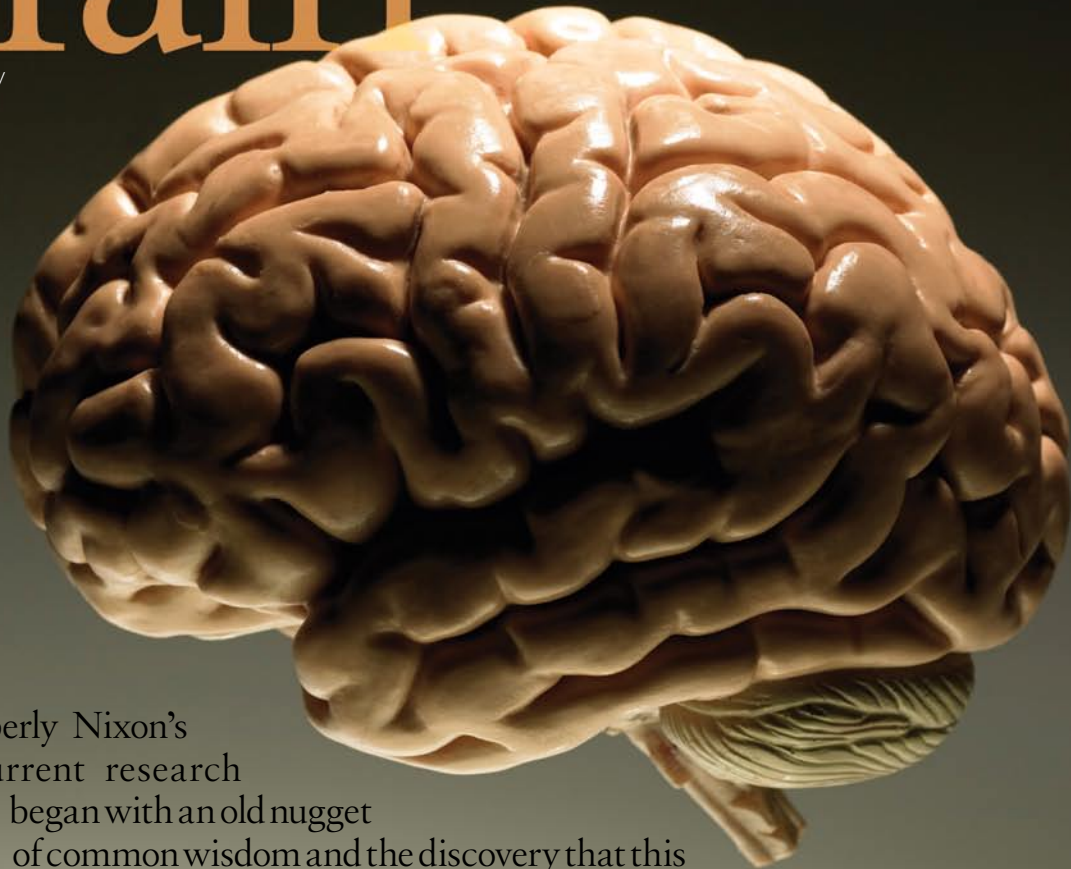


# Bringing Back the Brain

UK researcher working to trigger the birth of new neurons

By Jeff Worley



**W**imberly Nixon's current research began with an old nugget of common wisdom and the discovery that this long-held belief was dead wrong.

“Once upon a time, scientists thought that when longtime alcoholics destroyed brain cells, they were simply gone forever, even if the drinker jumped on the wagon and stayed there,” says Nixon, an assistant professor in the UK Department of Pharmaceutical Sciences. “We’ve known for some time now that when an alcoholic becomes abstinent, some brain mass recovers. What researchers in the field haven’t considered is that new neurons could be born and help in this recovery, which is the focus of our work right now.”

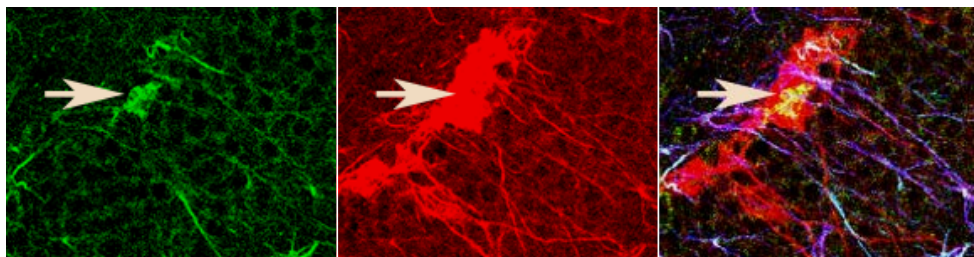
Discovering how new neurons aid in this process would be big news indeed. If they can be induced to form in the alcohol-damaged brain, some regions could be repopulated with healthy cells to stave off significant impairment.

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In this brain work, Nixon is focusing on two types of cells—glia, the supporting cells of the nervous system, and neural stem cells, which are able to become a range of cells found in the nervous system.

“We’re asking some very basic questions about how alcohol affects these different cell types,” states Nixon, whose energy and contagious excitement about her work have been stoked by three major federal grants she’s won in the past two years. “One of the important discoveries we’ve made is that alcohol may trigger interactions between microglial cells and neural stem cells, which only recently were

Through a process called immunohistochemistry, which uses colorized labels or markers to see proteins expressed by cells, Nixon and her team work to identify these cells from the brain of a rat who has not been given alcohol for four days. The arrow in these panels points to the same place in each image and shows that multiple markers overlap one another—a nonspecific stem cell marker called Nestin (green) and a newborn neuron marker called Doublecortin (red). When green and red are expressed in the same place, their colors combine to identify the yellow neural progenitor cell, which is likely in the process of becoming a neuron. This image was taken on one of the new Leica confocal microscopes in UK's Imaging Facility.



found to contribute to brain function. Now we're working to find out exactly how alcohol affects these cells." This project is supported by a five-year, \$1.6 million grant from the National Institute on Alcohol Abuse and Alcoholism.

This is very complex work, because in the regions of the brain that can regenerate—the hippocampus and the subventricular zone—several types of cells besides the glial and stem cells spew out various brain chemicals and growth factors. Trying to catalog and describe the functions of these cells and how these functions interact and lead to neurogenesis, the birth of new neurons, is a daunting task. The hippocampus is primarily involved in learning and memory; the subventricular zone serves as a source for neural stem cells in the front of the brain.

Nixon uses rats as her animal model in this work. Rats were the best choice, she explains, because the animal's brain is remarkably similar to the human brain, and a rat, when intoxicated, behaves very much like we do. "We chose this particular rat model based on previous research that showed clear evidence of brain damage, brain shrinkage, and neurodegeneration that mimics what we see in humans."

In the lab, Nixon and her staff of six—graduate and undergraduate students, a postdoc, and a technician—give rats alcohol through a tube three times a day at eight-hour intervals for four days, a dosage that results in a blood alcohol level of around 0.30—nearly four times the legal driving limit for humans. "This level of intoxication mimics the binge drinking of the true alcoholic," Nixon says. "Bingeing alcoholics are much more likely than other drinkers to have cognitive problems, learning and memory deficits, and physical brain damage."

The rat's behavior is monitored and noted at different dosage levels. "We give an injection of an agent called Bromo-deoxy-Uridine (BrdU), which is commonly used to detect proliferating cells in living tissues, before, during or after alcohol exposure so we can label dividing cells," Nixon explains. "Any cell that is in its DNA synthesis phase of cell division will incorporate BrdU into the DNA."

After a week in abstinence, the rat is sacrificed. Why a week?

"We chose the one-week time point based on evidence from other types of brain damage. The brain, it turns out, has a fairly consistent response to damage—regardless of how that damage occurred. You get a burst in microglia

dividing one to two days after neurons start dying, then you have this reactive increase in neural stem cell proliferation at six to seven days after cell death. This proliferation results in increased newborn neurons four weeks later."

Through a method called immunohistochemistry, Nixon attaches a color to proteins or "markers of interest" in the brain tissue sections that her team studies, and these colors announce themselves brightly through a microscope. "The cool thing about BrdU is that it permanently labels these dividing cells, so we can clearly see how many cells—and which ones—survive or remain," says Nixon, underlining "which ones" with her voice.

The research team is also characterizing these newborn cells to see what various stem cells have "grown up to become," as she puts it. To observe and describe the cells, Nixon uses Leica confocal microscopes, which allow her to optically "section" a fluorescent sample (such as a cell that has been stained with contrasting fluorescent dyes) with excellent resolution. Nixon explains that by collecting a series of such images through the depth of a sample, she is able to assemble a highly accurate three-dimensional reconstruction of the entire sample.

"These state-of-the-art microscopes are indispensable in this work," she adds, adamantly. "You can't lead or be a top-20 university without this kind of facility and equipment."

At this point in the project, Nixon and her lab have shown that the proliferation of neural stem cells leads to a twofold increase in the number of new neurons generated. “The next big questions we’re trying to answer are why and how this reactive neurogenesis occurs,” says Nixon, who came to UK in 2005 from the University of North Carolina. “We hypothesize that it is related to the fact that after two days in abstinence these microglia in the brain can release growth factors that promote neurogenesis. But we’ll see. We’re getting closer to the answers every day.”

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Excessive alcohol consumption is the third leading preventable cause of death in the United States, according to the Centers for Disease Control and Prevention (CDC), and is associated with a number of health problems, including liver cirrhosis, various cancers, unintentional injuries, and violence. In a report five years ago, the CDC estimated the number of deaths attributable to alcohol at nearly 76,000 and that alcoholics lose approximately 30 years of life on average. These sad stats emphasize the importance of adopting effective strategies to reduce excessive drinking.

“One strategy, I guess you could call it, is to let alcoholics know that if they stop drinking and remain abstinent, there’s a good chance—50-50 at least—that their brain will fully recover, that their ability to think and reason will come back,” says Nixon.

From the beginning of her alcohol-related work here, Nixon has been “buoyed up,” she says, by the wealth of expertise of so many UK colleagues who are proven leaders in the field. “The major thing that brought me here was the other alcohol researchers. I had always worked at major alcohol research centers across the country, so I knew several of the researchers here: Susan Barron and Mark Prendergast in psychology, John Littleton at the Kentucky Tobacco Research and Development Center, and Jim Pauly and Audra Stinchcomb in pharmaceutical sciences. This was an impressive research core, and there was just no doubt that this was a great fit for me.” Nixon was also drawn by the presence of UK’s Spinal Cord and Brain Injury Research Center, where she is a faculty associate.

“Dr. Nixon is an example of the gifted scientists we have been successful in recruiting to Kentucky who are making a difference with their work on a national and international level,” says Kenneth Roberts, dean of the College of Pharmacy. “We take great pride in her exceptional achievements in garnering NIH funding to advance research in treating alcohol abuse and alcoholism.” ■

The recipient of three major federal grants in the past two years, Kimberly Nixon says, “If alcoholics stop drinking and remain abstinent, there’s a good chance—50-50 at least—that their brain will fully recover, that their ability to think and reason will come back.”

