Scientists use gene therapy to improve memory and learning in animals

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Stanford University neuroscientists have designed a gene that enhances memory and learning ability in animals under stress. Writing in the Nov. 8 issue of the *Journal of Neuroscience*, the Stanford team says that the experimental technique might one day lead to new forms of gene therapy that can reduce the severe neurological side effects of steroids, which are prescribed to millions of patients with arthritis, asthma and other illnesses.

"Steroids can mess up the part of the brain involved in judgment and cognition," said neuroendocrinologist Robert Sapolsky, co-author of the study. "In extreme cases it's called steroid dementia. Ideally, if you could deliver this gene safely, it would protect the person from some of these cognitive side effects, while allowing the steroid to do whatever helpful thing it should be doing elsewhere in the body."

Sapolsky, the John A. and Cynthia Fry Gunn Professor of Biological Sciences and a professor of neurology and neurological sciences at Stanford, has conducted numerous experiments on the damaging physiological effects of stress and has written extensively on the subject, including a 1995 book, "Why Zebras Don't Get Ulcers."

Hormonal effects

In the Journal of Neuroscience study, Sapolsky and his colleagues focused on the effect of stress on the hippocampus, a part of the brain

that's important for learning and memory. Nerve cells throughout the hippocampus contain numerous receptors that respond to a group of steroid hormone called glucocorticoids, which are secreted from the adrenal glands in male and female rats during times of stress. When high levels of these corticoids bind to the hippocampal receptors, they can trigger a destructive biochemical cascade that damages nerve cells in the hippocampus and ultimately impairs memory and learning.

But not all hormones are bad. Estrogen, the primary female sex hormone, enhances memory and can therefore block the negative cognitive effects of the corticoids.

"Estrogen protects memory against stress," said former Stanford postdoctoral fellow Andrea Nicholas, lead author of the study, who was recently named an adjunct professor at St. Mary's College. "In women, there are long-term protective effects of estrogen in the brain. As people age, females often fare better than males cognitively, in part because they have that estrogenic protection."

In a 2004 study, Sapolsky and his co-workers showed that gene therapy could be used to neutralize the deleterious effects of stress in laboratory rats. The idea behind gene therapy is eventually to cure a disease or repair an injury by injecting a beneficial gene into the patient's DNA. For the experiment, Sapolsky and his team created what geneticists call a chimera--an experimental strand of DNA made with two genes stitched together, in this case a glucocorticoid-receptor gene from a rat combined with an estrogen-receptor gene from a human.

When this new chimeric gene was injected into the hippocampus of a rat, the result was dramatic. The gene produced new protein receptors that quickly converted stress-inducing glucocorticoids into beneficial estrogen signals.

"That experiment showed that gene therapy works at the molecular level," Nicholas said. "We then wanted to see if the chimeric gene would actually alter the behavioral effects that we know stress hormones cause in live rats."

Water maze

To find out, Nicholas and her colleagues set up a Morris Water Maze experiment, a procedure widely used by neuroscientists to test spatial memory in rats. The maze consists of a round pool about 5 feet wide and filled with about 2 feet of water. A hidden platform is placed just below the surface. When an untrained rat is released into the pool, it swims around looking for an exit, until it finally discovers the platform and climbs out of the water.

"When the animals first go in, they're pretty clueless," Nicholas said. "It usually takes them about a minute to locate the platform, but over time, they get very efficient at finding it. Once they learn where it is, they'll swim directly to it in about 5-10 seconds. Then we remove the platform from the water and watch what they do."

A key part of the water maze procedure involves counting the number of times a rat swims across the spot where the platform was originally located. "It's a measure of their persistence," Nicholas explained. "If they know it really well, they're going to keep going over and over the platform area, as if saying to themselves, 'I know it's got to be here.'"

Stress tests

The goal of the study was to see if rats treated with gene therapy would perform differently than normal rats during the water maze tests following exposure to stress. To administer gene therapy, the researchers

anesthetized the rodent, inserted a syringe into its hippocampus and injected a genetically engineered virus with DNA containing the chimeric gene.

Once injected, individual copies of the virus penetrate the hippocampal neurons, thereby delivering the chimeric gene and activating it in the rat's brain. The new gene then transforms harmful corticoids into helpful estrogens--a process that should hypothetically block the animal's negative behavioral response to stress.

To make sure that natural estrogen wasn't a factor, the experiment was restricted to male rats only. Every rat was trained to find the hidden platform. To raise corticoid levels in the animal's bloodstream, the rats were subjected to a variety of stresses, such as immobilization or cold temperature, then released into the water, where observers counted how quickly and how often they swam to the area above the missing platform.

Stress tests were conducted before the animal received training, immediately after training and 24 hours later. "This taps into three different domains and three different timings--the effects of stress on learning, on storing learned information as memory and on retrieving that memory," Sapolsky explained. The results were clear: When stress was applied 24 hours after training, the rats infected with the chimeric gene swam to the area of the missing platform faster, and spent significantly more time looking for it, than the normal rats did.

"These results are pretty fantastic, " Nicholas said. "They suggest that this gene therapy not only blocks the deleterious effects of glucocorticoids but actually enhances spatial memory and learning through estrogen-controlled events, even in the presence of stress. Seeing this enhancement effect was pretty exciting. It's the best we could have hoped for."

Gene therapy

These findings also demonstrate the potential value of gene therapy for people who suffer severe cognitive side effects from taking large doses of corticoids to treat multiple sclerosis, rheumatoid arthritis and other diseases, Sapolsky said.

"Potentially it could be used to protect the brain when you're taking tons of this stuff for some disease," he explained. "People who take high doses of these steroids can also get clinically depressed. In principle, you could use gene therapy to protect them as well."

However, this type of gene therapy will not be medically available until scientists figure out a way to safely deliver the chimeric gene to humans, Sapolsky said. He also noted that the treatment should be used to prevent severe neurological side effects caused by medication and should not be given to those who simply want to enhance their short-term memory and learning skills. "You can't drill into people's heads and inject a virus just because somebody has a big exam coming up, " he said.

Source: Stanford University