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Researchers Discover Gene that Controls Learned Fear

Researchers have discovered the first genetic component of a biochemical pathway in the brain that governs the indelible imprinting of fear-related experiences in memory.

The gene identified by researchers at the Howard Hughes Medical Institute at Columbia University encodes a protein that inhibits the action of the fear-learning circuitry in the brain. Understanding how this protein quells fear may lead to the design of new drugs to treat depression, panic and generalized anxiety disorders.

"These findings reveal a biological basis for what had only been previously inferred from psychological studies that instinctive fear, chronic anxiety, is different from acquired fear."

— **Eric R. Kandel**

The findings were reported in the December 13, 2002 issue of the journal *Cell*, by a research team that included Howard Hughes Medical Institute (HHMI) investigators Eric Kandel at Columbia University and Catherine Dulac at Harvard University. Lead author of the paper was Gleb Shumyatsky, a postdoctoral fellow in Kandel's laboratory at Columbia University. Other members of the research team are at the National Institutes of Health and Harvard Medical School.

According to Kandel, earlier studies indicated that a specific signaling pathway controls fear-related learning, which takes place in a region of the brain called the amygdala. "Given these preliminary analyses, we wanted to take a more systematic approach to obtain a genetic perspective on learned fear," said Kandel.

One of the keys to doing these genetic analyses, Kandel said, was the development of a technique for isolating and comparing the genes of individual cells, which was developed at Columbia by Dulac with HHMI investigator Richard Axel.

Shumyatsky applied that technique, called differential screening of single-cell cDNA libraries, to mouse cells to compare the genetic activity of cells from a region of the amygdala called the lateral nucleus, with cells from another region of the brain that is not known to be involved in learned fear. The comparison revealed two candidate genes for fear-related learning that are highly expressed in the amygdala.

The researchers decided to focus further study on one of the genes, *Grp*, which encodes a short protein called gastrin-releasing peptide (GRP), because they found that this protein has an unusual distribution in the brain and is known to serve as a neurotransmitter. Shumyatsky's analysis revealed that the *Grp* gene was highly enriched in the lateral nucleus, and in other regions of the brain that feed auditory inputs into the amygdala.

"Given finding that this gene was active not only in the lateral nucleus but also in a number of regions that projected into the lateral nucleus was interesting because it suggested that a whole circuit was involved," said Kandel. Shumyatsky next showed that GRP is expressed by excitatory principal neurons and that its receptor, GRPR, is expressed by inhibitory interneurons. The researchers then undertook collaborative studies with co-author Vadim Bolshakov at Harvard Medical School to characterize cells in the amygdala that expressed receptors for GRP. Those studies in mouse brain slices revealed that GRP acts in the amygdala by exciting a population of inhibitory interneurons in the lateral nucleus that provide feedback and inhibit the principal neurons.

The researchers next explored whether eliminating GRP's activity could affect the ability to learn fear by studying a strain of knockout mice that lacked the receptor for GRP in the brain.

In behavioral experiments, they first trained both the knockout mice and normal mice to associate an initially neutral tone with a subsequent unpleasant electric shock. As a result of the training, the mouse learns that the neutral tone now predicts danger. After the training, the researchers compared the degree to which the two strains of mice showed fear when exposed to the same tone alone — by measuring the duration of a characteristic freezing response that the animals exhibit when fearful.

"When we compared the mouse strains, we saw a powerful enhancement of learned fear in the knockout mice," said Kandel. Also, he said, the knockout mice showed an enhancement in the learning-related cellular process known as long-term potentiation.

"It is interesting that we saw no other disturbances in these mice," he said. "They showed no increased pain sensitivity; nor did they exhibit increased instinctive fear in other behavioral studies. So, their defect seemed to be quite specific for the learned aspect of fear," he said. Tests of instinctive fear included comparing how both normal and knockout mice behaved in mazes that exposed them to anxiety-provoking environments such as open or lighted areas.

"These findings reveal a biological basis for what had only been previously inferred from psychological studies — that instinctive fear, chronic anxiety, is different from acquired fear," said Kandel.

In additional behavioral studies, the researchers found that the normal and knockout mice did not differ in spatial learning abilities involving the hippocampus, but not the amygdala, thus genetically demonstrating that these two anatomical structures are different in their function.

According to Kandel, further understanding of the fear-learning pathway could have important implications for treating anxiety disorders. "Since GRP acts to dampen fear, it might be possible in principle to develop drugs that activate the peptide, representing a completely new approach to treating anxiety," he said. However, he emphasized, the discovery of the action of the *Grp* gene is only the beginning of a long research effort to reveal the other genes in the fear-learning pathway.

More broadly, said Kandel, the fear-learning pathway might provide an invaluable animal model for a range of mental illnesses. "Although one would ultimately like to develop mouse models for various mental illnesses such as schizophrenia and depression, this is very hard to do because we know very little about the biological foundations of most forms of mental illness," he said. "However, we do know something about the neuroanatomical substrates of anxiety states, including both chronic fear and acute fear. We know they are centered in the amygdala.

"And while I don't want to overstate the case, in studies of fear learning we could well have an excellent beginning for animal models of a severe mental illness. We already knew quite a lot about the neural pathways in the brain that are involved in fear learning. And now, we have a way to understand the genetic and biochemical mechanisms underlying those pathways."