

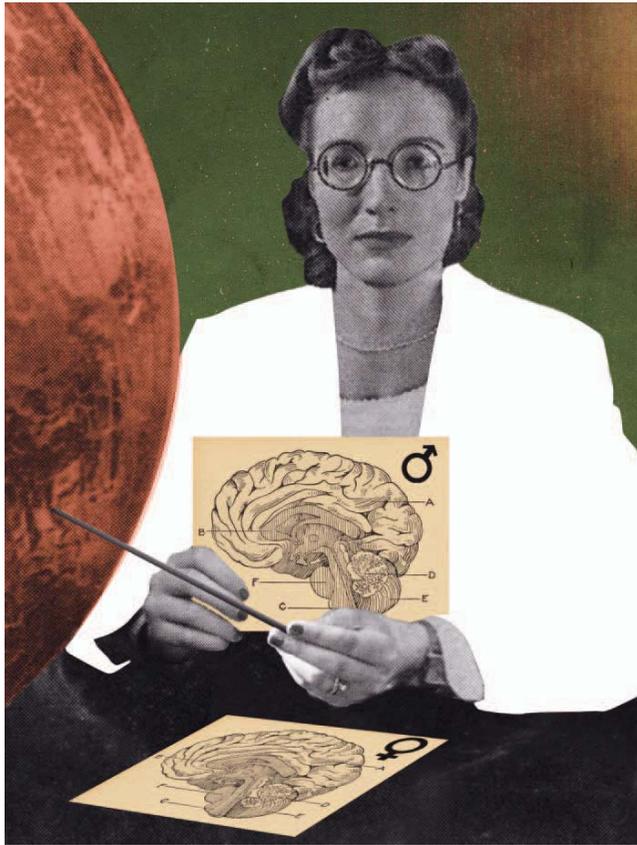
# MARS AND VENUS, REVISITED

OR, WOMEN REALLY ARE NOT SMALL MEN

BY ELAINE VITONE

WITH REID R. FRAZIER AND ERICA LLOYD

ILLUSTRATIONS BY JESSE LENZ



At first, the idea of essential biological differences between men and women—beyond obvious things like sex organs and Adam’s apples—might sound suspect, and perhaps for good reason. Misogyny has masqueraded as medicine all too often in our history. In 1873, the prominent physician Edward Clarke warned that education robs females of their vigor. *Girls lose health, strength, blood, and nerve*, he wrote, *by a regimen that ignores the periodical tides and reproductive apparatus of their organization.*

But once we separate societal influences from scientific fact, the evidence is compelling. Epidemiological studies have told us for decades that men and women and boys and girls are worlds apart in many diseases and disorders, from incidence to age of onset to severity. But historically, medical research has, for the most part, failed to analyze data by sex or to even consider it as a variable. For various reasons (expense, simplicity, potential risks in pregnancy, among oth-

ers), the overwhelming majority of studies has focused on males only.

We’ve seen an increasing urgency to change that in recent history. In 2001, the Institute of Medicine published guidelines to promote research on sex differences—particularly at the cellular and molecular levels, but also at every stage of life.

At Pitt, scientists are beginning to sort out sex-based disparities in the nervous, musculoskeletal, respiratory, cardiovascular, and immune systems. They’re finding strengths and vulnerabilities in each sex that further our understanding of certain illnesses overall. Such insights should eventually help to restore the “health, strength, blood, and nerve” of men and women alike.

—Elaine Vitone

## OF DIFFERENT MINDS

To say that men and women are of different minds is a tired punch line, a fact of life we accept so fully that it’s practically old hat. But if you try to talk about differences in terms of the structure and function of the brain—the organ itself—scientists stumble over the idea, hindered by certain hang-ups.

For one, throughout the history of our evolving understanding of human health, we’ve tended to chalk up every inconsistency between the sexes as a function of the circulating

hormones that are so powerful through our reproductive years. For another, if you point out a difference—even something as statistically strong as the slant toward women when it comes to major depression—people shrug it off. “Women are more likely to seek treatment than are men,” they say. “How can you be sure we’re so different?” People tend to get caught up in the social construct of gender if you try to talk about the sexes in terms of biology.

But in recent years, scientific circles have begun to accept the notion that socialization

isn’t all to blame. If it were, why would women in Sweden—a place where education and labor laws have gone a long way to foster equality among men and women—be just as prone to depression as American women? Nor can we explain it all away with circulating hormones. For example, Alzheimer’s disease—which emerges years after sex hormone levels wane—is more fatal in men than in women.

Investigators at Pitt and elsewhere are finding that we are built differently and wired differently, quite literally. The neurocircuitry that connects the parts of the brain has a very distinct way of evolving in males versus females.

It turns out, we are fundamentally different, even at the cellular level.

## SNAKES AND SNAILS AND OXIDATIVE STRESS

An investigator/critical care doc who works with children, Bob Clark has a quiet manner and a collection of pictures of his young, dance-recital-costumed daughters around his office in the new Children's Hospital of Pittsburgh of UPMC, where he's chief of the Division of Pediatric Critical Care Medicine. He's also a Pitt associate professor of critical care medicine and pediatrics and associate director for molecular biology in the Safar Center for Resuscitation Research. On a recent day, a single speck of gold glitter shines on his forehead, the mark of a parent of tiny dancers.

Clark explains that in the 1970s, researchers ran rodents through intense exercise tests and found that males burn protein to push them through, while females draw their energy from stores of fat—which women tend to carry more of, pound-for-pound.

A few years ago, Clark's team modeled starvation in brain cells. They grew neurons from male and female rodents in separate cultures, then denied them nutritional sustenance. The males' died more quickly, they found. The famished neurons from females used their outer membranes to form a snack of lipid droplets, and also gathered fatty acids from outside the cell walls—a veritable cellular smorgasbord of fat stores.

The guys' cells, however, did none of this. Instead, they were all about protein. Their neurons resorted to killing and eating their own organelles.

In human history, we've seen women also do better in times of hunger. When Nazis starved concentration-camp prisoners during World War II, the women who were subjected to this torture survived longer than the men did.

"If you look at it, it makes sense that during times of famine you would favor women," says Clark.

"Just because we store more fat in general?" asks this (female) writer.

"Because you're more important, from a teleological perspective," he says with a smile. "You need more women, and you need them at a certain period of time—during childbearing years. And the way we [pediatricians] look at it is: Well, you can't get to be of child-bearing

age unless you make it through childhood. So you have some mechanism that also protects you before puberty."

Like any scientist worth her or his salt, Clark doesn't like to speculate. But he'll humor a joke about the predominance of men as hunters, the fatherly fascination with barbecue grills. And if you look at dietary intake, Clark points out, "Clearly men eat more protein than women. Men have higher levels of carnitine, which you get from red meat." Maybe men are meat eaters by design, straight back to their earliest origins, straight down to their organelles.

The starving-neuron study generated some Internet buzz, with mentions in *Scientific American Mind* and elsewhere. It's the latest in a series of probing questions Clark has asked about the basic biology of various insults to the brain, each triggering many cascades of molecular ruination in varying degrees. He's found that how we weather the onslaught depends, in part, on our sex—regardless of whether we're in our hormone-laden reproductive years or not.

Seven years ago, Margaret Satchell—an assistant professor of pediatrics at New York City's Mount Sinai School of Medicine who was then a fellow working in Clark's lab at Pitt's Safar Center for Resuscitation Research—led a study of cytochrome C, a biomarker for apoptosis (or programmed cell death) in the spinal fluid of children with traumatic brain injury. Apoptosis is a normal cellular process; how-

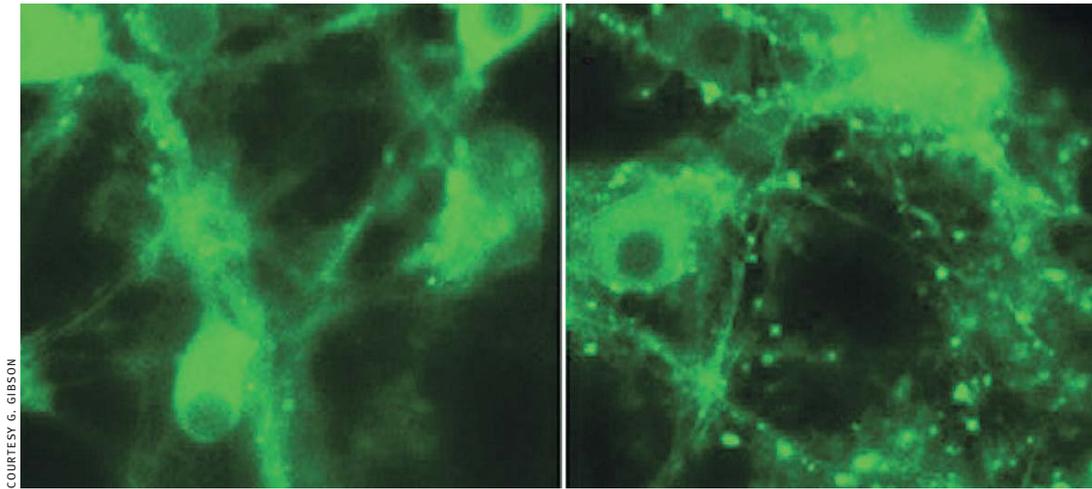
ever, after injury, it can spiral out of control, killing cells before their time. Satchell checked for sex differences in this process merely as a matter of course; the average age of the patients was just 6 years old—prepubescent by a long shot—so she had no expectation of finding a sex difference.

But there was. Girls suffered far more apoptosis than boys.

Intrigued, the team launched an animal study and eventually found that the brain cells of female rats were more susceptible to apoptosis than those of the males. Further, male rats were more vulnerable to another cell killer, oxidative stress. Satchell, Clark, and Lina Du, research associate at the Safar Center and Pitt's Brain Trauma Research Center, detailed in several papers differences between how young males and young females respond to traumatic brain injury, and this work later made ink in *Nature*.

The findings make sense, Clark says. Young girls with certain cancers respond better to chemotherapy, and the way these agents kill cancer cells is—apoptosis. Men are more likely to fall victim to Parkinson's disease, which is thought to be influenced by oxidative stress.

Still, it took a couple of years to get the results of the team's brain-injury study published. Initially, reviewers weren't too keen on the idea of cellular-level differences between male and female brains. But other labs began supporting this claim with mounting evidence in cell-culture, animal, and patient-observa-



COURTESY G. GIBSON

**Neurons from female (left) and male (right) mice respond differently to starvation. In females, the cells live for days longer, sustaining themselves on lipids. In males, the cells eat their own organelles within their lysosomes, seen here as bright green dots.**

tional studies. It takes careful, thorough work to challenge the dogma, Clark says. “The last thing people wanted to hear was that they might have to use both males and females in their studies. It doubles people’s workload.”

For the most part, labs use males—and only males—in rodent experiments because they are easier to work with. (More on that later.) But Clark says also studying females would be well worth it if it leads to more tailored therapies and better outcomes. For example, Clark and Mioara Manole, a Safar Center investigator and Pitt assistant professor of pediatrics, are working on an animal study on the effect of antioxidant medication in cardiac arrest.

“It seems to be effective only in males,” he says.

And perhaps something as simple as diet could be tweaked to the advantage of a recovering brain on the skids.

“If you’re in the ICU right now, we just pull a bag of IV nutrition off the shelf, and we don’t take into consideration whether you’re a boy or a girl. But maybe they need different fat content versus protein content. To echo what *Scientific American Mind* said, there are dietary preferences in men and women, and it’s probably for good reason.”

## THE BIRDS AND THE BEES

Bea Luna, Pitt associate professor of psychiatry and psychology and director of the Laboratory of Neurocognitive Development at the Western Psychiatric Institute and Clinic, studies brain development during adolescence. (This magazine did a cover story on her work in Fall 2007.) The number one question she’s asked when she speaks at conferences is: *Have you looked at sex?* “There’s this intuition that there are differences,” she says.

Although sex differences haven’t been a major focus in her studies, they are beginning to come to the fore. Early this year, Luna published in *Cerebral Cortex* a study examining sex disparities related to white matter—the connective fibers that enable the parts of the brain to talk to one another. At the start of puberty, males and females both have a growth spurt in their white matter tracts, sprouting more than anyone would ever need. As the brain matures,

white matter tracts become increasingly insulated with a protective, conductive layer of fatty material called myelin. The result is a quicker, more streamlined, and capable brain. In other words, an adult’s brain.

Luna looked at connections throughout the brain that change during human neurocircuitry development. She found that in the prefrontal-striatal connections—part of the neurocircuitry network that helps us control our impulses and act more like grown-ups—most females reached maturity in the window of ages 13 to 17. The males were still hemming things up, in terms of white matter, much later—in the 18-to-22 range.

What that means, really, we haven’t figured out yet, Luna says. There could be pluses and minuses for each sex. Point for the boys: Perhaps a longer period of myelination means a brain that’s more finely tuned and better adapted to his environment. Point for the girls: Perhaps if you’re early to complete one stage, then you’re sooner on to the next one—a stage of specialization, of learning to use the tracts you’ve developed with more practiced precision.

Luna is mapping out templates of normal brain development and of the abnormalities that cause psychopathologies. Her studies include ADHD and autism—overwhelmingly male disorders that hit in early childhood.

“But the interesting thing is we haven’t thought of looking at sex differences in autism,” Luna says. “It’s very difficult to do that because it’s hard to find the females that have it. To find a female with autism is so odd that you think maybe it’s something completely different. It’s not representative of the disorder.”

As she charts out the developmental progress of healthy young people in her long-term imaging studies, she’s struck by the way disorders like depression seem to creep in out of nowhere. “We have a lot who are in our normal population, and then all of a sudden, [sigh], depression,” she says. Though Luna’s team is not studying this disorder specifically, they are “keeping an eye on” these youths who stray from the healthy course, watching for any signs of what might distinguish them. Luna recalls a conversation she had with David Kupfer, Thomas Detre Professor of Psychiatry and professor of neuroscience and clinical and translational science, when she first came to Pittsburgh several years ago. Kupfer was head

of psychiatry at the time, and the number of young depressed women he’d been seeing in the clinic concerned him. He wanted to know why this was happening. What was going on?

Indeed, after puberty, females are two to three times more prone to depression than males, even after accounting for all possible alternate behavioral explanations.

## EVEN MOUSE GIRLS GET THE BLUES

Etienne Sibille, Pitt associate professor of psychiatry and principal investigator at Pitt’s Center for Neuroscience and the Pitt/Carnegie Mellon University Center for the Neural Basis of Cognition, says it’s no wonder no one has made any significant breakthroughs in the treatment of depression in the half century since monoamine oxidase inhibitors were discovered. Depression is a complex, multifactorial disorder, with possible roles for genetics, organ mechanics, and environment. Most research has focused on existing drugs—rather than the source of the disorder.

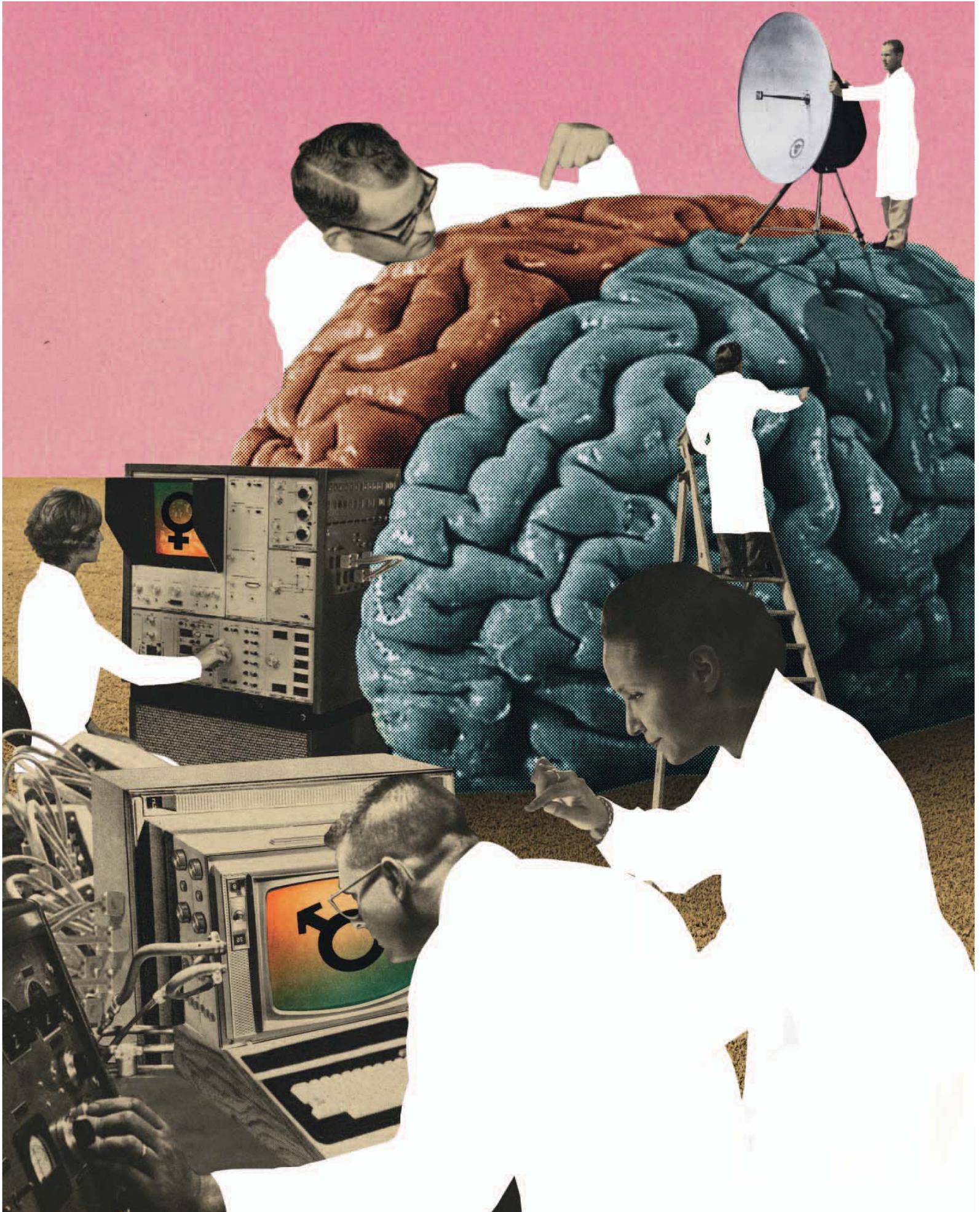
“There are a lot of variables, so it’s very difficult to study,” he says.

It certainly hasn’t helped that the overwhelming majority of animal studies focusing on this predominantly female disease have used male mice only. Female mice are more expensive and more difficult to work with because of their estrogen cycles, an added experimental variable that must be measured through regular vaginal smears. “We can do it; but if people aren’t well trained, then it’s very stressful to the mice,” Sibille says. And so there you are, performing a stressful procedure on the females that you don’t do on the males—and doing it in an animal model of psychological distress. “It gets complicated,” he says.

But nobody said this work would be easy.

Sibille suspects that, for each sex, there are several subgroups—distinct combinations of factors—that conspire to cause what appears on the surface to be the same disease: major depression. To disentangle all of that, an accurate animal model—with both sexes represented—is key.

For weeks on end—in an unpredictable, random fashion—Sibille’s lab exposes the male



The brains of men and women are built and wired differently.

and female mice to several mild stressors: the scent of a predator, changes in light that undermine their biorhythms, wet bedding, or no bedding at all. In time, certain mice lose weight, their coats thin out, and their stress-hormone levels climb.

“It mimics real-life stress in humans, without being the kind of acute stress that induces post-traumatic stress disorder,” he says.

And, just as in humans, female mice are far more likely to lapse into this depressive-like state than their male counterparts. Sibille’s results are beginning to characterize the biology of male and female “depressed” mice.

Our emotional lives depend, to a large extent, on the white-matter networks that connect our emotion-processing brain areas to our emotion-regulating brain areas. To better understand these networks, Sibille is conducting large-scale studies of humans postmortem and looking at the function of genes within an important emotion-regulatory tract, the corticolimbic network. In people with depression, the genes in this network are altered.

“We don’t know if the gene is creating this [altered] function, or if the function of the brain somehow modifies the genes,” Sibille says. He’s examining levels of RNA, a product of genes, as a measure of their function. So far in this ongoing study, it appears that for each sex, partially overlapping sets of at least 40 genes will be implicated in depression.

Sibille’s lab is also looking into the effects of hormones—both in the circulating-during-reproductive-years sense people usually think of, and in terms of what’s called the organizational effect—where hormones present during certain stages of development can influence the way our neurocircuitry sets up shop.

The organizational effect of hormones is well documented. It was first noted back in 1959, when a group of researchers at the University of Kansas found that a single dose of testosterone was enough to masculinize the brains of fetal female mice. But the study of how distinctly male and distinctly female wiring patterns lead to mood disorders—and exactly what mechanisms form those patterns in the first place—is a new frontier.

Incidentally, last year, Pitt’s Mary Phillips—professor of psychiatry and director of the Functional Neuroimaging Program—and Jorge Almeida, a postdoc in her lab, stumbled upon evidence of gender-specific wiring in the

living human brain, a finding that they expect will lead to a promising collaboration with Sibille. It happened as Phillips was using diffusion tensor imaging (DTI)—a kind of MRI technique that measures the diffusion of water to map out the structure of living tissues—to investigate the differences between major depression and bipolar depression.

As they analyzed the data, the researchers found that, consistently, in the brains of women with major depression—and only women—there were two abnormal tracts in the connections to the amygdala, a brain region involved in emotion processing. One was to the orbital frontal cortex and the other to the subgenual cingulate gyrus. Both of these brain regions are important for our ability to appraise emotional input, put it into context, and carry on. With these wiring abnormalities, however, this process is stymied. Instead of working with the amygdala, these two brain regions were dampening its effect.

Further, Phillips and Almeida found, these abnormal connections in the female patients

with depression were on the left side of the brain only—the hemisphere associated with positive feelings.

So, what does all that mean? Most likely: These women had brains that were hardwired to overlook—effectively eliminate—the positive.

“This could be really important,” Phillips says, “because it’s what cognitive therapists have been saying for years: that certain types of people who are depressed will ignore positive things—no matter what. The negative is all they see.”

This happy accident of a finding was included in the bipolar/unipolar study her team published in *Biological Psychiatry* this year, though not as a main focus. Phillips and Almeida have been awarded several new federal grants that will allow them to examine sex differences in the brains of depressed adolescents and adults in more detail.

“And we’ve replicated our findings on a completely new group of people, in a slightly different cognitive test, and in a different scanner. The only thing that’s the same is that it’s Pittsburgh, and it’s us,” Phillips says, laughing, “and yet we

## Hysteria!

**Before there was conversion disorder—the current *DSM IV* term for a kind of psychological stress with physical symptoms—there was hysteria.**

**But unlike conversion disorder, hysteria, for much of history, was unique to women. It first emerged in America during the industrial revolution. As housework suddenly became much simpler, scholars were plagued by the question of what women should do with themselves, and to this, the then-nascent field of modern medicine answered: as little as possible. In 19th century urban America, women were thought to be innately frail, and the best way to protect what doctors saw as their sole function—reproduction—was through a life of seclusion and rest. Popular culture idolized a pale, tightly corseted image of woman; her practically invalid condition became a kind of status symbol. Among moneyed women, an epidemic began to spread—episodic fits of excitability, crying, screaming, or laughter. In time, any woman’s act of defiance or dissatisfaction with her station would be dubbed “hysterical.”**

**In the Freudian era, the condition came to be classified as a mental illness. (Freud’s teacher, French neurologist Jean-Martin Charcot, penned the first accurate definition of hysteria in both men and women in the 1860s.) But prior to that, dating back to the early centuries, hysteria was considered a disease of the uterus. The Greeks thought that when a woman abstained from sex or engaged in excessive “male activities,” the uterus would “wander” and wreak havoc on other organs, including the brain. The root of the word hysteria, of course, means womb. —EV**

still show the same thing. So this suggests that it might be generalizable to female depression.”

For male patients with depression, a distinct signature in the circuitry will take time to sort out, she says. “There’s not a clear picture, but our findings suggest there may be something about processing negative emotions—anger, fear, threat, and things like that—rather than avoiding the positive.”

## DOUBLE TIME

In March, the Institute of Medicine hosted a workshop in San Francisco called Sex Differences and Implications for Translational Neuroscience Research, and Sibille gave a panel presentation on depression. (Pitt’s Katherine Wisner, professor of psychiatry as well as of obstetrics, gynecology, and reproductive sciences, was also on the panel.) The effort was organized in response to growing concern over the differences between men and women and boys and girls when it comes to physiological responses to drugs and the particular challenge of understanding these differences in complex mental, neurological, and substance abuse disorders.

Teasing out the logistics—and, indeed, even convincing everyone that sex differences are potentially significant—is still an “uphill climb,” Sibille says.

Jill Becker, president of the multidisciplinary Organization for the Study of Sex Differences, says the issue is at least beginning to show up on the radar for the scientific community at large, not just in neuroscience and psychiatry. The biggest challenge now is convincing everyone that it’s important enough to fund research on the topic, she says. “I think part of the problem is that people worry they’ll fail to advance science if they have to study both males and females. They think you have to use more animals, [yet] money isn’t spent advancing what we already know about males.

“But if you’re not learning about whether it’s also applicable to females, you’re really not advancing science in general. You’re really just advancing science for one half of the population,” she notes.

“What’s important,” says Sibille, “is that you assess whether sex has an influence on what you’re studying. It may, but it may not. And if it does, then you have to take it into consideration.” —EV

## IMMUNE DIVIDE

**W**omen live longer than men, on average, by about five years. The reason for this difference—as the old epidemiological saw goes, often uttered with a chuckle—is that women are smarter. Or, more precisely, men are more reckless. Men drink and smoke more. They drive faster. They eat more cheeseburgers at 3 in the morning. They get into fights more often with guns, knives, fists, rocks, and nunchaku. Call it the death-by-testosterone theory of life expectancy.

“The general notion is men ... are out there trying to kill themselves as much as possible throughout their lifetimes whereas women are far more cautious,” says Sachin Yende, assistant professor of critical care medicine at the University of Pittsburgh.

For a few years, however, scientists like Yende have known there may be more to it than the recklessness of boys and men. Men get more infections than women do, and when they get infections, they are more likely to die.

Theories abound about this discrepancy. It could be the effect of estrogen and testosterone in immune response, or the withering effect of all the chronic diseases men tend to accrue as a result of their risky behavior. Or it could be something else entirely.

Yende and his collaborators have performed one of the bigger tests to date exploring this question. The group took blood samples of more than 2,000 patients who’d come down with community-acquired pneumonia, one of the leading causes of infection-related hospitalizations in the United States.

Men had higher levels of inflammatory molecules and blood coagulators than women did in the study. And they were 35 percent more likely to be dead one year after diagnosis than women. Even when the researchers controlled for the higher rate of chronic disease among men, the men with pneumonia simply did worse than the women.

Because the mean age of patients in his study was about 65, and testosterone and estrogen production generally decreases with age, Yende tends to downplay the role hormones had in his results. One theory is that the X chromosome could play a role in the differ-

ences in the expression of interleukin-1 (IL-1), another immune trigger. Women, of course, have two Xs, while men have an X and a Y. The X is home to IRAK-1, which regulates transcription and production of the IL-1 gene. The thinking goes that having two Xs, and thus two copies of IRAK-1, could result in different gene expression for IL-1 for women than for men.

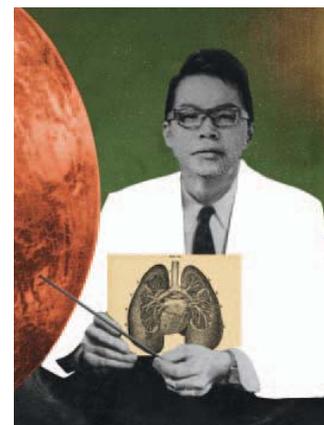
Do men have a heightened immune response because they are sicker than women? Or does the heightened response make it harder for them to recover? Consider the case of severe sepsis, in which the body’s own immune and inflammatory response becomes so strong that it can cause multiorgan failure and death. In sepsis, heightened immune response can actually be more harmful.

A study of 1995 data by Pitt’s Derek Angus, the Mitchell P. Fink Professor and Chair of Critical Care Medicine, found that women with severe sepsis had lower mortality rates than their male counterparts—their bodies behaved about five years younger than men’s.

Scott Watson—also a member of the critical care medicine faculty, as well as pediatrics—had similar findings when he examined a large cohort of young people with severe sepsis. His most significant finding was that boys ages 1 to 10 had about a 10 percent higher rate for the condition than girls. And Jason Sperry, assistant professor of surgery, found that male trauma patients had “excessive” levels of interleukin-6, an immune-triggering protein.

If the female immune response is less intense, shouldn’t girls get sick more often? They don’t. Girls’ immune systems seem to be able to fight infection off well with a lighter touch. Watson wonders whether something basic is at play: “[These differences] could be related to the fact that women are built to have this partially foreign creature growing inside them, and the immune system has to be tuned to not interfere with that,” he says.

The immune systems of women may be smarter after all. —Reid R. Frazier



## A MATTER OF HEART

**Y**ou don't have to look at the personal ads to know the hearts of men and women act differently. Just look at an EKG.

The QT interval—a measurement of the length of the electrical pulse that controls the contraction of the heart's ventricles before returning it to a “resting” state—is longer on a woman's EKG than on a man's. This is normal, but it exposes women to different kinds of risks. The longer a person's QT interval gets, the greater her risk for arrhythmia, a fluttering of the heart muscle that can lead to heart failure and sudden death. This is the case in a congenital disorder, long QT syndrome, which can produce a Torsade de Pointes, a highly lethal type of arrhythmia. The disorder occurs in about 0.1 percent of the population.

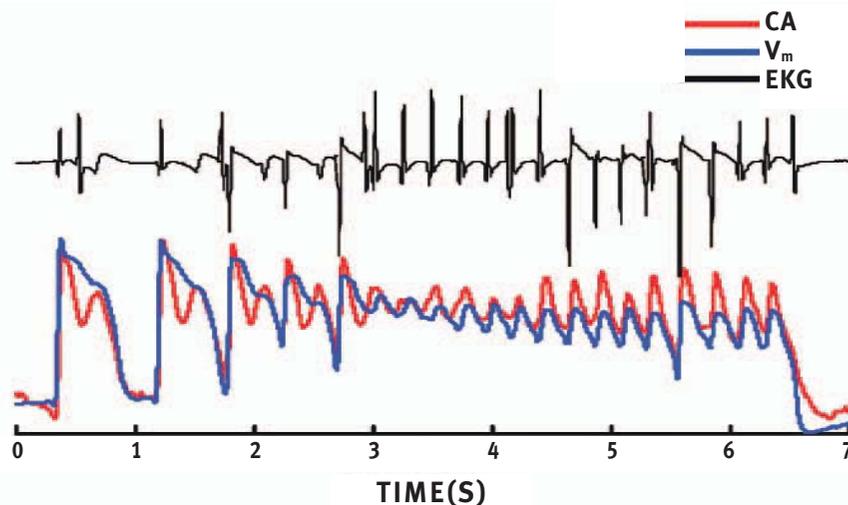
Why do male and female hearts behave so differently? Guy Salama, PhD professor of cell biology and physiology at the University of Pittsburgh, thinks it has something to do with the effect sex hormones have on the heart's electrophysiology. Ion channels are the heart's version of the World Wide Web: Ions pass through these cell membrane pores to send electrical impulses from cell to cell with instructions on how to pump blood throughout the body. Testosterone and estrogen can increase or decrease the number of available ion channels, amplifying or limiting different messages. In men, the potassium ion channel is busiest; the calcium ion channel activity is more robust in women.

What happens when drugs change “normal” ion channel activity? Things get dicey. The level of electrical activity surges at different times, and the QT interval can lengthen, especially in women, producing an arrhythmia. Salama and his collaborators found that when a drug that restricts the calcium ion channel was administered to rabbits, the adult females were much more susceptible to arrhythmia than were their male counterparts. In juveniles, with much lower levels of sex hormones, the effects were reversed.

“With the female rabbit, you add half a micromole per liter of a drug, and *Bang!* you get arrhythmia. In the male, you get nothing; they're fine. When you don't pay attention to how old they are, you get confused because the young females have no arrhythmia at all.”

This fine-grain understanding of the heart's circuitry could affect which drugs are considered safe. Currently, any new drug found to block the potassium current is almost always shelved. Yet if the relevant ion channels can be fine-tuned and perhaps targeted specifically according to the sex of the patient, then a drop in the potassium current might be tolerated.

“There could be good drugs that were thrown away that we don't know about because we didn't give them a second chance,” Salama says. —RRF



Hormones affect the heart's electrophysiology, making females more susceptible to a rare but lethal type of arrhythmia known as Torsade de Pointes. Here, measurements of intracellular calcium and voltage across the membrane are shown with an EKG just before an episode of arrhythmia in a rabbit.

COURTESY HEART RHYTHM, JULY 3, 2010

## “YOU’VE COME A LONG WAY, LUNG CANCER RESEARCH”

**O**ne in five women diagnosed with lung cancer has never smoked; in men, it's one in 12. Jill Siegfried—a PhD and coleader of the University of Pittsburgh Cancer Institute's Lung and Thoracic Malignancies Program—suspected estrogen might be a factor in the heightened risk for women, and she was right. Five years ago, she became one of the first to demonstrate that the estrogen receptor beta is expressed in the lungs and overexpressed in up to 85 percent of lung tumors. Surprisingly, Siegfried found, this is the case in both men and women. “We think the difference is how much estrogen you're exposed to in your lifetime,” she says.

Siegfried's team found that estrogen-dependent lung cancer cells thrive not only on the estrogen that naturally circulates through men and women (to different degrees), but also on a supply they make in-house. (Lung tumors express aromatase, an enzyme that synthesizes estrogen.)

When estrogen binds with its receptor on lung tumor cells, it not only kick-starts growth by interacting with the usual suspects—genes that are known players in cancer—but also through an infamous cancer-causing protein, EGF (epidermal growth factor). Siegfried found that estrogen actually makes the EGF receptor pathway more active.

EGF-targeting drugs help about 10 or 12 percent of lung cancer patients. Siegfried hopes that adding estrogen-targeting therapies will boost the efficacy of these drugs. A 100-patient clinical trial that started this summer will test that hypothesis.

Recently, her group has been studying progesterone, and as it turns out, progesterone receptors can also be overexpressed in the lung tumors of both sexes. But unlike estrogen, progesterone hinders tumor growth rather than helping it. Now, Siegfried is looking at what different levels of all three receptors mean for patients in the long run. She's been able to outline outcomes for several distinct patient profiles. For example, patients whose estrogen and EGF receptors (cancer helpers) are high and progesterone receptors (cancer hinderers) are low are five times more likely to die.

These insights hold promise for a more personalized approach to the second-most common cancer in both men and women. In a few short years, the field has, truly, come a long way. —EV

# SEXUAL (RESEARCH) REVOLUTION

The University of Pittsburgh's Johnny Huard and Bridget Deasy are quietly fomenting another sexual revolution. This one starts at the cellular level.

A decade or so ago, Huard made a big splash. His and other labs showed that adult stem cells—that is, cells that rebuild and repair tissue throughout our lives, not just the precious cells that build bodies from embryos—could regenerate tissue outside of their supposed preordained realms. For instance, if you put them in the right place under the right conditions, muscle-derived stem cells not only regenerate muscle, they regenerate blood, bone, even nerve. *Wow*, scientists realized. *All along we've been carrying around the potential to heal ourselves from all sorts of injury and disease. Now we just need to figure out how to manipulate these cells in the right way for therapeutic uses.*

But amid all the buzz, there was a sticking point for Huard, who holds appointments in a number of Pitt departments and is the Henry J. Mankin Professor of Orthopaedic Surgery Research and director of Pitt's Stem Cell Research Center. "There was a huge variability," he says.

Sometimes the stem cells his lab took from musculoskeletal tissue regenerated heart and other damaged muscle well, and other times they didn't. Naturally, he wanted to find out why.

So he designed a system that would track what happened to the cells after they were put into a mouse. His plan: Tag the Y chromosome in male stem cells and inject the cells into female animals. This way his lab colleagues would be able to track the stained Y chromosome to find out what the stem cells were up to. Sounded good. One problem: All the stem cells they had been using in their muscle studies turned out to be from female mice. They hadn't thought much about it, but the stem cells that had been working nicely had come from the critters without a Y chromosome.

"Is it possible we are working with female stem cells because they repair muscle better?" Huard recalls wondering. A study, run by Deasy, an assistant professor of orthopaedic surgery and bioengineering, showed that was exactly what was happening. When Deasy injected muscle stem cells from females into mouse models of muscular dystrophy, the mice were more successful regenerating tissue than when she administered male stem cells. Huard's team is seeing the same pattern

in humans. (And Deasy hopes to soon publish new research showing that stem cells from baby girls' umbilical cords repair muscle better than cells from boys' cords.)

"Maybe estrogen is good for stem cells and testosterone is not that good," Huard conjectured. But trying to stimulate muscle stem cells with estrogen didn't seem to make a difference.

So what's going on? No one knows, but Huard offers some other possible explanations.

"Maybe our genetic constitution is

different," he says. Perhaps that extra chromosomal limb (XX v. XY) contains genetic information that gives females a muscle-building advantage.

Alternatively, because a man's muscle mass is typically larger than a woman's, "maybe the woman has more [stem cell] reserves or 'less-tired' stem cells," offers Huard.

It does appear women end up with more reserves. At first, the male stem cells actually do a good job. Then they peter out. "At 48 hours, male cells are doing better," explains Huard. "But at 10 days, the female cells are doing better." As it turns out, the female stem cells take time to proliferate before regenerating muscle fiber. The male stem cells build muscle quickly but not much of it; they max out after a couple of days.

The sex of the host matters, as well. When Deasy put female stem cells into female mice, they regenerated muscle beautifully. What about female stem cells in male mice? Those results were pretty good. Male stem cells in female mice? So so. Male stem cells in male mice? Not promising.

But female stem cells are not always the best choice. Not if you want to regenerate bone or cartilage.

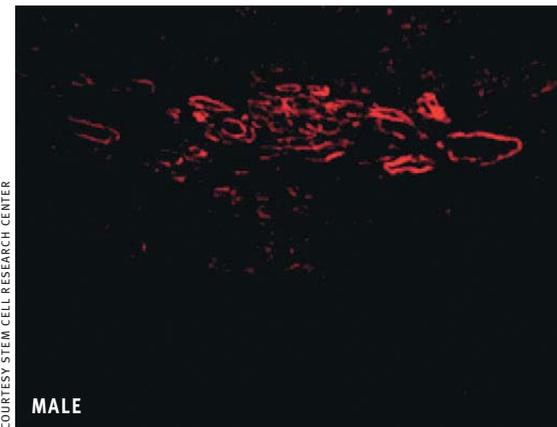
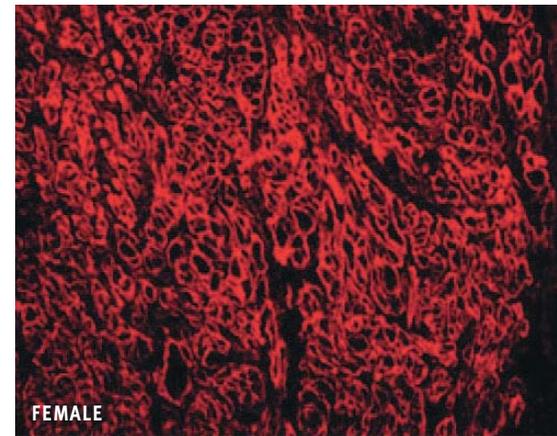
"I thought females would be able to regenerate everything better, but that's not the case," Huard says.

He knew that muscle-derived stem cells could build bone and cartilage, as well as muscle. When his colleagues tried coaxing muscle-derived stem cells to regenerate bone or cartilage, the stem cells from male mice performed the best.

"We can look for 20 years to find the reason for this," says Huard.

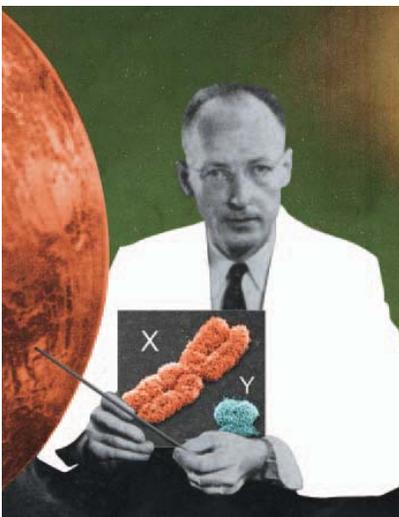
In the meantime, the stem cell community's reaction to these sorts of findings may be tepid, notes Deasy. She points out that if she and Huard are on the right track, it means scientists like them should be doing their experiments on stem cells from both male and female donors—and studying how those cells perform in both male and female hosts. That takes money and time.

But few revolutions come easily. —Erica Lloyd



COURTESY STEM CELL RESEARCH CENTER

**Arnold Schwarzenegger, watch out. Turns out, muscle-derived stem cells from females are much better at rebuilding muscle than stem cells from males. Here the cells regenerate tissue in mouse models of muscular dystrophy.**



## WORLDS APART?

**T**hroughout their lives, men are more vulnerable than women in many ways. As children, boys are more susceptible to neurobehavioral disorders, neurodevelopmental disorders, and genetic disorders. At puberty, boys start to become more vulnerable to schizophrenia (for adolescent girls, it's depression and bipolar disorder). As adults, men typically suffer heart attacks five years earlier than women. In old age, men are more likely than women to die from Parkinson's and Alzheimer's diseases. And telomeres—the ends of chromosomes that relate to the life spans of cells—are shorter on older men than on older women. Add to that these recent findings from Pitt researchers:

When neurons from males and females are exposed to stroke-related compounds in culture, both die, but they do it via different mechanisms and to different degrees. Overall, more cells from males die than from females.

Neurons from men and women also differ in how they die of oxygen deprivation. In a cell-culture model of this process, levels of glutathione crashed in cells from men, which has possible relevance to suffocation, heart attack, and severe bleeding. Glutathione levels did not crash in females.

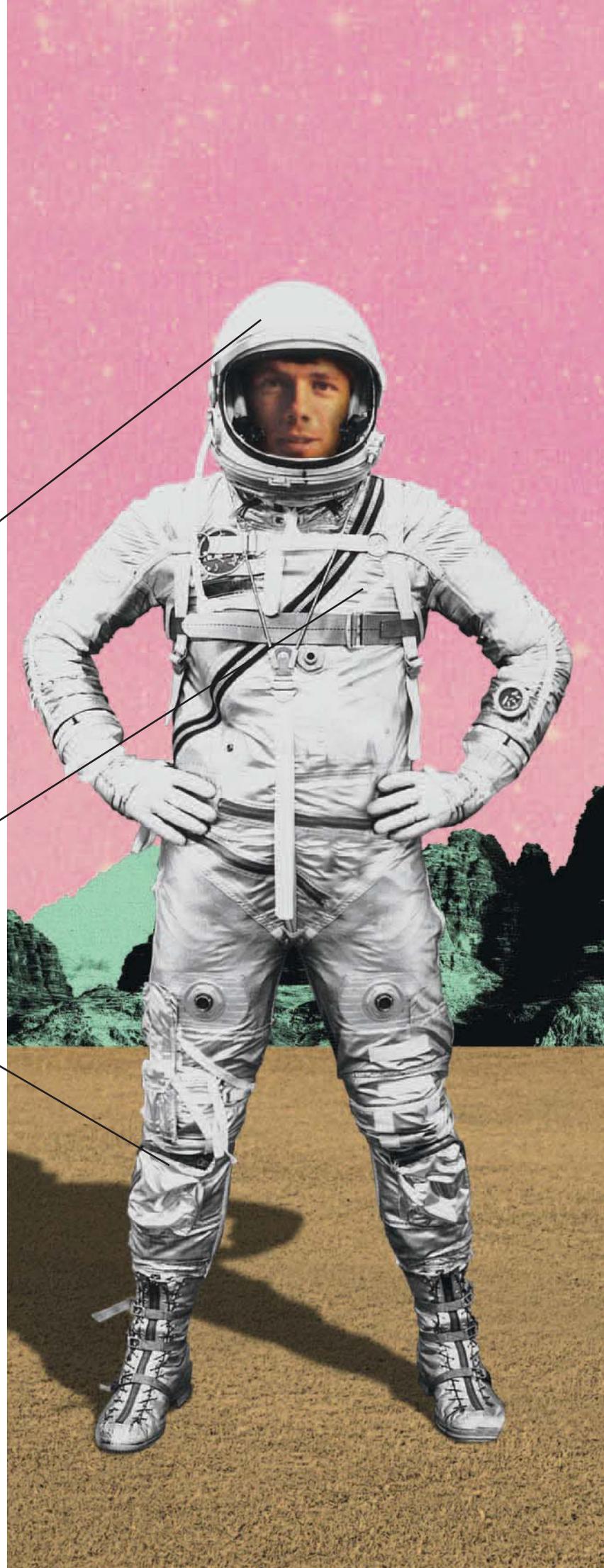
Omega-3 fatty acids help stave off cardiovascular disease in men with type 1 diabetes, but not in women with the disease.

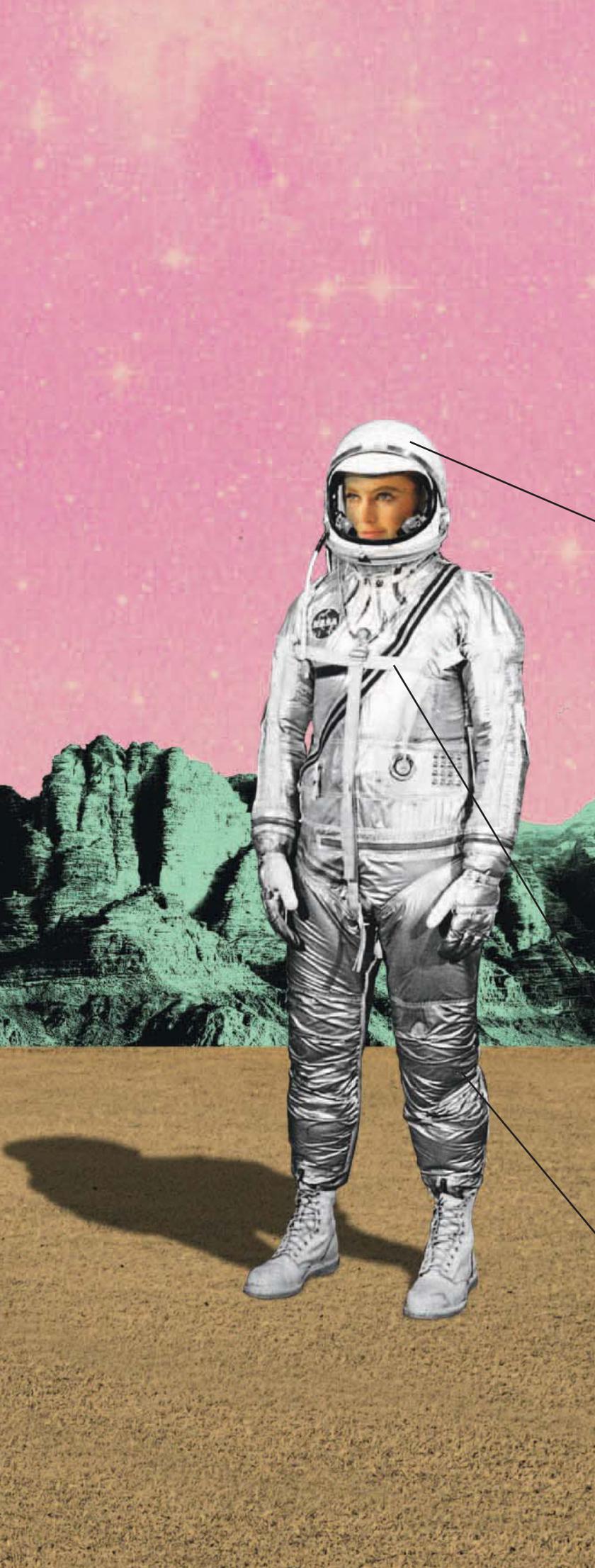
“Good” cholesterol (HDL) is cardioprotective in men but not women with type 1 diabetes. In women, HDL actually increases risk.

Men are better at rebuilding bone and cartilage with muscle-derived stem cells in therapeutic studies. They are also better at building cartilage with bone marrow stem cells; this ability declines with age in males, but not in females.

Males and females have very different immune responses to foreign invaders. Newborn boys are more likely to develop sepsis, to require mechanical ventilation, and to land in the neonatal intensive care unit.

Older men hospitalized with community-acquired pneumonia are more likely to succumb to the disease. They seem to generate a stronger inflammatory and coagulation response and, perhaps, break up blood clots more quickly than women do in response to infection.





**T**here are plenty of reasons to enjoy being a girl. Women are healthier during their reproductive years, when estrogen levels are highest. The hormone is known to protect them from strokes, heart attacks, and head trauma, and to ward off oxidative stress. Of course, being female is a mixed bag. We've long known women are more prone to rheumatoid arthritis, lupus, multiple sclerosis, eating disorders, chronic fatigue syndrome, mood disorders, asthma, and breast cancer (men get that too, don't forget). After menopause, women are just as susceptible to heart attacks as men, though they do experience them differently (pain in the shoulder and jaw, a queasy stomach, and heartburn). So Professor Higgins, there are many reasons a woman can't be more like a man. And Pitt researchers keep unearthing more and surprising findings:

The neurons of female mice in culture survive starvation for days longer than those of male mice. Ladies' brain cells subsist on fat stores; the gents', however, nosh on their own organelles.

The neurocircuitry networks that help us control our impulses and act more like grown-ups reach maturity years earlier in females than they do in males.

In brain scans, depression looks very different between the sexes. For women with the disorder, the neurocircuitry appears to be hardwired to block positive emotions at certain points. In contrast, males with depression may have trouble processing negative emotions like anger and fear.

Though protective in some situations, estrogen may also contribute to electrical disturbances leading to certain types of arrhythmia, including a rare but lethal condition known as Torsade de Pointes.

Most lung tumors—in both men and women—have estrogen receptors, meaning they grow in the presence of this hormone. The fact that women have more estrogen on board throughout their lives may explain why they're at higher risk for lung cancer, regardless of smoke exposure. (One in five women diagnosed with lung cancer has never smoked; in men, it's one in 12.)

Adult stem cells from women are better at rebuilding muscle in therapeutic experiments. Somehow, women's bodies also give muscle-building stem cells a needed boost that men's bodies don't, no matter the sex of the cell donor.

—Compiled by Elaine Vitone