NEUROBIOLOGY

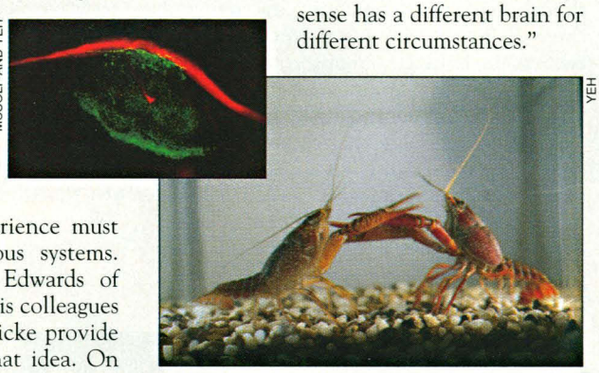
Social Status Sculpts Activity Of Crayfish Neurons

Like rival gunslingers in the Old West, two male crayfish living in the same territory are compelled to fight it out to determine who's the boss. First they circle and size each other up. Then their well-choreographed skirmish escalates into violent combat as they try to tear each other limb from limb. The winner of that clash becomes dominant, strutting his stuff confidently through the territory, while the loser skulks about, trying to stay out of his rival's way.

Once those social lines have been drawn, the behavior of the two crayfish is so different that researchers suspected that the animals' experience must somehow change their nervous systems. Now, neurobiologist Donald Edwards of Georgia State University and his colleagues Shih-Rung Yeh and Russell Fricke provide the first direct evidence for that idea. On page 366, they report that they have found a neuron in crayfish whose response to the neurotransmitter serotonin differs dramatically depending on the animal's social status. In dominant animals, serotonin makes the neuron more likely to fire, while in subordinate animals serotonin suppresses firing.

The finding's implications go beyond crayfish. For example, it complements work from Stanford University neurobiologist Russ Fernald and his colleagues, who showed several years ago that a change to dominant social status alters the brains of male cichlid fish, causing the enlargement of neurons that release hormones that stimulate the sexual organs. But the Edwards group's finding is "the first time that one has been able to link a social phenomenon to a change in a particular identified synapse," says neuroscientist Allen Selverston of the University of California, San Diego. And that is something researchers have suspected might occur throughout higher animals, but have never seen. "Even though you are seeing something that in a sense you always knew had to be there," says Brandeis University neuroscientist Eve Marder, "it is incredibly powerful to actually see it."

What's more, the activity change that the Edwards team has linked to social status takes place in a very well-studied neural circuit—a set of nerve cells that controls the escape reflex called the tail-flip. As a result, the team is in an ideal position to unravel the molecular and cellular events by which the change occurs, as well as to ask how changes in this neuron and others combine to alter the animal's behavior. "There are going to be a constellation of [nervous system] changes that go along with this change in social status, each of which will endow the animal with different new abilities and serve it in some situation," says Fernald. "One way to think about it is that the animal in some sense has a different brain for different circumstances."



MUSOLF AND YEH

YEH

Face off. Crayfish in combat. (Inset) Serotonin-releasing neurons *(green)* are located near the lateral giant neuron *(red)*.

The present work grew out of a set of findings made 15 years ago by Harvard University neuroscientist Edward Kravitz and his then-student Margaret Livingstone. They found that serotonin injections caused lobsters and crayfish to assume the aggressive postures characteristic of dominant animals. That finding spurred researchers in several labs to study serotonin's effects on behaviors like the tail-flip reflex, which the animals use in both fighting and escaping.

In 1985, one of those researchers, Russell Fricke, then an assistant professor at Emory University, got a perplexing result. When he injected serotonin into young crayfish, it inhibited the tail-flip reflex in some animals, while enhancing it in others. As he tried to make sense of this, Fricke realized that the crayfish in which the reflex was enhanced had either been raised alone or were the biggest in their cage. That suggested the animals' social status might have been influencing his results.

Before going further in the work, however, Fricke left Emory to become a physician, and his observation "lay there for a long time," says Edwards. Then, in 1994, Yeh, a graduate student with Edwards, decided to pursue it. Yeh paired crayfish in cages, allowing them to fight and settle who was dominant. He then dissected the animals, and, in a culture dish, tested the effect of serotonin on the lateral giant neuron, which triggers the tail-flip reflex. He found that serotonin enhanced the excitability of the giant neurons from dominant animals, while it suppressed the activity of the neurons from subordinate animals.

Edwards and Yeh wondered what might be happening inside the lateral giant neuron to switch its response so dramatically. One possibility was that there was some sort of change in the receptors through which serotonin exerts its effects. There are three known types of serotonin receptors in crustaceans, which can exert different effects even within an individual cell.

The researchers' hunch proved correct. By using different compounds that activate different classes of serotonin receptors, Yeh and Edwards found that activation of one type of serotonin receptor makes the lateral giant neuron less excitable, while a second receptor type boosts its excitability. Neurons from subordinate and dominant crayfish respond differently to serotonin, says Edwards, because the subordinates seem to have "more, or more effective," receptors of the former type, while in dominants, the latter type prevails.

That state of affairs isn't permanent, however. Just as social status can change in the course of an animal's lifetime, the responsiveness of the lateral giant neuron can change as well. When Yeh placed two previously subordinate crayfish together, one would become dominant. Two weeks later when he tested the dominant animals' lateral giant neurons, the neurons' responsiveness was enhanced rather than inhibited by serotonin.

Dominant animals, in contrast, let go of their dominant physiology much more slowly, perhaps because of the advantages that dominant animals enjoy in access to food and mates. When Yeh paired dominant crayfish with each other, forcing one of each pair to become subordinate, the new subordinates continued to be truculent, provoking fights and getting themselves killed by their rivals at an unusually high rate. When he tested their lateral giant neurons more than a month after the new pairing, serotonin still enhanced their firing, as if "the animals are reluctant to go from being dominant to being subordinate," Edwards says. Fernald's group sees similar results with their male cichlid fish. Dominant males, even when forced to become subordinate, are very slow to give up their dominant physiology.

One question that's still unanswered is how the change in the lateral giant neuron might help explain the aggressive behavior of dominant males. Harvard's Kravitz offers a possible explanation. In lobsters, his group has found that the lateral giant neuron not only triggers the tail-flip reflex, but also activates other neurons that squirt out a burst of serotonin. And serotonin is the substance

Kravitz and Livingstone had linked to aggressive behavior in lobsters and crayfish 15 years ago.

Edwards's group doesn't know yet whether the neuron also triggers a serotonin burst in crayfish, but they suspect that it might. If it does, then a "feed-forward loop" would operate in dominant animals, says Kravitz, in which triggering the lateral giant neuron would cause a burst of serotonin, which would make the neuron even more likely to be triggered again, causing more serotonin to be released. All that serotonin would pump up the animals' aggressive behavior. In contrast, subordinate crayfish are better served by not acting truculent and inviting a fight they are likely to lose, so it is adaptive for them to put the brakes on that cycle, which is what happens when the burst of serotonin caused by the lateral giant's firing makes the neuron less likely to fire again and trigger the release of more serotonin.

Despite the appeal of that explanation, Kravitz and others point out that the lateral giant neuron alone is unlikely to be the full explanation for the behavioral changes. "You can't say this particular [neuron] ... is causally responsible for any behavioral changes," says Brandeis's Marder. "It is probably only a piece of the story."

To fill out the remaining pieces of that story, Edwards and others are eager to learn what other neurons may be influenced by the switch in social status, and whether some of those neurons show a change in serotonin receptors, or in receptors for other molecules. One target for study is the neurotransmitter octopamine, which Kravitz's group has shown to have the opposite effect to serotonin, producing submissive rather than aggressive behavior.

In addition, the researchers plan to take a closer look at the lateral giant neuron itself, focusing on the specific pathways through which social position gets translated into cellular and molecular changes in the neuron. With all these possibilities for future work, says Kravitz, the Edwards group has opened "a potentially incredibly exciting area of investigation."

–Marcia Barinaga

DEVELOPMENTAL BIOLOGY

Choreographing the Bacterial Cell Cycle

A bacterial cell's life cycle resembles a carefully choreographed dance, with particular movements, such as cell division or the development of particular cell structures, occurring only after previous steps are completed. But while scientists reason that this dance must depend on a few master choreographers—molecules that trigger several steps, ensuring that they proceed in proper sequence—researchers have been unable to find them. Now, however, biologists have come upon a gene that may coordinate several developmental events with the progress of the cell cycle in the aquatic bacterium *Caulobacter crescentus*.

In the 12 January issue of *Cell*, developmental biologists Lucy Shapiro and Kim Quon of Stanford University and Gregory Marczynski of McGill University describe the identification of *ctrA*, a gene whose product regulates not only the formation of a propellerlike flagellum but several other critical events in the *Caulobacter* life cycle, including DNA replication.

CtrA, the protein encoded by the gene, is part of a system of sensor enzymes and so-called "response regulator" proteins that are thought to respond to external and internal events in these cells. "This is the first demonstration of a direct role for a response regulator in global cell cycle events" in any bacterium, says Shapiro. Researchers in molecular biologist Austin Newton's lab at Princeton University reported last year that a response regulator called DivK influences a late cell division event in the *Caulobacter* cell cycle, but Shapiro's group is the first to document a mechanism for how such a protein might perform its integrative functions.

Other scientists are impressed. "Here the protein is being used to drive things the cell does all day long," says Richard Losick, a developmental biologist at Harvard University, and it unveils an important part of the cycle's "underlying pathway." And once molecules that activate *ctrA* have been identified, says Bert Ely, a bacterial geneticist at the University of South Carolina, "we can then ask how general this mechanism is. Does it work for other kinds of bacteria? Does it apply in eukaryotic cells [those with distinct nuclei] as well?"

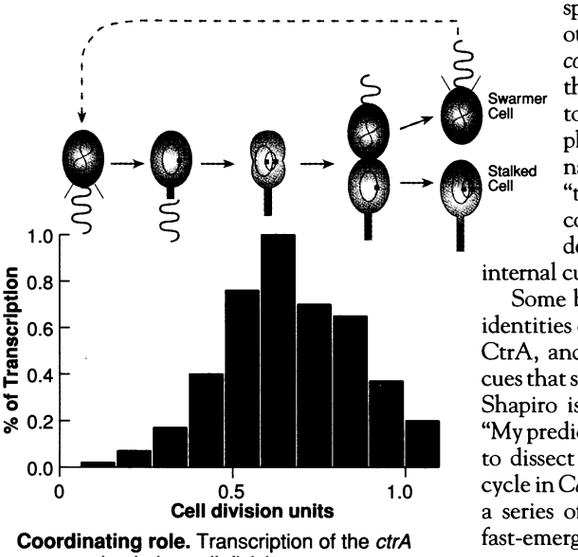
Shapiro's group found *ctrA* while studying *Caulobacter*'s flagellum, which forms at a crucial point in the bacterium's life cycle. Only after its assembly at one pole of a cell does the cell divide in two. The propeller-driven progeny cell then swims off in search of a new home in which to settle down and reproduce, while the other progeny cell stays in place and produces more swarmer cells. Although Shapiro's group has spent years studying mutations affecting flagellar assembly in *Caulobacter*, "we could never find the top of the hierarchy, the gene that turns the thing on," she says. Quon reasoned that this top-level gene might have been so difficult to find because it has some additional essential function. In that case, mutating the gene might kill off the bacteria. So 2 years ago the group began to look for heat-sensitive mutations that might be just slightly perturbed by altered temperatures, upsetting transcription of genes affecting flagellar assembly without killing the cell.

The strategy succeeded. The group found one mutation, in *ctrA*, that not only caused overproduction of FliQ—a protein required for the initial steps of flagellar synthesis—but also prevented cell division. And CtrA, the protein product of normal *ctrA*, binds to a specific DNA sequence in one of the regions that control *fliQ* gene transcription. That same sequence is present in regulatory regions for other genes, including one that contributes to the control of the initiation of DNA replication at different times in both new progeny cells.

The researchers then found that CtrA's amino acid sequence resembles that of response regulator proteins found in other bacteria, including *Escherichia coli*, and that it is readied for action in the same way as these other regulators: through the addition of a phosphate group by so-called "sensor kinases." The work helps confirm that "these two-component systems are controlling essential cell-cycle and developmental events in response to internal cues," says Newton.

Some big unknowns remain, such as the identities of the enzymes that phosphorylate CtrA, and the exact nature of the cellular cues that send these enzymes into action. But Shapiro isn't daunted by these challenges. "My prediction is that we are going to be able to dissect the complete control of the cell cycle in *Caulobacter*, and that it's going to use a series of these response regulators. It's a fast-emerging field."

–Wade Roush



SOURCE: L. SHAPIRO

Coordinating role. Transcription of the *ctrA* gene peaks during cell division.