

NOVEMBER 18, 2005

Loss of Fear Factor Makes Timid Mouse Bold

Researchers have identified a fear factor - a protein the brain uses to generate one of the most powerful emotions in humans and animals. The molecule is essential for triggering both the innate fears that animals are born with - such as the shadow of an approaching predator - as well as fears that arise later in life due to individual experiences. Eliminating the gene that encodes this factor makes a fearful mouse courageous. The finding, the researchers say, suggests new approaches for drugs designed to treat conditions such as phobias, post-traumatic stress disorder, and anxiety.

Working in mice, the scientists, led by Howard Hughes Medical Institute investigator Eric R. Kandel at Columbia University, found that the protein stathmin is critical for both innate and learned fear. Mice without stathmin boldly explore environments where normal mice would be hesitant, and, unlike their normal counterparts, fail to develop a fear of cues that have been associated with electric shock. The scientists also found physiological changes in the brains of mice lacking stathmin that correlate to the behavioral changes they observed.

The work, published in the November 18, 2005 issue of the journal *Cell*, was carried out by lead author Gleb Shumyatsky, a postdoctoral fellow from Kandel's lab who is now at Rutgers University, and other scientists from Columbia, Rutgers, Harvard Medical School, and Albert Einstein College of Medicine.

"It was localized not only in the pathway of the learning process, but also in the pathway of instinctive fear."

- Eric R. Kandel

Both humans and animals are born with an innate fear of certain threatening stimuli. As an example, Kandel said, "If you see a train heading right at you, you get scared and run away. This is built into the genome - the capability to respond to natural threat." Furthermore, when researchers pair a naturally

frightening stimulus, such as an electric shock, with a neutral signal, such as a tone, animals develop fear of the neutral tone. “That is called learned fear - that's acquired, it's a form of learning,” Kandel explained. In humans, stage fright, phobias, and post traumatic stress disorders are examples of learned fear.

In previous work, Kandel and his colleagues set out to determine the underlying mechanisms that encode fear in the brain. “We knew from other people's work about the neural pathways involved,” Kandel said, “but there was little knowledge of the key genes or the detailed neural circuitry involved. So we thought we would tackle that problem.”

The researchers began their studies by searching for genes that were particularly active in the amygdala, a region deep within the brain known to contribute to fear and other emotions. They zeroed in on the lateral nucleus, the portion of the amygdala that receives information from the rest of the body about fearful stimuli. They dissected out individual pyramidal cells, the principal cells in the lateral nucleus, and found two genes, known as gastrin-releasing peptide (GRP) and stathmin, that were much more active in the lateral nucleus than in a part of the brain not thought to be involved in fear, which the researchers analyzed for comparison.

Several years ago, Kandel, Shumyatsky, and their colleagues studied the first of these genes, GRP, in detail and found that it encodes a protein that inhibits the fear-learning circuitry in the brain. GRP does not, however, play a role in innate fear—demonstrating that the two fear pathways are genetically distinct.

When the scientists moved on to study stathmin, they had few clues as to what role it might play in fear - if it was involved at all. “When you go after a gene like this, you have no idea what behavior or biological process it may be involved in,” Kandel said. “I think it's the mystery of the thing that creates part of the excitement. Except for thinking that the amygdala was very likely to be involved, we had no way of knowing what the outcome would be.”

An indication that stathmin might contribute to fear came when they mapped the parts of the brain where the gene was most active. They found that stathmin was highly expressed not only in the amygdala, but also in other parts of the brain's fear circuitry. “It was localized not only in the pathway of the learning process, but also in the pathway of instinctive fear,” Kandel noted.

To investigate stathmin's role in more detail, the researchers created mice lacking that gene, and examined the brain activity in the lateral nucleus of their amygdalas. Recent work from other labs had shown that during fear learning, the connections between the neurons in this part of the brain strengthen. In stathmin-deficient mice, however, the connections between these neurons remained virtually unchanged, despite repeated stimulation.

These results were good indications that stathmin might play a role in learned fear. To determine whether a lack of stathmin actually altered animals' behavior in situations likely to trigger fear, the scientists used several standard laboratory tests. Mice were trained to associate an electric shock with either an auditory tone or a particular location in a cage. After the training period, normal mice would freeze when they encountered the tone or location that they'd learned was likely to accompany a shock. Stathmin-deficient mice, on the other hand, seemed unnerved by those stimuli, carrying on their normal activities boldly, without fear.

From these experiments, it was clear to the scientists that stathmin was needed for fear learning. To find out whether it might also contributed to innate fear, the scientists took advantage of mice's natural fear of open spaces. Unlike normal mice, which cower on the edges of an open field and stay near the center of a plus-shaped maze, mice without stathmin were much more adventurous, readily exploring exposed areas.

The authors concluded from their experiments that stathmin is required for both innate and learned fear. Together with his lab's previous work on GRP, Kandel said, the work advances the understanding of learned fear versus instinctive fear in several ways. "It shows genetically there's a fundamental difference between the two; it gives you some insight into the neural circuitry; it shows that there's an inhibitory constraint to fear; and it gives you the potential of thinking of therapeutic targets."

As drug targets, Kandel said, GRP and stathmin each present unique opportunities. "One would be for learned anxiety, the other would be for instinctive. They both, I think, are reasonable - no one has worked on those as targets before." While drugs targeting stathmin would likely affect both types of fear, Kandel expects that with further work, researchers should also be able to identify genes that act exclusively on instinctive fear.