Researchers enhance neuron recovery in rats after blood flow stalls

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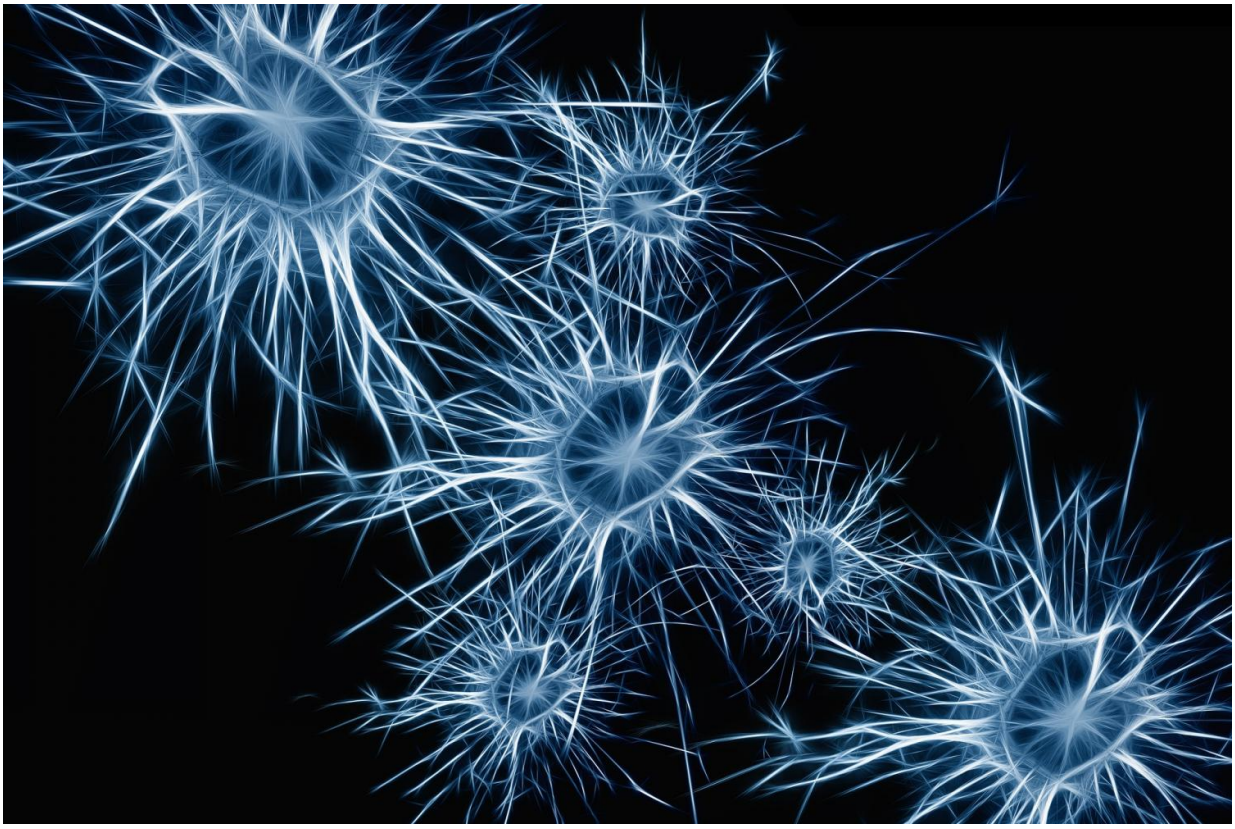

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Researchers at the Stanford University School of Medicine report in a new study that they found a way to help rats recover neurons in the brain's center of learning and memory. They accomplished the feat by

blocking a molecule that controls how efficiently genetic instructions are used to build proteins.

If the approach described in the study can be applied to humans, it may one day help patients who've suffered a stroke, cardiac arrest or major blood loss and are thus at higher risk of memory loss.

In the study, to be published online Aug. 19 in *eNeuro*, researchers induced extremely low blood pressure—as would happen when the heart stops beating—in rats. These rats lost neurons in a specific region of the hippocampus critical to learning and memory, but the researchers improved the animals' recovery of the cells by injecting a molecule that blocks a microRNA: a short molecule that tweaks gene activation by preventing the conversion of genetic blueprints into proteins. Interestingly, the scientists found that a microRNA blockade potentially causes astrocytes—cells that support neurons and make up 50% of the cells in the brain—to turn into neurons.

The findings demonstrate that neurons, with some assistance from their astrocyte neighbors, recover in a region of the hippocampus not known to have a local stem cell population that can replenish lost neurons. Enhancing this recovery in humans could help those who've suffered a temporary loss of blood flow to the brain.

"There's currently no treatment to improve brain function in patients with heavy blood loss, cardiac arrest or stroke," said Creed Stary, MD, Ph.D., assistant professor of anesthesiology, perioperative and pain medicine. "This is the first study to show that the natural process of post-injury hippocampal recovery can be substantially improved with a pharmaceutical microRNA-based therapy."

Stary is the study's senior author. Lead authorship is shared by postdoctoral scholar Brian Griffiths, Ph.D., and senior research scientist

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Under (low blood) pressure

When fresh blood stops flowing through the brain, cellular waste piles up, and neurons starved of oxygen and glucose eventually die. This can occur when a person has a stroke, loses a significant amount of blood or suffers a cardiac arrest.

Amid the damage, levels of a microRNA known as miR-181a soar. In an earlier study, the researchers blocked miR-181a with a molecule designed to stick to and inactivate the microRNA. They found that blocking miR-181a before reducing the flow of blood to the brains of rats stopped neurons from dying.

"If you want to find a therapy for an injury, one approach is to look for disruptions that occur in cells and try to reverse them. The first step was asking, 'Is reversing the increase in this specific microRNA protective?'" Stary said.

But while the prior findings were encouraging, they didn't reflect how such an intervention would probably be used in a clinical setting; it's more likely that a patient would receive a microRNA blockade after an injury.

To test whether miR-181a blockade helped rats recover hippocampal neurons, the researchers decreased the rats' blood pressure dramatically by siphoning off much of their blood and reinfusing it 10 minutes later. Similar drops in blood pressure can occur in people during cardiac arrest, after a major loss of blood or during certain surgeries.

The blood pressure drop caused nearly 95% of neurons in a region of the hippocampus known as CA1 to die off. By around two months after the

procedure, those neurons bounced back to nearly 50% of normal levels.

The researchers then tested the effects of a microRNA blockade by injecting the blocking molecule directly into the hippocampus of rats either two hours or seven days after the animals experienced a drop in blood pressure. These rats had significantly higher neuronal recovery than those injected with a control molecule that didn't target any known microRNAs. In earlier studies, the researchers showed they could deliver the blockade intravenously, making it well-suited for clinical use.

Solving a puzzle

But the fact that there was any recovery was puzzling. The hippocampus is one of the few brain regions that harbors neural stem cells, which can form new neurons in adults, but not in the CA1 region the researchers were studying.

"If you don't have new neural stem cells and you don't have any evidence of cell division, then how are CA1 neurons being repopulated?" Stary said.

The researchers had one important clue: When CA1 neurons were at their nadir, specialized neuronal support cells known as astrocytes moved into the damaged region. Typically, astrocytes sit above and below the neuron-containing layer of the CA1 and support the metabolism and connectivity of their neuronal neighbors.

To figure out what the astrocytes were up to, the scientists tracked them with fluorescent molecules that labelled astrocytes green and neurons red. When they looked under the microscope, they found cells that glowed yellow—meaning the cells expressed both green and red markers. These yellow cells were found at higher levels in rats in which miR181a had been blocked.

The observation strongly implied that some of the astrocytes were beginning to turn into neurons. While the researchers are planning further experiments to confirm the finding, astrocytes have been shown to turn into neurons in other animal models of brain injury. Whether this phenomenon occurs in humans after loss of blood flow to the brain has not yet been fully established, but if verified, it could open a new realm of astrocyte-based gene therapies for survivors of cardiac arrest and stroke.

The researchers next plan to verify whether blocking miRNA-181a helps the rats recover their memory, learning and other cognitive abilities linked to the hippocampus. If so, the approach is one step closer to aiding recovery from brain injuries in which blood flow gets cut off.

"This paper shows that you can effectively augment the normal recovery the brain tries to do on its own by blocking this specific microRNA across injury models and across species, something of a holy grail for a gene therapy," Stary said. "It points toward blocking the microRNA being a protective agent itself, but also provides insight to identify new therapeutic gene targets, opening the possibility for combinatorial or adjuvant pharmaceutical therapies."

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