Scientists describe the delicate balance in the brain that controls fear

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The eerie music in the movie theater swells; the roller coaster crests and begins its descent; something goes bump in the night. Suddenly, you're scared: your heart thumps, your stomach clenches, your throat tightens, your muscles freeze you in place. But fear doesn't come from your heart, your stomach, your throat, or your muscles. Fear begins in your brain, and it is there -- specifically in an almond-shaped structure called the amygdala -- that it is controlled, processed, and let out of the gate to kick off the rest of the fear response.

In this week's issue of the journal *Nature*, a research team led by scientists at the California Institute of Technology has taken an important step toward understanding just how this kickoff occurs by beginning to dissect the neural circuitry of fear. In their paper, these scientists—led by David J. Anderson, the Benzer Professor of Biology at Caltech and a Howard Hughes Medical Institute investigator—describe a microcircuit in the amygdala that controls, or "gates," the outflow of fear from that region of the brain.

The microcircuit in question, Anderson explains, contains two subtypes of neurons that are antagonistic—have opposing functions—and that control the level of fear output from the amygdala by acting like a seesaw.

"Imagine that one end of a seesaw is weighted and normally sits on a garden hose, preventing water—in this analogy, the fear impulse—from flowing through it," says Anderson. "When a signal that triggers a fear

response arrives, it presses down on the opposite end of the seesaw, lifting the first end off the hose and allowing fear, like water, to flow." Once the flow of fear has begun, that impulse can be transmitted to other regions of the brain that control fearful behavior, such as freezing in place.

"Now that we know about this 'seesaw' mechanism," he adds, "it may someday provide a new target for developing more specific drugs for treating fear-based psychiatric illnesses like post-traumatic stress disorder, phobias, or anxiety disorders."

The key to understanding this delicate mechanism, Anderson says, was in uncovering "markers"—genes that would identify and allow for the scientists to discriminate between the different neuronal cell types in the amygdala. Anderson's group, led by postdoctoral fellow Wulf Haubensak, found its marker in a gene that encodes an enzyme known as protein kinase C-delta (PKCδ). PKCδ is expressed in about half the neurons within a subdivision of the amygdala's central nucleus, the part of the amygdala that controls fear output.

Along with fellow postdocs Prabhat Kunwar and Haijiang Cai, Haubensak was able to fluorescently tag neurons in which the protein kinase is expressed; this allowed the researchers to map the connections of these neurons, as well as to monitor and manipulate their electrical activity.

The studies, Anderson says, "revealed that PKCδ+ neurons form one end of a seesaw, by making connections with another population of neurons in the central nucleus that do not express the enzyme, which are called PKCδ− neurons." They also showed that the kinase-positive neurons inhibit outflow from the amygdala—proving that they act as the end of the seesaw that rests on the garden hose.

Still, a key question remained: What happens to the seesaw during exposure to a fear-eliciting signal? Anderson and his colleagues hypothesized that the fear signal would push down on the opposite end of the seesaw from the one formed by the PKCδ+ neurons, removing the crimp from the garden hose and allowing the fear signal to flow. But how to test this idea?

Enter neurophysiologist Andreas Lüthi and his student Stephane Ciocchi, from the Friedrich Miescher Institute in Basel, Switzerland. In work done independently from that of the Anderson lab, Lüthi and Ciocchi had managed to record electrical signals from the amygdala during exposure to fear-inducing stimuli. Interestingly, they had found two types of neurons that responded in opposite ways to the fear-inducing stimulus: one type increased its activity, while the other type decreased its activity. Like Anderson, they had begun to think that these neurons formed a seesaw that controls fear output from the amygdala.

And so the two teams joined forces to determine whether the cells Lüthi had been studying corresponded to the PKCδ+ and PKCδ− cells Anderson's lab had isolated. In what Anderson refers to as a "sophisticated experiment," the two teams performed electrophysiological recordings while simultaneously turning the PKCδ+ neurons on or off using a genetic method developed by Henry Lester, Caltech's Bren Professor of Biology.

The results of the experiment were "gratifyingly clear," says Anderson. The cells that decreased their activity in the face of fear-inducing stimuli clearly corresponded to the PKCδ+ neurons Anderson's lab had isolated, while those that increased their activity corresponded to the PKCδ− neurons.

"These results supported the hypothesis that PKCδ+ neurons were indeed at the opposite end of the seesaw from the one that the fear signal

'presses down' on, consistent with the finding that PKCδ+ neurons crimp the 'fear hose,'" says Anderson.

The marriage of molecular biology and electrophysiology created by the collaboration between Anderson's and Lüthi's laboratories has revealed properties of the fear circuit that could not have been discovered in any other way, Anderson says. "The functional geography of the brain is organized like that of the world," he notes. "It's divided into continents, countries, states, towns and cities, neighborhoods and houses; the houses are analogous to the different types of neurons. Previously, it had only been possible to dissect the amygdala at the level of different towns, or of neighborhoods at best. Now, using these new genetic techniques, we are finally down to the level of the houses."

And that, he adds, is what will make it possible for us to fully understand the networks of communication that exist between neurons within a subdivision of the brain, as well as between subdivisions and different areas. "While these studies shed light on only a small part of the picture, they are an important step in that direction," Anderson says.

More information: *Nature* paper: "Genetic dissection of an amygdala microcircuit that gates conditioned fear"

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