Sex-typical behavior of male, female mice guided by differences in brain's gene activity

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Male and female mouse brains differ in important ways, according to a new study led by Stanford Medicine investigators.

These differences are likely reflected in the brains of men and women, the researchers say.

The scientists probed four tiny structures within mouse brains that are known to program "rating, dating, mating and hating" behaviors. These behaviors—for example, males' quick determination of a stranger's sex, females' receptivity to mating, and maternal protectiveness—help the animals reproduce and their offspring survive.

Analyzing tissue that was extracted from these brain structures and enriched for cells responsive to sex hormones, the scientists found more than 1,000 genes that are substantially more active in the brains of one sex versus the other. Genes are the blueprints for proteins, which do virtually all of a cell's work. Gene-activation levels—the rate at which the information genes contain is copied and converted into proteins—determine a cell's functions.

The findings, described in a paper to be published online Jan. 21 in *Cell*, help explain behavioral sex differences in mammals.

"Using these genes as entry points, we've identified specific groups of brain cells that orchestrate specific sex-typical behaviors," said the study's senior author, Nirao Shah, Ph.D., professor of psychiatry and behavioral sciences and of neurobiology.

Joseph Koestler, Ph.D., a postdoctoral scholar in Shah's lab, is the lead author of the study.

The researchers also pinpointed more than 600 differences in gene-activation levels between females in different phases of their estrous cycle. (In women, this is referred to as the menstrual cycle; female mice don't menstruate.)

"To find, within these four tiny brain structures, several hundred genes whose activity levels depend only on the female's cycle stage was completely surprising," said Shah, who has devoted his career to understanding how sex hormones regulate sex-typical behaviors.

The brain structures the researchers focused on are shared among mammals, including humans.

"Mice aren't humans," Shah said. "But it's reasonable to expect that analogous brain cell types will be shown to play roles in our sex-typical social behaviors."

Insight into neurological and psychiatric disorders

Some of the genes the researchers catalogued are established risk factors for brain disorders that are more common in one or the other sex. Of 207 genes already known to confer high risk for autism spectrum disorder, which is four times as common in men as in women, the researchers identified 39 as being more active in the brains of one or the other sex: 29 in males, 10 in females. They also identified genes linked to Alzheimer's disease and multiple sclerosis, both of which tend to afflict women more than men, as being more activated among female mice.

The researchers speculate that males need some genes to be working harder, and females need other genes to be working harder—and that a mutation in a gene that needs high activation may do more damage than a mutation in a gene that's just sitting around.

"The frequency of migraines, epileptic seizures and psychiatric disorders can vary during the menstrual cycle," Shah said, "and our findings of gene activation differences across the cycle suggest a biological basis for this variation."

Sex-typical social behaviors

Sex-typical social behaviors have been built into animals' brains over millions of years of evolution.

Male mice, for example, quickly distinguish the sex of strangers infringing on what they've deemed their turf. If the intruder is another male, they immediately attack it. If it's a female, they, to put it politely, initiate a whirlwind courtship.

Female mice exhibit maternal rather than territorial aggression, attacking anything that threatens their pups. They're vastly more inclined than males to guard their youngsters and retrieve any that stray. Their willingness to mate varies powerfully depending on the stage of their cycle.

"These primal behaviors are essential to survival and reproduction," Shah said, "and they're largely instinctive. If you need to learn how to mate or fight once the situation arises, it's probably already too late. The evidence is pretty clear that the brain isn't purely a blank slate just waiting around to be shaped by environmental influences."

Previous attempts to find gene-activation differences between male and female rodent brain cells have come up with only about 100 of them—seemingly too few, Shah's group mused, to generate the numerous profound differences in known instinctual behavior.

"We wound up finding about 10 times that many," Shah said, "not to mention the 600 genes whose activity levels in females vary with the stage of the cycle. In all, these add up to a solid 6% of a mouse's genes being regulated by sex or stage of the cycle."

Needles within needles in a haystack

Shah likened the methods his team used to finding needles within needles in a haystack.

"The cells we identified as mission-critical for these sex-typical rating, dating, mating or hating behavioral displays account for probably less than 0.0005% of all the cells in a mouse's brain," he said. Determining what made these cells tick required separating them from their surrounding cells and examining their genetic contents, one cell at a time.

The researchers vastly improved their prospects by zeroing in on scarce but crucial cells that are responsive to estrogen—that is, cells that have receptors for this major female sex hormone. (Estrogen is also present in males, although in lower levels.) Women's estrogen levels and those of another hormone, progesterone, wax and wane on a roughly monthly basis, like phases of the moon—as do corresponding female-sex-typical behaviors in many mammals. In mice, ovulation and maximal sexual receptivity, known as estrus stage or heat, is marked by peaks in both hormones' levels; the polar opposite, or diestrus stage, by troughs in the hormone levels.

Shah was able to purify tissue from each of the four key brain structures in a way that enriched the resulting brain-cell population for estrogen-responsive cells—the "needles," in Shah's analogy. Comparing males, females in estrus and females in diestrus, the researchers discerned 1,415 genes with activity levels that varied among the groups.

The estrogen-responsive cells were far from alike. In one brain structure called the bed nucleus of the stria terminalis, they could be classified into 36 separate cell types distinguished by the genes in each cell type that were especially active in one or another of the mouse groups. (The

bed nucleus of the stria terminalis, or BNST, is also found in human brains.)

Of these 36 estrogen-responsive cell types in mice, the scientists showed that just one was essential to male mice's ability to rapidly recognize the sex of an unfamiliar mouse and then behave characteristically toward it.

Another brain structure, called the ventromedial hypothalamus, or VMH (also found in the human brain), contained 27 estrogen-responsive cell types distinguishable by different patterns of gene activation. Knocking out the performance of just one of those cell types—but not of the other 26—transformed females who would ordinarily be sexually interested into ones who rejected sexual advances even when they were in heat.

Those BNST and VMH cell types that regulate males' recognition of gender and females' sexual receptivity, respectively, were the "needles within needles" in the haystack that's the brain. What tasks each of the other 35 sex-hormone-responsive cell types in the BNST and the other 26 equivalent cell types in the VMH are carrying out, whether sex-differentiated or not, is a mystery, Shah said.

'Tip of the iceberg'

"This is probably just the tip of the iceberg," he said. "There's likely to be many more sex-differentiated features to be found in these and other brain structures, if you know how to look for them."

Stanford's Office of Technology Licensing has filed for a patent on intellectual property associated with the study.

Other Stanford co-authors of the study are research scientists Sayaka Inoue, Ph.D., and Taehong Yang, Ph.D.; postdoctoral scholars Daniel Bayless, Ph.D., and Nicole Leung, Ph.D.; graduate students Adarsh

Tantry and Chung-ha Davis; undergraduate student Grace Wang; life science research professional Maricruz Alvarado; laboratory manager Charu Ramakrishnan; Lief Fenno, MD, Ph.D., instructor of psychiatry and behavioral sciences; and Karl Deisseroth, MD, Ph.D., professor of bioengineering and of psychiatry and behavioral sciences and the D. H. Chen Professor. Researchers at Accent Therapeutics and at Columbia University contributed to the work.

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