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An Essential Regulator of Body Weight Revealed

Scientists are one step closer to unraveling the complex mechanisms in the brain that regulate body weight. Working with mice—whose appetites are controlled by systems very similar to those in humans - they have identified a specific type of neuron that is essential for feeding behavior. Without these neurons, adult mice stop eating and undergo rapid weight loss.

Remarkably, the researchers found that absence of these neurons only influenced eating behavior when they were removed from adult mice. If the neurons were eliminated in newborn mice, their developing brains found a way to compensate for the deficiency, and the mice grew up eating normally. The research, conducted by Serge Luquet at the University of Washington in the laboratory of Howard Hughes Medical Institute investigator Richard D. Palmiter, will be published in the October 28, 2005 issue of the journal *Science*.

The task of sorting out the body's diverse and sometimes conflicting signals about hunger and satiety falls to a small cluster of about 5,000 cells in a region of the brain known as the arcuate nucleus. Hormones such as insulin, leptin, and ghrelin deliver information to the arcuate nucleus about whether the body has sufficient calories and nutrients. The brain, in turn, uses this information to decide whether to eat or expend energy.

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- Richard D. Palmiter

Two types of neurons that recognize and respond to these signals are found in the arcuate nucleus. The first of these, known as POMC (pro-opiomelanocortin) neurons, send signals to other parts of the brain to reduce appetite. Mice with defects in POMC neurons eat excessively and become obese.

The other neurons that make up the arcuate nucleus are NPY/AgRP neurons, named for two proteins they produce, neuropeptide Y (NPY) and agouti-related protein (AgRP). Researchers have long suspected NPY/AgRP neurons to be important regulators of feeding behavior, acting in opposition to POMC neurons to stimulate appetite. But genetic experiments designed to tease out exactly how NPY/AgRP neurons triggered feeding had failed to show any direct relationship between eating behavior and the molecules scientists thought NPY/AgRP neurons might use to regulate it.

“So I think it's fair to say the POMC neurons gained in stature during the last 10 years as being really important to feeding; meanwhile, the importance of the NPY pathway diminished. To some extent, the field began to think these cells were supporting players, but not really important,” said Palmiter.

Now, however, Palmiter's lab has taken a different approach, and demonstrated definitively that NPY/AgRP neurons are essential for regulating eating behavior and the regulation of body weight. “We've put these neurons back on the map as being very, very important for feeding,” he said.

Scientists first became interested in NPY/AgRP neurons in the early 1980s, when they found that one of the proteins they produce, NPY, triggers voracious eating behavior when injected into the brains of rats. High levels of NPY in the brain of both hungry and obese mice provided further evidence that NPY was likely to stimulate feeding.

Ten years ago, Palmiter's lab created mice that lacked the NPY protein, expecting that they would eat less than normal mice. “We thought they might chronically eat less than normal mice, and hence be lean - maybe even starve,” Palmiter said. But in fact, the feeding behavior of mice without NPY seemed no different than that of their normal counterparts. Something else, they decided, must be mediating NPY/AgRP neurons' ability to stimulate appetite.

“We were delighted,” Palmiter said, “when, a few years later, agouti-related protein was discovered.” That protein, which is found only in NPY/AgRP neurons, blocked appetite-suppressing signals from POMC neurons - suggesting it could stimulate appetite through a mechanism unique from that of NPY. It seemed to be the molecule they'd been searching for. “We thought, this is great: NPY is gone, AgRP takes over - how simple could it be?” Palmiter said. But when scientists at Merck created mice that lacked AgRP, and then mice that lacked both NPY and AgRP, they too had normal weight and fed normally.

Speculation continued as to what molecule or molecules might be responsible for triggering feeding behavior. But, says Palmiter, “we realized this could go on forever. So we thought, let's just make sure the neurons are important.” To determine whether NPY/AgRP merited further study, they decided to

eliminate them entirely in mice.

To do this, the team took advantage of the fact that, unlike humans, mice are not susceptible to the toxin produced by diphtheria bacteria. They generated genetically modified mice whose NPY/AgRP neurons produced the molecule needed to recognize and take up that toxin. Then, by administering diphtheria toxin to these animals, the scientists could destroy NPY/AgRP neurons without causing other harm.

Once they'd eliminated those neurons, the scientists monitored precisely how much the animals ate by keeping them in a cage equipped with a device that measures each time a mouse licks the bottle containing its liquid diet. The effects of eliminating NPY/AgRP neurons in adult mice were dramatic. After treatment with diphtheria toxin, the animals began to eat less and less; by the fifth day, they had stopped eating entirely. Within 6-8 days, they had lost 20 percent of their body weight.

Interestingly, the researchers found that if they administered the toxin when the animals were less than eight days old, while NPY/AgRP neurons were still developing and forming connections with neighboring cells, the mice ate normally and maintained a normal body weight. Their findings indicated that if NPY/AgRP neurons are eliminated before they become fully functional, then animals somehow compensated, maintaining feeding behavior through some other mechanism. The compensatory mechanism persists, as animals treated with diphtheria toxin early in life and then again as adults continued to eat normally.

“Killing these neurons before they're functionally engaged gives the developing mouse brain a way to compensate,” Palmiter said. “But it's still a tall challenge. You're asking some other neuron, presumably, to either increase what it normally does, or to do something that it never did before. How does that happen?”

While the current study focused on feeding behavior in mice, Palmiter said, “everybody in the field believes that NPY/AgRP neurons and POMC neurons are undoubtedly doing the same thing in humans as they do in rodents. So I would predict that if you could do the experiment in humans, this result would be the same, because the circuits are the same.” And he speculates that mutations in human genes that affect the survival of these neurons or their ability to respond to hormonal signals could alter eating behavior and body weight regulation.

Now that his lab has demonstrated that NPY/AgRP neurons are critical for feeding in adults, Palmiter said the next step will be to investigate what makes them so important. He and his colleagues have already begun experiments to test specific molecules that might contribute to their essential role.