NEUROBIOLOGY

# Enzyme Linked to Alcohol Sensitivity in Mice

In the movie *Days of Wine and Roses*, the drunken stupors of the alcoholic couple add to the pathos of the story. But in the laboratory of Hiroaki Niki at the RIKEN Brain Science Institute in Wako City, Japan, the drunken stupors of certain genetically altered mice are cause for celebration: These mice are providing new insights into how alcohol affects brain neurons, and hints of why some people are more susceptible to alcohol addiction than others.

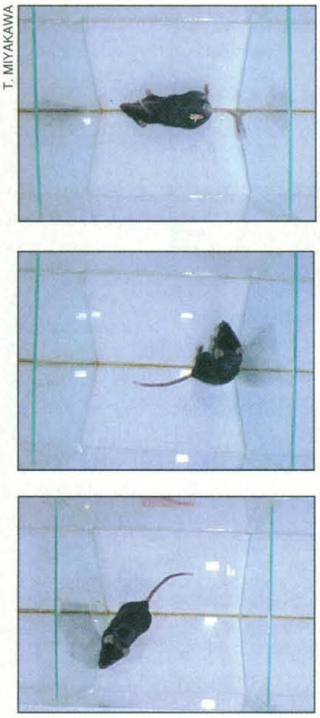
The mice Niki and his colleagues are studying lack a functional gene for an enzyme called Fyn tyrosine kinase, which previous work had already linked to memory and learning. The researchers found that animals without the kinase stay drunk longer than normal mice, apparently because the enzyme normally counteracts alcohol's depressive effects on neurons, causing acute tolerance. When it is missing, the neurons—and the animals—take much longer to recover.

In the past, one theory held that sensitive individuals simply break down alcohol more slowly, but this study suggests that sensitivity to alcohol is determined at least in part by levels of Fyn tyrosine kinase. If so, individual differences in enzyme levels could explain why some people are more prone to have trouble with alcohol than others.

"Initial insensitivity [to alcohol] seems to be a strong predictor of alcoholism later in life," notes pharmacologist Adron Harris of the University of Colorado Health Sciences Center in Denver. And because too little Fyn kinase increases alcohol sensitivity, it could be that too much of the enzyme may lead to lowered sensitivity. If that is the case, he adds, the Fyn kinase gene may be a target for researchers searching for genes that might predispose people to alcoholism. "Any gene encoding a protein that influences alcohol action becomes a candidate gene for alcoholism," Harris says.

Fyn kinase first became a center of attention 5 years ago. By producing mice lacking the Fyn kinase gene, Eric Kandel's team at Columbia University College of Physicians and Surgeons in New York City had showed that the enzyme is needed for spatial learning and long-term potentiation, a long-lasting alteration in nerve-cell excitability thought to underlie memory (*Science*, 18 December 1992, p. 1903).

Independently, geneticist Takeshi Yagi of the National Institute for Physiological Sciences in Okazaki had made a similar knockout mouse strain to learn more about the enzyme's function in brain development. Niki then teamed up with Yagi to look at how the mice responded to various drugs, including sedatives such as ethanol. To assess alcohol sensitivity, Niki's RIKEN collaborator Tsuyoshi Miyakawa injected ethanol into the mice, put them on their backs in V-shaped troughs, and measured the time it took for the inebriated animals to stand upright.



T. MIYAKAWA

A. COOPER/THE KOBAL COLLECTION

**Smashed.** Drunk mice have trouble righting themselves; in *Days of Wine and Roses*, Jack Lemmon and Lee Remick can't make their lives right.

They found that while mice not given alcohol stand up immediately, normal injected mice take anywhere from 3 to 40 minutes, depending, Niki says, "on how much alcohol they were administered." But for each blood level of alcohol tested, the mice lacking Fyn kinase took about twice as long to get up as the mice with a functioning Fyn kinase gene, the Japanese team reports.

To try to find out how the tyrosine kinase affects neuronal responses to alcohol, Miyakawa and Yagi then assessed the enzyme's activity in knockout and normal mice both before and after alcohol exposure. They already knew that a biochemical effect of the tyrosine kinase is to add phosphates to a component of the NMDA receptor, a multiprotein complex in the nerve cell membrane that receives a specific chemical signal from adjacent cells.

As expected, Niki's team found that giving ethanol to the knockouts didn't change NMDA receptor phosphorylation in their brains. But in normal animals, the researchers found that within 5 minutes of ethanol administration, phosphorylation of the receptor increased in a brain structure called the hippocampus, then dropped off slowly.

This phosphorylation appears to be correlated with the neurons' recovery from suppression by the alcohol. When Nobufumi Kawai's group at the Jichi Medical School in Minamikawachi-machi monitored the electrical activity of nerve cells from the hippocampus in culture, they found that, at first, alcohol made the nerve cells less responsive and less likely to fire. Soon, however, the effect diminished in mice with Fyn kinase, presumably because the NMDA component became phosphorylated. After about 5 minutes, the cells were just as active as they had been initially, despite continued exposure to ethanol. In contrast, nerve cells from mice lacking Fyn kinase were still only half as responsive as normal after 15 minutes.

Alcohol researchers are impressed with Niki's effort. "There's a nice correlation between the electrophysiology, phosphorylation, and the behavioral effects," notes Harris's Colorado colleague Paula Hoffman. "It's quite convincing." They note that this new role for Fyn kinase also fits well with what Kandel's group first discovered using knockout mice. "Tolerance, like learning, is an adaptation of the brain to an external stimulus," Hoffman adds.

Both Harris and Hoffman caution, however, that the work still leaves many questions unanswered. "I don't think you can explain all of intoxication through the NMDA receptor," agrees neurobiologist David Lovinger, who does alcohol research at Vanderbilt University in Nashville, Tennessee. Even so, these researchers agree that Niki's results do offer another clue to the brain chemistry behind an important societal problem, one that, in life as in *Days of Wine and Roses*, can have all too tragic outcomes.

–Elizabeth Pennisi