His and Hers: Sex Differences in the Brain

By Catherine S. Woolley, Ph.D.

While the 1990s bestseller *Men Are from Mars, Women Are from Venus* addressed behavior, the neurobiological sex differences in the male and female brain remain largely a mystery. Our author—an acclaimed neuroendocrinologist at Northwestern University—tells us what we know and why we don't know more.

Sex differences in the brain are real, but they are not what you might think. They're not about who is better at math, reading a map, or playing chess. They're not about being sensitive or good at multi-tasking, either. Sex differences in the brain are about medicine and about making sure that the benefits of biomedical research are relevant for everyone, both men and women.

You may be surprised to learn that most animal research is done in males. This is based on an erroneous view that hormonal cycles complicate studies in female research animals, and an assumption that the sexes are essentially the same down at cellular and molecular levels. But these beliefs are starting to change in neuroscience. New research shows that some fundamental molecular pathways in the brain operate differently in males and females, and not just by a little. In some cases, molecular sex differences are all-or-nothing.

Recognition that male and female brains differ at a molecular level has the potential to transform biomedical research. Drugs act on molecular pathways. If those pathways differ between the sexes, we need to know how they differ as early as possible in the long (and expensive) process of developing new medicines and treatments for disease.

The Brain's Sex Differences: Not What You Think

The bulk of public attention to brain sex differences is focused on structural differences and their purported relationship to behavior or cognition. Yet structural sex differences are actually quite small, and their interpretation is often based on gender stereotypes with little to no scientific justification.

Reports of sex differences in the brain often make headlines. For example, a large 2014 study used a type of magnetic resonance imaging (MRI) called diffusion tensor imaging to show what the authors called "conspicuous and significant" sex differences in brain connectivity; it generated 87 news articles and 162 discussions in blogs in the first month after its publication. Tellingly, most media attention focused on potential behavioral manifestations of the anatomical differences that were reported, even though the researchers did not look at behavior in the study. This may be because the university press release announcing the study suggested that its findings could help provide a neural basis for why men excel at certain tasks,

"like cycling or navigating directions, whereas women… are better equipped for multi-tasking and creating solutions that work for a group."

The urge to link structural sex differences to brain function seems almost irresistible. This common pattern in reporting led to a suggestion in the *New York Times* in 1912 that men devote 6.5 times more gray matter (areas where brain cells are concentrated) to intelligence-related tasks than women do (which is not true, in case that needs to be said). The now infamous 2017 "Google's Ideological Echo Chamber" memo (whose author was subsequently fired) drew on studies of sex differences to make a case against efforts to achieve gender balance in the technology workforce. The neuroscience of sex differences has also been interpreted incorrectly to promote single-sex education based on purported brain differences between girls and boys that don't exist.

The proponents of these and other stretches of the imagination have been opposed by a vocal group of neuroscientists and scholars who claim that there are no meaningful sex differences in the brain. The latter group's arguments center on the role of experience in shaping brain structure and connectivity, and the idea that everyone's brain is a mosaic of male-typical and female-typical characteristics. Indeed, a recent large-scale analysis of brain regional volumes found statistically significant sex differences throughout the brain, but also that these differences are small, with a great deal of overlap between men and women.

Scientists often measure the size of a difference with a statistic called "Cohen's d." In the study mentioned above, sex differences in brain regional volume had an average Cohen's d value of 0.33, which means that men and women actually overlapped by 86.9 percent (ranging from 75.3 percent for the largest differences to 90.8 percent for the smallest ones). So even though there are many sex differences when you compare male averages to female averages, brains don't fall neatly into two categories based on their physical structure. And even the differences in averages are pretty small.

For perspective, consider the familiar sex difference in height: on average, men are taller than women, but there are also some women who are taller than some men. Here, the comparison

has a Cohen's d value of 2.0, corresponding to only a 31.7 percent overlap: the sex difference in height far exceeds any of the sex differences reported in human brain structure.

So, what *do* structural sex differences in the brain mean for function? The reality is that no one knows. Except in cases of brain disease or injury, or in very rare instances, there is no way to predict what a difference in the size of a certain brain region means for its function. While we can say that a particular part of the brain contributes to functions like memory, language, or even empathy, our understanding of *how* that brain region contributes to a specific function is still in its infancy. There is no basis to say, for example, whether bigger is better or worse for function. A brain region could vary in size for any number of reasons, including the number or size of neurons, glial cells, blood vessels, or differences in the amount of extracellular space. The underlying sources of size disparities cannot be resolved from brain scans.

Finally, it is worth noting that for the relatively few brain functions for which there is evidence of a difference between sexes, the neural basis of the difference is unknown. For example, the largest and most reliable cognitive sex difference is in mental rotation of three-dimensional shapes. But this too shows a high degree of overlap between the sexes, 79.9 percent, with a Cohen's d value of 0.51. Complicating the issue even further, spatial skills like mental rotation are known to improve with practice. This makes it possible that the types of activities boys and men are more likely to engage in, from sports to video games, give them more opportunity to practice spatial skills leading to better scores on spatial tasks.

So, if sex differences in brain structure are so small, so mixed, and so hard to connect to what the brain does, couldn't we just dispense with the issue of sex when it comes to the brain? Some in the field have suggested that we should. The flaw in these arguments, however, is that structure may be the wrong thing to focus on when it comes to brain sex differences. New research shows robust sex differences at a much deeper level, where no one expected them: in molecular interactions that regulate neural activity.

Out With the Old, In With the New

The billions of neurons in the brain are wired into circuits through trillions of tiny junctions called synapses. At most synapses, neurons communicate when neurotransmitter molecules are released by one neuron and activate receptor molecules on another neuron. The type of neurotransmitter receptor determines whether a synaptic connection is excitatory, stimulating the next cell in line, or inhibitory, silencing a downstream neuron. The effectiveness of each synapse, or its strength, is variable and changes with differences in the amount of neurotransmitters released and/or its sensitivity to neurotransmitters. This is analogous to adjusting the volume settings for a speaker or a microphone.

Changes in synapse strength are the basis of learning and memory and are involved in disease—in addiction, for example. The molecular machinery that controls synapse strength is finely tuned by a host of molecules, including enzymes, lipids, and small molecules that carry messages from one part of a cell to another. Scientists study these molecular interactions both to better understand the brain and because drugs often work by altering neurotransmission. Each molecule that affects how synapses work is a potential target for new drugs.

In 2012, we discovered a sex-specific molecular mechanism for tuning synapse strength, quite by accident, while studying the action of estrogens in the hippocampus (a part of the brain important in learning and memory), responses to stress, and neurological disorders such as epilepsy. Although estrogens are commonly thought of as reproductive hormones important mainly in females, they are also synthesized in the brain—of both sexes—where they exacerbate seizures and can improve memory.

Using female rats, we found that estrogens weaken critical inhibitory synapses in the hippocampus. In the search for a key to this effect, our initial experiments pointed us toward molecules called endocannabinoids, which decrease neurotransmitter release. (Endocannabinoids get their name because they activate receptors also activated by tetrahydrocannabinol, the principal psychoactive component of cannabis.) However, as we probed the connection between brain estrogens and endocannabinoids, our findings didn't replicate previous results from the scientific literature.

Although confusing at first, we quickly realized that those earlier studies had been done exclusively in males. When we compared males and females directly, we found that the estrogen regulation of inhibitory synapses that was so clear in females was absent in males. That meant that a drug based on the molecular effects of brain estrogens or endocannabinoids could have different effects in each sex.

Sure enough, when we tested an inhibitor of fatty acid amide hydrolase (FAAH, an enzyme that breaks down endocannabinoids), it suppressed inhibitory synapses in the hippocampus of females but had no effect on the same synapses in males. This indicated that females, but not males, produce FAAH-sensitive endocannabinoids continuously. As a result, applying the FAAH inhibitor caused endocannabinoids to build up in females, weakening inhibitory synapses in a way that didn't occur in males.

Endocannabinoids influence diverse aspects of physiology and behavior, including learning and memory, motivational state, appetite, responses to stress, and pain. They are also involved in seizures. Because of these effects, enzymes that regulate endocannabinoid levels are targets for drug development. Indeed, at the time our study was published, the same FAAH inhibitor that we used had already been tested in human clinical trials, presumably without knowledge that it could affect the brains of males and females differently.

Recognition that molecular mechanisms controlling synapse strength differ between males and females prompted my lab to start using both sexes in all our animal experiments and to compare the sexes in every case. We have found a mixture of sex-based similarities and differences. One important concept emerging from this research is the existence of latent sex differences, in which the same functional outcome in males and females arises through different underlying mechanisms in each sex. This means that sex differences can exist at a molecular level and *not* at the level of behavior or physiology: there are two routes to the same result. It also means that some sex differences won't be apparent until the system is perturbed, for example, with a drug that targets one of the molecules that differs between the sexes.

Latent sex differences can also explain apparent inconsistencies in the scientific literature. For example, in contrast to their suppression of inhibitory synapses specifically in females,

estrogens strengthen excitatory synapses in the hippocampus of both sexes. Initial studies aimed at understanding the molecular mechanism(s) of this excitatory effect were done in different sexes and came to different conclusions. The group that studied males found that estrogens strengthen excitatory synapses by increasing neurotransmitter sensitivity, whereas our group studied females and found that estrogens strengthen excitatory synapses by increasing neurotransmitter release. Both groups reported that estrogen receptor β was the critical receptor involved in the different effects they observed.

To resolve the discrepancy, we compared males and females using a technique that can distinguish changes in neurotransmitter release from neurotransmitter sensitivity. This showed that both groups were right: activating estrogen receptor β strengthens excitatory synapses in both sexes, but through different mechanisms in each sex. The apparent conflict was due to a sex difference. As with FAAH inhibitors, this is especially significant in the context of drug development. Estrogen receptor β activators are another class of drugs tested in human clinical trials. If results of the animal studies translate to humans, these drugs could have different effects in men and women.

Some latent sex differences have been hiding in plain sight. One is the lasting increase in synaptic strength caused by brief patterns of neural activity called long-term potentiation, or LTP. Discovered in 1973, LTP is thought to underlie the formation of new memories. We found that, although there is no difference between males and females when LTP is tested under control conditions, LTP in females requires a well-studied enzyme, protein kinase A (PKA), whereas in males it does not. This was very surprising because, while LTP has been the subject of intense research with over 10,000 scientific papers published over the last 40-plus years, no one was aware of this profound sex difference in its molecular underpinnings. Apparently, no one had looked.

Molecular sex differences are now found in many areas of interest in neuroscience, including in mechanisms of pain and effects of stress, how an autism-linked gene regulates neurophysiology, and how an intellectual disability-linked gene affects the biochemistry of synapses. Even with this increased awareness, though, what we know now is likely just the tip

of the iceberg. The only way to find out which brain mechanisms are similar and different between the sexes is for more scientists to explicitly compare males and females in their studies. While there has been some progress toward this, the majority of animal research still ignores the issue of sex.

Thinking Differently about Sex Differences

One of the most widely cited reasons for studying the brains of both sexes is that the incidence of many neurological and neuropsychiatric disorders varies by sex. For example, autism spectrum disorders are more common in boys than girls, whereas women are more likely to develop major depressive disorder, post-traumatic stress disorder, and anxiety disorders. Schizophrenia tends to develop at an earlier age in men than women and its symptoms can differ between the sexes. But such differences in prevalence and presentation haven't persuaded the majority of neuroscientists who investigate molecular mechanisms in the brain to get serious about how sex might affect the outcomes of their studies.

In 2009, more than five times as many neuroscience studies in animals were done using males only as were done using females only. We found a similar imbalance when we analyzed brain studies, specifically in rats and mice, published in five top journals from mid-2011 to mid-2012: 32 percent studied exclusively males, 7 percent exclusively females, and only 4 percent studied both sexes, with only the latter noting whether there were any differences between them. The rest either used both sexes without saying whether or not there were any differences (29 percent) or failed to mention the sex of the animals studied (28 percent).

This bias toward males prompted the National Institutes of Health, the largest funder of biomedical research in the U.S., to issue a new policy in 2016 requiring that grant applicants explain how they would consider sex as a biological variable in animal research. At about the same time, many scientific journals also started requiring researchers to state the sex of animals used in studies they publish. But neither policy requires comparison of the sexes, and researchers often fail to note how many males or females were involved in published studies.

By 2017, more scientific papers reported using both sexes, but studies comparing males and females increased only slightly, from four to eight percent.

Why Has the Field Been Slow to Catch On?

One reason neuroscience has been slow to understand the need to compare male versus female research results might be that sex differences in the incidence of human disease, like differences in brain structure, are apparent when considering averages across large populations. This gives the impression that differences between males and females are simply quantitative variations on a common theme: each sex has or does something, but one sex has or does more of that thing than the other sex. If that were true of all sex differences, then comparison of males and females at a molecular level might not matter much because results from one sex would apply, perhaps with minor differences, equally well to the other. But sex-specific molecular mechanisms and latent sex differences change that calculation.

The existence of latent sex differences makes it clear that molecular mechanisms targeted for drug development can be sex-specific, even in the absence of differences in behavior or disease. It follows that drugs derived from molecular studies in only one sex could be ineffective or have unanticipated consequences in the other.

The next time you hear about a sex difference in the brain, consider whether claims about its implications for brain function have really been tested. And the next time you hear about a new brain study in animals, find out whether the results apply to both sexes. It may be that the best way to persuade scientists to get serious about sex differences is for non-scientists—who, after all, pay the bills for federally funded research—to demand that they do.

Bio

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