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## Mapping the Brain's Food-Intake Circuitry

Researchers have used a genetically altered virus to map the neural inputs that project into regions of the brain that control food intake. According to the scientists, these mapping experiments, which were done in mice, represent an exciting step in understanding the neural circuitry that executes decisions about whether or not to eat.

Howard Hughes Medical Institute investigator Jeffrey M. Friedman and colleagues at The Rockefeller University, Princeton University and the University of California, San Diego (UCSD), used pseudorabies virus to create an elaborate biological tracer that only propagates itself in neurons that express the leptin receptor or neuropeptide Y (NPY), an appetite-stimulating substance found in neurons. The virus, which travels upstream from the site of infection, jumping from neuron to neuron, was engineered to carry a gene for green fluorescent protein. The presence of the fluorescent protein enabled the scientists to trace the path of the virus as it moved through the brain. "The results indicated that a number of factors, including the blood levels of leptin as well as inputs from emotional and higher centers of the brain, contribute to the decision about whether or not to eat," said Friedman.

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The researchers published their findings in the March 30, 2001, issue of the journal *Science*. Lead author of the research article is Jeff DeFalco in Friedman's laboratory, and co-authors include Lynn Enquist and Mark Tomishima at Princeton and Jamey D. Marth, an HHMI investigator at UCSD.

Leptin, which was discovered by Friedman and his colleagues in 1994, is produced by fat tissue and secreted into the bloodstream, where it travels to the brain and other tissues, causing fat loss and decreased appetite. In the brain, leptin affects food intake by acting on distinct classes of neurons in the hypothalamus that express the leptin receptor. However, said Friedman, mapping how the higher centers of the brain affect these neurons is crucial to understanding appetite and food intake.

"It's obvious that the decision of whether or not to eat has some conscious input," he said. "For example, there is higher cortical input involved in making the decision about whether or not we're going to skip a meal, try to diet or eat less." If the brain mechanisms behind such decisions were better understood, he said, we might be in a position to better understand the behavioral bases of food intake.

"While our study is only a beginning and doesn't address such behavioral issues, it's pretty clear that people differ in how much willpower they have," he said. "And willpower is not a metaphysical thing; it's a bunch of neural connections and neural circuits. And so, it's not inconceivable to me that individuals who have greater conscious ability to consume less food might have slightly different neural circuitry or more powerful neural connections that might ultimately be visualized through mapping studies.

"So, now we need to learn how this neural system is organized. And then we can begin to think about what is different about this system in obesity versus leanness; and how the higher circuitry interacts with the circuitry that responds to basic physiological drives."

In mapping the feeding circuitry, Friedman, DeFalco and their colleagues drew on earlier studies by Enquist and other scientists who had used the Bartha strain of pseudorabies virus (PRV) to trace neural circuits. The Bartha strain of PRV can travel "upstream" in neural circuits and it can propagate across neural junctions, called synapses.

However, the scientists wanted to develop a viral tracing system that would specifically label only those hypothalamic neurons expressing the leptin receptor, or those producing neuropeptide Y, an appetite-stimulating peptide found in abundance in certain types of neurons. The scientists found that they could achieve such specificity by building an "off" switch into the virus that was controlled by a protein named Cre. In the engineered virus, Cre is required for PRV to begin replicating. They then targeted Cre to neurons that express either the leptin receptor or NPY. "Once the virus infected these—and only these—cells, the presence of Cre triggers viral replication," said Friedman.

The scientists made certain that they could trace, or follow, the virus by including a green fluorescent protein that would act as a beacon in PRV-infected neurons. "Once the virus is turned on, it's turned on forever. We traced it backwards to find out which nerve cells send signals to the cells that receive leptin signals," said Friedman. Thus, when the scientists examined slices of mouse brain treated with the virus, they could see which

regions of the brain send neurons into the brain's areas known to regulate feeding behavior. "We could see inputs from a number of other regions to the hypothalamus, which is where basic drives for feeding are controlled," he said. "We could see inputs from brain centers that control emotion and from others that receive olfactory inputs. We also saw inputs from centers in the mouse that are the equivalent of centers that control higher cortical or cognitive functions in humans."

"It was not completely unexpected that we would find connections from centers in the brain, such as the amygdala that deals with emotion, that would have an impact on feeding centers in the hypothalamus," said DeFalco. "But the viral tracer also revealed indirect projections—sites that project to sites like the amygdala—which in turn project to the leptin-receptor-expressing neurons in the hypothalamus. That's where the real power of this technique lies."

While these findings suggest how the system is wired, said Friedman, they are still indirect. "The connections we see suggest that there are inputs and that there will be modulatory effects on feeding from these higher brain regions," he said. "But now we need to understand more about what type of cells these are, what molecules they make and how those molecules might influence the activation state of these neurons that also receive leptin signals."

DeFalco, Friedman and their colleagues are beginning studies using other PRV strains that can follow the connections downward from the higher levels, as well as combinations of viruses engineered with different markers to trace multiple pathways simultaneously. They also plan to explore the hierarchy of the circuitry by using advanced microscopy and computer systems to generate three-dimensional reconstructions of the labeled cells.