiple small sex differences that occur during the course of an acute pathologic event. Furthermore, the many exogenous sex steroid hormones and their congeners used in medicine (eg, in contraception and cancer therapies) may add to these effects.

Conclusions: The studies reviewed provide evidence that sex and gender are determinants of many outcomes in life science research. To embed the gender dimension into basic scientific research, a broad approach—gender mainstreaming—is warranted. One example is the use of review boards (eg, animal ethical review boards and journal peer-review boards) in which gender-related standardized questions can be asked about study design and analysis. A more fundamental approach is to question the relevance of present-day laboratory models to design methods to best represent the age-related changes, comorbidity, and variations experienced by each sex in clinical medicine. (*Gen Med.* 2007;4[Suppl B]:S64–S74) Copyright © 2007 Excerpta Medica, Inc.

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INTRODUCTION

The exclusion and lack of sex recognition of males and females in scientific studies are often based on beliefs and funding decisions that are supported by little evidence. Biases may be visible or invisible and may be factors in the entire scientific research process, from selecting a species to reviewing a publication that merges male and female results without subgroup analysis. Fortunately, unconscious assumptions are beginning to be challenged, and evidence is building to reduce biases and encourage innovative methods to identify sex and gender influences. This review aims to challenge assumptions and to develop opportunities to mainstream sex and gender in basic scientific research.

It is important to recognize that even if sex or gender differences are not identified, they may still exist. During an experiment or clinical experience, changes may occur that are then reversed by other mechanisms, with the result being that the overall effect appears to be the same for males or females. Recognition of these processes and their manipulation can potentially enhance therapeutic effects.¹

In the past decade, scientific research to identify sex and gender differences has been aimed at explaining biological variations and disease presentations and identifying fundamental mechanisms relevant to human health. One of the key reviews was published by the Institute of Medicine in Washington, DC, after a series of high-level, nationally led discussions.² In the introduction, the authors emphasized the need for clear definitions of sex and gender. *Sex* was defined as, "The classification of living things, generally as male or female according to their reproductive organs and functions assigned by chromosomal complement." *Gender* was defined as, "A person's self-representation as male or female, or how that person is responded to by social institutions on the basis of the individual's gender presentation. Gender is rooted in biology and shaped by environment and experience." The dichotomy between the terms was strengthened by their application to human health and by resources offered by profession-

al groups relating gender to culture and social roles (**Table I**). Arguments were used to support this separation. It was reasoned that humans are sexually dimorphic (except for those individuals whose sex chromosomes are not XX or XY), and that gender is a continuum that can change over time. One result has been to discourage interchangeable use of the terms *sex* and *gender* and to label research in animals as the study of sex differences and in humans as the study of gender differences, because gender is a "uniquely human trait."² These distinctions, however, are not realistic, because throughout life, sex- and gender-related factors interact and change as individuals age. In addition, the emphasis on the dichotomy may be detrimental to developing innovative research designs, not only because it can generate confusion in the minds of policy makers, but also because researchers may assume that biological differences predominate in basic science and neglect the effects of the environment and social interactions that are present during animal housing and experimentation.

Consider the word *female*. Every cell in the body and those used by molecular and cell biologists have a male or female chromosome complement that may determine their activity and chemical profile.³ Female hormonal influences throughout life are never static, and the variability they cause is far greater than that measured in males. In general, females used in animal research are young, have a regular reproductive cycle, and have not been pregnant. It is important to recognize these restrictions of parity and age, because they may influence the results of experiments, making the findings

Table I. Dichotomy between the terms *sex* and *gender*.

Sex	Gender
Genetic	Psychological
Molecular	Environmental
Cellular	Social
Physiological	Cultural

inapplicable to human populations in which older individuals may predominate and child-bearing is normal. Another issue is female variability resulting from normal responses to sex steroid hormones, which range from organizational effects during early prenatal development to the activational effects of circulating reproductive hormones on genomic and non-genomic mechanisms.^{4,5} One consensus group's interesting approach to this issue has been to advocate demasculinization or defeminization to negate some of these effects and to create clinically relevant models in animals.⁵ A timely experiment illustrating this practice involved determining the effects of environmental toxins on the differences in brain development between females and males.⁶

The statement that "researchers from a myriad of disciplines are beginning to appreciate the importance of considering sex differences in the design and interpretation of their studies, this is an area that is full of pitfalls" supports the purposes of this review and relates to an influential article from the Society for Women's Health Research, Washington, DC.⁵ On behalf of the Society, a North American team of experts in endocrinology and related disciplines aimed to provide guidance on basic scientific and clinical studies of sex differences. The article is a valuable resource that has addressed biological details and identified practical tools for sex- and gender-based research, including how to determine if a sex difference exists and if an effect is related to sex hormones; how to determine the mechanisms for the different effects (eg, estrous cycle, gonadectomy, hormone replacement); what factors to consider in pharmacologic effects; how to measure hormones; and how to assess the effects of stress. Now we are faced with what other gender issues can be identified and how to implement best practice across a wide research field.

Can Gender-Related Factors Be Excluded in Basic Scientific Research?

The comparative neglect of social, environmental, and other nonbiological influences in

basic science as opposed to clinical research may have arisen through terminology and strict definitions. This neglect may also be the result of animal housing in institutions, a lack of adequate animal models, and inappropriate study design.

The influences of environment and social interactions on experimental results generally have been documented separately for animals and humans. In animal research, after in utero organizational effects have occurred, there are a variety of other influences throughout life. For example, in rat studies of maternal behavior, mothers have been observed grooming the perineum of juvenile males more than those of females, with consequent effects on open field behaviors being recorded.^{7,8} Another example is that female rodents synchronize their estrous cycles if housed together.⁹ This effect may either assist in the identification of sex differences or negate the differences if synchronization is prevented by using other types of housing or other animal suppliers.

However, in addition to animal-to-animal interaction, the interactive role between the animal colony or individual animals and the handler or investigator should be considered. Basic evidence has accrued from behavioral studies in humans. For example, it is now acknowledged in the human volunteer research community that the gender of the investigator is associated with changes in pain responses.¹⁰ These effects have not been clearly identified for animals, but they are believed to occur and to be related not to competition or beliefs, as in humans, but to odors, sounds, and handling differences.

The complex nature of the environment in which animals are housed has been identified by animal welfare groups as affecting their well-being, but less attention has been paid to its influence on the outcome of experiments.¹¹ A call has been made to document the social environment, although not specifically for research on the differences between males and females. When changes are made to improve the environment for animals, there is inevita-

bly the potential for introducing variation into experiments. In addition, when results are compared across institutions (eg, to assess sex differences), conflicting findings are often obtained. A confounding factor may be the laboratory milieu (eg, food, the presence of animals of the other sex, light, stress, discomfort, isolation, noise, temperature, the sex of the laboratory staff, odors). Because standardization may be inappropriate across institutions and specialties, other opportunities to allow comparisons or to combine results should be sought. Thus, it is important to begin to disclose such factors in publications that have the potential to report variations in results between males and females.^{12,13}

How Are Small Changes Recognized?

One of the key findings from the study of coronary artery disease in humans is that differences between males and females appear to be small if measured separately, but when an entire episode is considered from presentation with chest pain through diagnosis and treatment, the overwhelming finding is that these differences compound to change the outcome.¹⁴ Therefore, consideration should be given to assigning scientific value to small changes in laboratory-generated results, because these may be part of a larger event. In the guise of protecting animals and attempting to minimize their use in experiments, these small changes are sometimes lost if sample sizes are too small. In some circumstances, there is a fine balance between recognizing small changes and meeting ethical principles without compromising scientific progress.

The following methodologic approaches may support this focus.

Reduce Variation

The direction of sex- and gender-based differences in healthy animals depends on a number of factors. If the variation in these factors can be reduced, it is likely that more reliable and reproducible results can be obtained. Potential factors and mechanisms that influence variabil-

ity warrant systematic examination to assess their relevance. These influences can be divided into physical, cyclical, and other determinants in experimental design.

Physical Factors

When stimuli or chemicals are applied to animals, their body size, composition, or physical response may be relevant to the outcome of the test. Because male rodents tend to be larger than females, a particular physical technique may not fit the anatomy of one or the other sex. Drugs and chemicals are often given on the basis of weight, but the effect of other physical factors may be neglected, especially those influenced by sex hormones, such as protein binding. Variations in response occur through pharmacokinetic differences generated by sex-related differences in fat, protein, blood flow, and weight. Finer details (eg, skin thickness, bowel motility, and autonomic responses) may also be sex dependent and may alter measured outcomes. Careful assessment during study design and peer review should help minimize physical differences.

Biological Rhythms

Becker et al⁵ clearly defined the reproductive cycle and its identification in rodents. In female animals, if sex differences are being measured, it is not mandatory to conduct experiments at each phase of the cycle. Nevertheless, the phase of the animal should be determined for comparative purposes and so that appropriate times during the cycle can be identified. For example, if it is important to identify a female group with stable hormone levels, the study could be performed during the diestrous II (metestrus) phase, or gonadectomy and hormone supplementation could be used to produce stability artificially.

One question that has been raised previously but should be considered again is cycle irregularity. Can animals that do not exhibit the normal pattern of cyclical activity be included in experiments? The answer to this question lies partly in the importance (to the investigation) of including all females in a particular study or

selecting a predefined group to reduce variation. In either case, it is in the planning as well as in the writing of the study that the design should be clarified and justified.

Other biological rhythms can also alter behavioral and physiologic responses. The main system influencing sex differences is corticosteroid secretion, which has a circadian rhythm and influences stress responses. Body temperature also fluctuates with time of day; therefore, if temperature is a factor in the rodent responses being measured, it should be taken into account in study methodology.

Experimental Design

The designs and methods for research on sex differences have been extensively reviewed by Becker et al.⁵ Because their stated goal was to improve experimental design, different methods of endocrine manipulation were examined. In addition to this hormonal mechanistic approach, other techniques to reduce variability should be considered. Several years ago, we had 2 issues to consider in my laboratory: (1) reducing the number of animals used in our experiments; and (2) developing techniques to measure nociceptive responses to visceral stimulation in females. At that time, all the published data were from awake male rodents, because results from females and from anesthetized animals were too variable.¹⁵ We developed a technique using accurately controlled anesthesia that allowed us to limit the number of animals, not only because we could use repetitive testing but also because the effect of anesthesia on stabilizing cardiovascular effects may have reduced variability.¹⁶

We tested the hypothesis that we could reduce the effects of stress by anesthetizing the animals. We measured stress using arginine vasopressin (AVP) because it is directly released into the circulation.¹⁷ The AVP levels were normal after induction of noninterventional inhalational anesthesia, and our results after visceral stimulation demonstrated that the hypothalamic axis is more reactive in females than in males. Becker et al⁵ stated that surgery causes lasting effects on the hypothalamic-pituitary-

adrenal axis. Therefore, if gonadectomy is the method of choice, then the control group should undergo surgery.

Study Size and Analysis

A major criticism of studies of sex differences in both animals and humans has been the statistical power calculation of the investigation and the possibility of a type II (β) error (ie, failure to reject the null hypothesis) because an insufficient number of subjects have been studied and an error in the results may have occurred by chance.¹⁸ The possibility of a type II error decreases with increasing sample size.¹⁹ In clinical research, best practice has been achieved through the CONSORT (Consolidated Standards of Reporting Trials) statement, which identified the essential statistical analyses.²⁰ A similar statement would encourage basic scientists to use adequate power for their studies.

Meta-Analyses

A number of solutions can be considered, particularly in view of the difficulties experienced in funding large studies and the ethics of using large numbers of animals. The re-use of animals should be considered, and the example given previously of repeat measures in the same animal is another possible method. In addition, if agreement on study design is achieved, it should be possible to combine results so that a meta-analysis could be performed. The diversity and lack of consistency between menstrual cycle studies in humans have recently been reviewed.²¹ Of relevance to basic scientific research are the differences between studies in nomenclature, the timing of experiments, and the different female populations sampled. The nomenclature has been clarified in the endocrinology literature⁵; the other factors are part of study design and should be amenable to a cooperative approach. Hence, there is the threat that if females are not studied systematically, sex differences may be lost in the multitude of study designs. Making this statement, however, alerts the scientific community and might suggest ways to use appropriate group data for comparisons between groups.

When to Measure Differences

The literature abounds with examples from animal and human studies in which positive results are obtained but are limited by factors such as population or species studied, time, site, and dose. Although these factors are enumerated in the following sections, 1 example comes from dental pain studies using nalbuphine for pain relief. A key finding was that sex differences were only observed late in the study and at a particular dose of analgesic.^{22,23} These results raise issues about loss of data: if study measures are not applied appropriately, errors in data analysis may result from poor study design.

An analytic approach to sex differences can hypothesize that if males and females are distributed normally and have the same variation around the mean, but their distribution means differ (even slightly), then 1 tail of the population distribution will be predominantly male and the other female. Consequently, if differences are sought around the mean values, sex differences are unlikely to be found. However, if they are studied at either extreme, sex differences will be identified.

These examples suggest the importance of both using a study design that allows adequate breadth of measures or interventions and of analyzing data carefully so that any differences are identified. For example, merging data may not be appropriate and trends for time may provide useful group comparisons.

What Hormonal Interactions Occur at the Molecular Level?

Neurochemicals and Receptors

Most neurochemicals and receptors involved in biological processes are influenced by hormonal changes, such as those that occur naturally during parturition or the reproductive cycle. The results of a MEDLINE search for neurochemicals and receptors involved in nociception are listed in **Table II**.²⁴ Many of the neurochemicals and receptors listed in the table were investigated in only a small number of studies; they did, however, demonstrate similarity across species. Furthermore, if a

molecular mechanism is not listed in the table, it is more likely that no relevant studies have been conducted rather than that no interaction exists.

Hormone manipulation can also be artificial and be applied at any time of life. When such tests are conducted in young animals, they can create effects that persist in later life (ie, organizational effects). Alternatively, there are direct effects on cell function of the sex chromosome genes.⁵ At present, such genetic testing is limited to mice.²⁵

Differences across species also apply to cyclical sex-hormone changes in females. The best example is lactation, which is common in women of reproductive age and may last for years. However, the blood hormone profile differs greatly between humans and animals.²⁶ Lactation has rarely been created artificially, and there is a dearth of mechanistic studies relating to this period of female reproduction. In contrast, studies of humans during pregnancy and of the effects of parity are more common.²⁷ These hormonal-change scenarios are not confined to females, and they should be considered to be relevant to sex differences. There are now a wide variety of hormonal manipulations available for human contraception, cancer treatments, and other therapies. Thus, the use of a relevant history of life events that apply to humans has the potential to be reproduced in animals.

The effect of pregnancy on receptors is illustrated by gestational analgesia. Many studies have provided strong evidence for sex steroid activity on multiple independent receptor systems (eg, central nervous system activation leading to greater inhibition through descending pathways). The benefits of such hormonal effects include synergistic effects with endogenous opioids, which may be exploited through pharmacologic manipulation.²⁸⁻³⁰

Hormones, Pharmacokinetics, and Pharmacodynamics

Drug availability at the receptor site is influenced by pharmacokinetic properties that are dependent on the physical factors described

Table II. Neurochemicals and receptor types involved in nociception that are influenced by sex steroid hormone manipulation.²⁴

Animals	Humans
Arginine vasopressin	Adrenocorticotrophic hormone
Bombesin	α -Melanocyte-stimulating hormone
Bradykinin	Angiotensin II
Cannabinoids	Arginine vasopressin
Cholecystokinin	Bradykinin
Choline acetyltransferase	Cholecystokinin
Dopamine	Corticotropin-releasing hormone
Enkephalins	Galanin
Galanin	γ -Amino butyric acid
γ -Amino butyric acid	Glucagon
Glutamate	Growth hormone
Insulin-like growth factor	Growth hormone-releasing hormone
Nerve growth factor	Motilin
Neurokinin A	Luteinizing hormone
Nitric oxide	Nerve growth factor
N-methyl D-aspartate	Insulin
Opioids	Opioids
Oxytocin	Prolactin
Preproenkephalin	Somatostatin
Serotonin (5-hydroxytryptamine)	Thyrotropin
Substance P	Thyrotropin-releasing hormone
Vanilloid receptor (VR1)	Vanilloid receptor (VR1)
	Vasoactive intestinal peptide

previously. The receptor or effector sites may themselves be influenced by hormonal activity, thereby altering pharmacodynamic mechanisms. If sex differences are not factored into experiments, pharmacodynamic sex differences may influence results. For example, the anesthetic propofol and opioid analgesics demonstrate potential pharmacodynamic effects in addition to pharmacokinetic differences.³¹ Drugs may also have different adverse effects in males and females. These adverse effects are often reported by sex in human studies but not in basic science experiments.³²

What Sex- or Gender-Related Issues Do Animal Ethical Review Boards Recognize?

Animal ethical review boards have been established nationally using peer review so that study design and methodology can be discussed within the scientific community to improve animal welfare, to eliminate unneces-

sary procedures, and to advocate the most efficient, reliable, and cost-effective ways to conduct experiments.

There are currently no guidelines to encourage disclosure of information that affects sex or gender differences. For example, the same strains of animals may differ based on which breeding supplier provides them. These differences may be due to the environment in which the animals are raised and to the transport systems used. One such example was given by Mogil et al,¹ who identified differences in pain responses in rats that had been transported using air freight compared with those that had been shipped via road transport for varying lengths of time.

One of the ethical principles for scientific consideration has been stated as follows: "The scientific community and the institutions that support it must ensure that scientific advances benefit all people, regardless of gender...."³³ If

male rodents are used for the majority of basic scientific research, then clinical research will lack information on essential mechanisms and the full range of their effects.

What Limitations Do Sex- and Gender-Based Studies in Animals Present?

Lack of Comorbidity

In clinical practice, many diseases occur with different frequency in males and females. However, this is often not reflected in basic scientific research. One reason for developing our techniques for colonic balloon distension and behavioral measures of visceral nociception in female rats was the prevalence of bowel disorders, such as irritable bowel syndrome (IBS), in females compared with males.¹⁶ IBS is a condition in which >1 disorder is often present in humans; to replicate this in animals, a composite experimental model is most appropriate. Because such a model has been devised for ureteric pain and endometriosis, the interrelation of the 2 disorders can be measured.³⁴ This mixed basic science and clinical approach can be useful in detecting additional relevant factors that may determine the presentation of a condition in one sex or the other.

Strain Variations

Some of the best evidence for the effect of rodent strain on sex-difference responses comes from Mogil's group.³⁵ In a systematic study using supraspinal morphine injections, the authors found that the analgesic response depended on the rodent genotype (**Table III**³⁵).

Age

Sex- and gender-related differences are closely associated with age-related effects, partly because sex hormone effects change over time (from organizing to activational effects). In humans, this has been documented in epidemiologic and acute clinical studies as the population ages (**Table IV**).³⁶⁻⁴³ Age is rarely identified as a factor in laboratory experiments, except during the neonatal or adolescence phase, and few studies have been conducted in

Table III. Rodent strain (mice) variability in analgesic response to morphine.³⁵

Male > Female	Female > Male
AKR/J C57BL/6J SWR/J	CBA/J

elderly rodents. Although Becker et al⁵ recognized age as a limitation to laboratory studies, the authors did not provide any answers as to how to analyze age-related data. One approach is to identify relevant endocrine groups or hormonal states; another is to test for trends over years. Longitudinal studies are rare in rodents but may provide clues to determine the relevance of environmental history or reproductive history. In this context, it is important to be sensitive to the findings and mechanisms of sex differences.

RECOMMENDATIONS

Use of the term *sex* or *gender* should be justified. The age and weight of male and female animals should be recorded. In addition, their reproductive status and the ovarian cycle phase of females should be determined as accurately as possible. The sex of biological research materi-

Table IV. Age effects and sex differences in studies of morphine consumption using patient-controlled analgesia after surgery.

Study	N	Age Effects	M:F
Burns et al ³⁶	100	Inverse correlation	M > F
De Kock and Scholtes ³⁷	200	None	M > F
Tsui et al ³⁸	1233	None	M > F
Macintyre and Jarvis ³⁹	1010	Best predictor	M > F
Sidebotham et al ⁴⁰	276	None	M > F
Glasson et al ⁴¹	150	Significant	M > F
Chia et al ⁴²	2298	None	M > F
Joels et al ⁴³	481	Inverse correlation	M > F

M = male; F = female.

als, including individual cells, should be determined and disclosed on publication.

In study design, reporting, and peer review, the possible variations and impact of sex differences on all aspects of the experiment should be considered and be based on evidence relevant to the strain, species, and environmental conditions. Results for sex- and gender-difference studies have to be applicable to all sectors of the population, rather than being limited to individuals with stable reproductive cycles or to young adults.

A collaborative statement should be made by journal editors to guide gender mainstreaming for laboratory and experimental studies (eg, through appropriate statistical methodology).

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