of mice,

and

men

IT WAS 2012, AND SOME OF AMY BRAUN'S LAB MICE WEREN'T BEHAVING NOR-MALLY. THIS WAS VERY EXCITING. • Rather than scampering about investigating their surroundings and introducing themselves to other mice, whiskers quivering, they eschewed social interactions and ran in circles. Placed into a water tank, they appeared disoriented and confused. Unlike their peers, they swam hesitantly along the walls, unable to find and remember the location of a platform hidden underneath the surface. They were pale shadows of their mousey selves.

It was exactly what the researchers, who were studying brain development in autism and schizophrenia, had expected to see. But there was just one — major — hitch.

"We realized, when we looked more closely," says Braun, "that we were seeing this aberrant behavior only in male mice. The females behaved like our control animals. We thought, 'Well, this is sort of weird.' Later we realized that the weird thing was that we actually looked at the female animals at all."

At the time, many researchers focused their studies only on male lab animals, under the belief that the normal hormonal cycling of female animals would render them more biologically variable. They worried that this perceived variability would make it harder to achieve statistical significance in their studies. A 2010 study in *Nature* found bias toward the use of male animals in eight out of 10 research disciplines.

The problem was particularly egregious in Braun's field of neuroscience, in which studies of male animals outnumbered those of female animals nearly sixfold. Colleagues who saw Braun's data counseled a simple fix: Leave out the results on the

WOMEN MAKING RESEARCH MORE INCLUSIVE

BY KRISTA CONGER

ILLUSTRATION BY GÉRARD DUBOIS



females. That didn't sit well with Braun, a graduate student in the laboratory of Theo Palmer, PhD, an associate professor of neurosurgery.

"I thought 'Wait, what? This is all biology. Let's instead figure out what is going on."

t'S OBVIOUS THAT SEX is important in health, health care and medical research. Women are more likely to suffer from autoimmune diseases, have osteoporosis and be diagnosed with depression and anxiety; men are more likely to develop Parkinson's disease and cardiovascular disease early in life. Knowing predispositions like these can help physicians make a diagnosis or researchers develop new therapies. Yet medical research has usually left females out.

And an increasing body of research suggests that the influence of a person's biological sex on their health is just the tip of the iceberg. Hovering just beneath the surface is a mixture of behaviors, expectations, cultural norms and attitudes that together define a given individual's gender. Gender is inextricably linked to sex, but not defined by it. And it indisputably affects health.

For example, a 2016 study from a group of Canadian researchers suggested that successful recovery from acute coronary syndrome (a term describing a blockage of blood flow to the heart, as happens during a heart attack) was dependent not on whether the patient was male or female, but rather, on each person's gender characteristics: Patients with more research studies, but it has no such requirement for gender. Yet.

"Both sex and gender influence human health and disease," says Janine Clayton, MD, the director of the National Institutes of Health's Office of Research on Women's Health. "It is increasingly clear that it is both an ethical and scientific imperative to conduct research and report on the results for both men and women."

There's just one tiny little problem, points out Stanford professor of medicine Marcia Stefanick, PhD.

"We don't know how to measure gender," says Stefanick, director of the Stanford Women and Sex Differences in Medicine, or WSDM (pronounced "wisdom"), Center. "Sex is generally assigned at birth, based on external genitalia, after which a broad range of biological, particularly reproductive, sex differences are assumed. Individuals are then, usually, forced into a binary model of gender — with distinct masculine and feminine categories — when the possibilities are much broader and more expansive."

Stefanick and Stanford's Londa Schiebinger, PhD, argue that gender is instead a point on a continuum with infinitesimal gradations. Together they are developing a way to measure gender in such a way that it can be accurately correlated to health outcomes. If successful, their approach could transform how medicine is practiced.

"Basically, we want to blast the standard attitudes about masculinity and femininity out of the water," says Schiebinger, who is the John L. Hinds Professor of History of Sci-

WE WANT TO BLAST THE STANDARD ATTITUDES ABOUT MASCULINITY AND FEMININITY

traditionally feminine traits, such as responsibility for caregiving, were more likely than those with more traditionally masculine traits, such as being the primary income earner for their households, to suffer another coronary episode or die within the following year, regardless of their biological sex.

Governmental and funding agencies are taking note of this and other examples of gender and sex disparities. The World Health Organization urges the incorporation of gender into health care policy worldwide and, in 2010, the Canadian Institutes of Health Research established policies requiring health and medical researchers to include both sex and gender as critical variables in any planned studies or clinical trials, as did the European Commission in 2013. In 2016, the U.S. National Institutes of Health began requiring that grant proposals include information as to how sex will be incorporated as a biological variable in ence and the former director of Stanford's Clayman Institute for Gender Research. "We want to get rid of the notion that you can assume there is a prepackaged set of characteristics that belong to men or to women. We want to develop a new instrument to measure gender that will allow us to better understand how gender and sex interact to impact health."

It would be difficult to argue that Braun's lab mice have a gender. Most researchers would agree that their

behavior is governed primarily by biology, rather than by the societal expectations of their furry cage mates or their own mouseconceived ideas of "self." But in people, sex and gender together make up a complex stew of biology and behavior that can be difficult to swallow for researchers, who want simple answers.

As Braun's experience shows, although it's much easier to



AMY BRAUN'S COLLEAGUES SUGGESTED OMITTING HER RESULTS ON FEMALE LAB MICE. INSTEAD, SHE DECIDED TO FIGURE OUT WHY THEY BEHAVED DIFFERENTLY THAN THE MALES.

OUT OF THE WATER.'

forge ahead with blinders on, doing so can vastly compromise a study's findings and overlook critical aspects of biology.

So how to proceed?

To begin with, it's important to define the terms accurately to avoid confusion. Even many research articles, and researchers, refer to gender when they mean sex.

Sex is a biological trait that is determined by the specific sex chromosomes inherited from one's parents. In humans, male sex is determined (with a few exceptions) by the presence of the Y chromosome. A gene on the Y chromosome directs the differentiation of the fetal gonads into testes, resulting in the production of testosterone — which affects many of the body's tissues — early in development. People with one X and one Y chromosome, or variants like XXY or XYY, are typically male, while those who have solely X chromosomes are usually female. People have a sex; animals have a sex; all tissues, including the fetal placenta, have a sex; even individual cells have a sex.

Gender, on the other hand, is socially, culturally and personally defined. It includes how individuals see themselves (gender identity), how others perceive them and expect them to behave (gender norms), and the interactions (gender relations) that they have with others. Often one's gender aligns with one's sex: Men tend to assume more masculine behaviors and traits, and to be seen as masculine by others around them, for example. But not always. Increasingly, researchers like Stefanick and Schiebinger are realizing that both men and women exhibit a spectrum of gender traits that aren't purely masculine or feminine.

Stefanick and Schiebinger refer to these characteristics as "gender variables" that are distinct from the overly broad and less helpful concepts of masculinity or femininity. They include, among others, consideration of the degree of responsibility for caregiving a person assumes; whether a person describes himself or herself as competitive or communal, empathetic or expressive; and the degree of social support one receives.

"We want to get rid of the notion that gender can be reduced to 'masculine' or 'feminine' based on a prepackaged set of characteristics," says Schiebinger. "Instead we want to open that package to find ways to measure each characteristic individually."

HERE ARE MANY EXAMPLES of the effect of gender, and gender norms, on health. It's becoming well-known that the high-heeled shoes favored by many women can cause lasting damage to the wearer's feet. Others examples are more subtle, such as the fact that American men who conform to what are perceived as traditionally masculine traits may avoid well-care visits with their physicians, drink and drive, engage in risky sexual practices or take on inherently dangerous occupations.

As a result of the interaction of these gender variables with biological factors, American men can expect to live about five fewer years than American women. They experience higher rates of mortality than do women for the majority of the 15 most common causes of death, including heart disease, cancer, accidents, suicide and homicide.

Conversely, "feminine" traits traditionally encompass the emotionally and physically stressful caregiving for children and elderly parents. Women frequently assume a greater portion of household tasks regardless of whether they work outside the home, leaving little time for exercise or other forms of self-care. Overall, they are often lower in social status, which may help explain why they are more likely to suffer from depression, recover more poorly from cardiac events and live with higher rates of osteoporosis.

The researchers conducting the Canadian study of acute coronary syndrome went beyond "masculine" and "feminine," and instead asked the patients to answer a series of questions to derive a composite measure of gender on a scale of 1 to 100. To do so, they used a measure developed in 1974 at Stanford called the Bem Sex Role Inventory.

The lower the score, the more traits the patient displayed that are typically considered to be more masculine, such as assertiveness. Higher scores included traditionally more feminine traits, such as being more expressive. The use of the scale allowed each person to be assigned a gender on a continuum.

The researchers found that, despite the fact that men overall tend to die younger than women, those cardiac patients with more feminine roles and personality traits fared much more poorly during the yearlong recovery period than those with more masculine identities — regardless of their biological sex.

"Basically it showed that if you are a masculine man or a masculine women, you recover more readily than if you are a feminine man or a feminine woman," says Stefanick. "But this study used a set of questions that we feel are too limiting and based on older ideas of gender roles. Our concepts about gender have been evolving so fast that the definitions can't keep up."

"We really need a big rethink on gender and health," says Schiebinger. "Sex and gender interact; gender behaviors such as a person's choice of shoes — can shape biology and biology certainly influences gender."

Like gender, the influence of sex on health and biology runs deep. At the most basic level, it controls

whether, when and how our genes are made into proteins. These proteins control how a cell functions, interacts and communicates with its neighbors. There's a hormonal aspect to sex differences, as well. Varying levels of testosterone and estrogen can affect the biology of many tissues throughout the body.

"Sex differences are important from the cellular level up," says Stefanick. "We really need to investigate the genetics and cell biology to truly understand the implications of these differences."

OUR CONCEPTS ABOUT

Or, in Braun's case, the level of the placenta. She and her colleagues in Palmer's lab were analyzing the effect of infection during pregnancy on the placenta and its role in supporting fetal development, brain structure and function of the resulting offspring. They were interested because there's a correlation in humans between maternal illness during early pregnancy and the development of neurodevelopmental disorders like autism and schizophrenia in the child.

Fascinatingly, both sexes of the mice she was studying had similar structural brain abnormalities, but only the males displayed behaviors that mimicked the human disorders — a biological quirk that would have been missed if female mice had been excluded.

"I wanted to know why; I wanted to understand this," says Braun. "What if we have accidentally mimicked something that is relevant to the human disorder? You can't automatically extrapolate your results to both sexes if you don't even test the females."

For decades, women were largely excluded from clinical trials intended to test the safety and efficacy of potential therapies. Like female lab mice, they were considered to be too complex due to monthly hormonal fluctuations. In 1977, the Food and Drug Administration issued guidelines urging against the inclusion of women of childbearing age in clinical trials to avoid unintentionally administering unproven medications to a developing embryo.

Ironically, however, these same researchers assumed that medications or interventions that got the thumbs-up after testing in men would work the same way in women. It was an insidious, and dangerous, double standard. Of 10 drugs recently recalled after approval due to adverse effects in humans, eight have been found to affect women more severely than men.

Things have been changing, but slowly. In 1994 the National Institutes of Health mandated the inclusion of women and minorities in clinical research that they fund. But although women now make up more than half of clinical trial participants, many published studies still fail to stratify their results to identify sex-specific side effects or outcomes. These problems also extend to the preclinical research on laboratory animals, tissues and cells that precede clinical trials. Often researchers neglect to even record or report the sex of the animals or cells they've used in their studies. concluded in the report's executive summary.

"We need to debunk the myth that females are mysteriously complex," says Braun, "and we need to increase the literacy around the concepts of sex and gender. They are not the same. Frankly, I think a lot of researchers just don't want to say the word 'sex.' 'Gender' is more comfortable, and more fancy-sounding. But gender is its own biological variable, and we have to understand that and think critically about it."

Stefanick and Schiebinger have been

working to integrate sex and gender into research at

Stanford for several years, originally with a program in the Department of Obstetrics and Gynecology that focused on women's health. The WSDM Center was launched in 2013, and the center has awarded 22 seed grants of between \$20,000 and \$35,000 to Stanford researchers to encourage them to incorporate questions about sex and gender differences into their research.

"I want Stanford to be the leader in the world on this topic," says Stefanick. "We have the potential to achieve this."

In 2017 the WSDM Center awarded eight grants to researchers to investigate topics as diverse as the impact of eye disease and visual function on women's health, the differences in normal immune function between men and women, and the effect of sex on treatment strategy and decision-making in patients with inflammatory bowel disease.

The center is also raising awareness of the roles of sex and

GENDER HAVE BEEN EVOLVING SO FAST THAT THE DEFINITIONS CAN'T KEEP UP.'

"We rely on preclinical research as the foundation for translation to clinical studies; consequently, the prevailing gaps in knowledge about female biology may hinder efforts to turn discovery into health benefits for women. By studying both sexes and reporting on the results for women and men separately, scientific questions will be more fully answered, driving the development of sex-appropriate treatments," says the NIH's Clayton.

In 2001, the Institute of Medicine published a report titled "Exploring the Biological Contributions to Human Health: Does Sex Matter?" The authors of the report recommended that the effects of both sex and gender on biology and health should be studied along the human life span, and urged researchers and publishers to be clear in their language. "There is inconsistent and often confusing use of the terms sex and gender in the scientific literature and the popular press," they gender in health among medical and graduate students. A recently piloted 90-minute training on sex, gender and sexuality will be required of Stanford medical students next year; however, Stefanick believes much more discussion on the impact of sex and gender on health outcomes is needed. Research scientists are rarely taught about it.

"Education about these topics is vital," says Schiebinger. "Information about sex and gender needs to be fully integrated into the medical curriculum. Right now it isn't."

For her part, Braun, who received a seed grant from the WSDM Center in December 2015, is continuing her investigation into the effects of maternal infection on the prenatal brain. Her results suggest that perhaps something was happening in utero to which the male fetuses were more vulnerable. They also contrast with the sometimes-floated idea

FEATURE

Of mice, men and women

CONTINUED FROM PAGE 11 that males are more vulnerable to developing autism spectrum disorders because their brains are already tilted toward the types of thinking associated with the condition, such as systematizing data, and away from other, more interactive, attributes such as empathizing with others.

"Our perspective zooms out from any preconceived notions of differences between male and female brains, and looks at the contributions of sex differences in the placenta," says Braun. "It's a fresh approach that's free from any gendered assumptions about the tissue's nature and function." Braun's results suggest that the risk factors that affect boys may be fundamentally different from those that affect girls, and that those differences may arise where one would least expect.

"Amy brings an unusual depth and breadth of understanding to her work on sex differences in our mouse models of autism," says Palmer, her adviser.

Braun is spending increasing amounts of time talking with other researchers about the importance of including sex and gender as critical variables in their work. But it's not always well-received.

"Researchers who have already decided they are going to ignore sex as a variable don't really want to hear about it," she says. "People can get defensive. But sometimes I feel like a broken record, asking over and over again when people present their research, 'Did you analyze by sex?' 'Did you look at females?'"

"More than once, Amy has stopped me in mid-sentence to say 'Theo, sex is a genotype, gender is an identity!" says Palmer. "Unlike many other researchers, Amy effortlessly reconciles these ideas in her personal and scientific life."

"Some biologists are so far behind in their social science literacy that the distinction between sex and gender often isn't clear," says Braun. "I didn't get any education about this earlier in my grad school career. I kind of stumbled sideways into this issue and now I can't stop seeing it everywhere." **SM**

> — Contact Krista Conger at kristac@stanford.edu

FEATURE Two minds

CONTINUED FROM PAGE 17 goings-on in at least some of the brain's neural circuits and in whatever little piece of behavior each of these neural circuits manages.

"We think gender-specific behavior is a composite of all these modules, which, added up, give you your overall degree of maleness and femaleness," says Shah.

Consider the genes Shah has isolated whose activity levels differ significantly in the brains of male and female mice. "Almost all of these genes have human analogues," he says. "We still don't completely understand their function in human social behavior. But when we looked at publicly available databases to find out what we do know about them, we found a surprising number that in humans have been linked with autism, alcoholism and other conditions."

Bigger imaging studies and imaginative animal research now in the works promise to reveal much more about humanity's inherent — although by no means uniform, and often not substantial — sex-associated cognitive differences and vulnerability to diseases.

Trying to assign exact percentages to the relative contributions of "culture" versus "biology" to the behavior of free-living human individuals in a complex social environment is tough at best. Halpern offers a succinct assessment: "The role of culture is not zero. The role of biology is not zero." SM

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FEATURE Pursuing parity

CONTINUED FROM PAGE 31 "I was like, 'What are you talking about?'" Harris says. "And I came over to look at the pictures and I was like, holy crap, you're absolutely right." In the photo from 2003, when Harris finished her residency, there is one other woman, and she's a neurologist rather than a neurosurgeon. In the 2009 photo, from Harris' first year on the faculty, there are a smattering, but again, most of them are not surgeons. In certain surgical specialties, women remain rare.

"The sense of isolation is pretty overwhelming if you don't have the sounding board and the mentorship," Harris says, emphasizing that she herself has felt strongly supported by the senior members of her department. "Everyone needs a sense of community to be able to thrive in this environment." In collaboration with the school's Office of Faculty Development and Diversity, she is spearheading a new program that will create small, supportive groups of women. The office also offers a monthly networking luncheon for all female faculty.

Outside of her work in neurosurgery, Harris has made it her mission to improve access to careers like hers, primarily through science outreach to children. "There are few jobs where you can take the time outside of work to serve on the board of a Boys' and Girls' Club," she says. "Where you can bring in an entire all-girls school to volunteer for a year at the VA when you're a neurosurgeon and some may think your time is better spent in the operating room. I have amazing bosses who authentically support that kind of vision.