NEUROBIOLOGY

Does Alcohol Damage Female Brains More?

As they begin to probe this question, researchers are finding surprising and sometimes contradictory evidence that gender matters

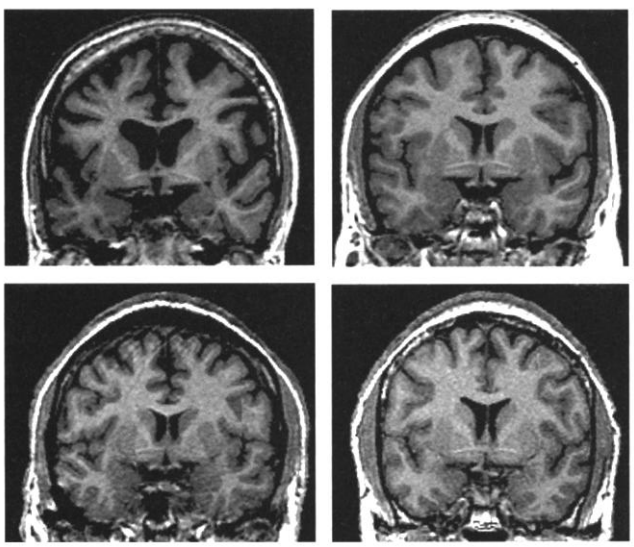
In the blockbuster film *Traffic*, newly appointed drug czar Judge Robert Wakefield and his wife Barbara argue about who drinks more alcohol. The judge swills a nightly Scotch and soda to take the edge off his boredom at home, while his wife quaffs at least three times as much. But their nasty spat over who drinks more begs a nagging scientific question: Does alcohol affect the brains of men and women differently, perhaps causing even more damage to the female than the male brain?

The answer has been surprisingly hard to pin down, despite anecdotal observations that women alcoholics suffer more severe motor problems and cognitive impairment than men do. Alcoholism has been traditionally thought of as a "male disease" because of its higher prevalence among men: There are about three times as many male as female alcoholics. In addition, researchers have often turned to the mostly male Veterans Administration hospitals for their research subjects; only recently have studies been published that compare men and women. Methodological problems are rife, too, especially controlling for alcohol consumption.

For these reasons, those sex differences that studies have turned up, such as increased damage to women's livers and hearts, have been largely attributed to the different ways in which men and women metabolize alcohol. Because women tend to be smaller and have more body fat than men—and because women may have less of a stomach enzyme that digests alcohol—women's blood alcohol levels (BALs) tend to be higher after imbibing the same amount as a man. Higher exposure simply translates into more severe effects, the argument goes.

Over the past few years, however, researchers have found tantalizing clues suggesting that more is at work than higher BALs. Using increasingly precise molecular and brain imaging techniques, researchers have been scrutinizing brains of both men and women, as well as male and female rats. From these studies are emerging new—although sometimes contradictory—evidence that when it comes to alcohol, one's sex matters. The findings reinforce the idea that, in addition to higher BALs, biological differences between male and female brains contribute to increased damage in women.

"It's still controversial, but more of us are starting to recognize the likelihood that the female brain is indeed more sensitive to the deleterious effects of alcohol," says neuroscientist Mark Prendergast of the University of Kentucky, Lexington, who is examining the issue.



More makes less. MRI scans of the brains of alcoholic men and women and controls show striking differences in brain shrinkage (female alcoholic, top left; male alcoholic, bottom left; controls, right).

"Sex differences in alcohol-induced brain damage is certainly an area that has to be explored," agrees David Lovinger, a neurophysiologist specializing in brain damage caused by alcohol at Vanderbilt University in Nashville, Tennessee.

The shrinking brain

Magnetic resonance imaging (MRI) studies conducted in male alcoholics have consistently shown that excessive alcohol consumption shrinks male brains, particularly the white matter, and that there is a corresponding increase in the volume of cerebrospinal fluid (CSF). In spring 1999, psychiatrist Daniel Hommer of the National Institute on Alcohol Abuse and Alcoholism in Bethesda, Maryland, got a surprise when he began looking for similar effects in females' brains. Specifically, Hommer compared the entire brains of 43 alcoholic men and 36 alcoholic women to those of nonalcoholic men and women of the same ages. The contrast was startling. "After reviewing the first dozen people, we thought, 'Wow, there's something here,' " Hommer recalls. Alcoholic women lost about 11.1% of their gray matter compared to healthy women, whereas alcoholic men lost only about 5.6% of their gray matter. Alcoholic women lost 8.2% of their white matter verses 5.3% for men. At the same time, the volume of space filled with CSF increased by 24.1% in alcoholic women over healthy women, and by 10.5% in alcoholic men relative to their controls. This latter difference was most striking in the intrahemispheric fissure, a gully that runs through the top center of the brain and separates its two hemispheres. Like a stream that erodes the landscape, the fissure widened and deepened in alcoholic women. In women as in men, brain shrinkage was evident by the time an alcoholic had reached her early 30s.

Hommer's MRI results, published in the February issue of the *American Journal of Psychiatry*, are the first to show that alcoholic women have reduced gray and white matter volumes and correspondingly greater CSF volumes than healthy women—and that their damage is greater than that of men. Already, the data are being challenged.

Indeed, a study published in the same journal found decided sex differences—this time, suggesting men are the hardest hit. In their MRI study, neuroscientists Edith Sullivan of Stanford University School of Medicine and Adolf Pfefferbaum of SRI International, a research and consulting firm in Menlo Park, California, compared 44 alcoholic men and 42 alcoholic women with healthy controls. The California team imaged a slab of brain, concentrating on the cortex, the brain's outermost layer and home to most of its gray matter. The images included the white matter just below and the lateral ventricles, the largest fluid-filled space in the middle of the brain.

They found a striking decrease in brain volume in men, especially in the frontal cortex—but not in women. The size of the ventricles, however, increased in both men and women to about the same extent, Sullivan says. This provided the only hint of brain shrinkage in women. The researchers

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also found that the women who had been abstinent for longer periods had larger amounts of white matter than women who were more recently sober, implying as other studies have that brain structure and volume can recover, at least partially.

"The new, contradictory results underscore the complexity of studying this problem," explains clinical psychologist Terry Jernigan of the University of California, San Diego. The discrepancies could stem from differences in either methodology or in patient groups, she says. In terms of methodology, the studies used subtly different MRI resolutions, leaving both research groups prone to different kinds of error. In addition, Hommer's alcoholic subjects were all recruited from an inpatient program, whereas the men in Sullivan's study were inpatient military veterans while the women were outpatients, and inpatients tend to have more severe disease than outpatients. In addition, the participants in Hommer's study had been sober for just 3 weeks, while Sullivan's participants had been sober for an average of several months, allowing more time for brain recovery. Finally, both studies could have been more rigorous in controlling for alcohol consumption, Jernigan says.

The studies raise more questions than they answer, including possible mechanisms of damage in the female brain. Brain imaging studies cannot determine specific causes, only effects. Nor can human studies probe actual molecular changes in the brain. For that, researchers turn to rats.

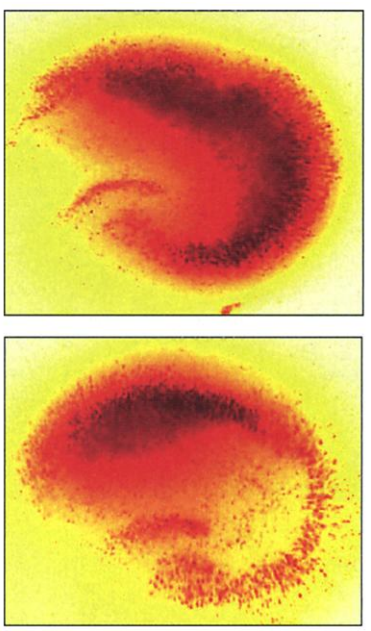
Molecular suspects

At the University of Kentucky, Prendergast and colleague John Littleton recently identified a molecular suspect in sex-specific brain damage: spermidine. Unrelated to its namesake, spermidine is a polyamine, a natural substance that plays a role in cell growth, differentiation, and death. Produced in cells throughout the body, polyamines can modulate the functions of RNA, DNA, and protein synthesis.

Studies by Peter Wilce of the University of Queensland in Brisbane, Australia, in 1998 had shown that spermidine is released in rat brain tissue during alcohol withdrawal. Wilce and colleagues also reported in the journal *Alcoholism: Clinical and Experimental Research* that spermidine's presence is accompanied by an increase in the seizure activity of neurons, in which they fire nonstop. Such seizures can kill nerve cells.

Subsequently, Prendergast and Littleton looked for possible sex differences in this effect, comparing slices of the hippocampus from male and female rats. They first dosed the brain slices with alcohol and then compared the amount of neuronal death during withdrawal, the brain's most sensitive time. They saw no difference between males and females—until they added spermidine to the slices. As reported in the December 2000 issue of the same journal, the presence of spermidine during alcohol withdrawal caused 15% to 20% more neuron death in female than male rats. Prendergast and Littleton speculate that, in living brains, nerve cells damaged by alcohol release spermidine as part of an intended repair process. But in females, the process goes haywire, and spermidine increases the seizure activity of neurons already agitated by alcohol.

Previous research established that spermidine alters the neurons' NMDA receptors in a way that enhances the activity of glutamate, an excitatory neurotransmitter. Upon binding to the NMDA receptor, glutamate normally opens a pore that allows a measured flow of calcium to enter the cell, where it contributes to an array of activities. But transformed by alcohol and, subsequently, by spermidine, the tap stays open, allowing more and more calcium to flood the neuron. This riptide of ions activates enzymes that destroy the cell. This basic process takes place during withdrawal, causing cell death; now, with Prendergast and Littleton's findings, there is evidence that spermidine exacerbates it in females.



Cold turkey. During alcohol withdrawal, female rat brains *(top)* showed 15% to 20% more neuron death (stained dark) than male brains did when the natural compound spermidine was present.

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Fulton Crews, a pharmacologist specializing in alcohol studies at the University of North Carolina, Chapel Hill, notes that the finding challenges another presumption about alcohol and the brain: that any differential damage would be due to hormonal differences in the male and female brains. "By putting the hippocampus in culture and taking it out of the brain's hormonal milieu, it implies that the difference is inherent to the system," he says.

Hormones with a new twist?

Throughout her lifetime, a woman's brain is exposed to a more variable hormonal milieu than a man's brain is. But researchers have had difficulty establishing a definitive link between those differences and the brain's response to alcohol. Now, one hint of such differences comes in the work of endocrinologist Catherine Rivier of the Salk Institute for Biological Studies in San Diego.

In a series of rat studies over the past 10 years, Rivier has found that alcohol overstimulates the hormonal cascade produced by the hypothalamic-pituitary-adrenal (h-p-a) axis far more in females than in males. One of the last hormones in the cascade is cortisol, and several studies have shown that the chronic release of cortisol can produce mild brain damage.

Although Rivier's studies strongly suggest that hormonal differences influence alcohol's effects on the brain, she cautions that they don't explain it entirely. When she removes female circulating sex steroids by removing the ovaries, alcohol still overstimulates the h-p-a axis, although to a much lesser degree.

Looking beyond the usual hormonal suspects, Leslie Devaud, a neuropharmacologist at Idaho State University in Pocatello, is exploring sexual dimorphism in brain circuitry and pathways. "Gender differences in the response to alcohol may arise from subtle differences in molecular responses," she says.

Devaud has found initial indications of such molecular differences by studying minute changes in both NMDA and $GABA_A$ receptors in male and female rats. (γ-aminobutyric acid or GABA is the main chemical messenger that inhibits the firing of neurons, and the $GABA_A$ receptor plays an important role in alcohol dependence and tolerance.) Devaud found that chronic alcohol exposure predominantly wrought changes in brain $GABA_A$ receptors in male rats, whereas in female rats, the most pronounced changes were in the NMDA glutamate receptors. But the impact of these differences remains a mystery.

Regardless of sex, the brain responds to alcohol by trying to counteract its depressing effects on the central nervous system. Devaud and others are continuing to search for molecular responses that vary between males and females as the brain attempts to compensate for the changes wrought by alcohol use. Says Devaud: "Big questions remain, but if we can identify relevant gender differences, it could ultimately affect how we treat alcohol dependence in men and women."

–BERNICE WUETHRICH

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