

for both hunger and satiety. It also sets the body's circadian clock, and receives sensory data: sights, smells and tastes. Visceral cues from our stomach and intestines, and reward and motivational triggers from elsewhere in the brain, also converge here. "It's a tangle of circuits that looks like a Jackson Pollock painting," says Lowell.

A picture of the anatomical configuration of this complex web of neurons is of little value, however, if the function of a particular neuron, and where it fits in the wiring diagram, is unknown. In the 1980s, there were few ways of doing mechanistic studies in the brain other than making cuts. But in 1989, the development of knockout mice and related *Cre/lox* conditional knockout technologies made it possible to understand the function of a neuron. In the past few years, optogenetics and chemogenetics — which use light or designer drugs to switch off individual neurons — have made it feasible to map the neural networks that control eating.

UNDERSTANDING APPETITE

To tease apart the hunger circuits, researchers initially focused on a tiny section of the hypothalamus called the arcuate nucleus. "It's an amazing structure," explains Lowell. "It exerts powerful control over hunger." The region contains two key neurons: AgRP neurons, which sense a fasted state and stimulate appetite, and POMC neurons that inhibit appetite. Together, these neurons monitor the nutritional state of the body and whether it has a surplus or deficit of calories by sensing chemicals in the blood and signals from upstream neurons. But Lowell isn't alone in being fascinated by the power of neurons to govern appetite.

The AgRP neurons were also being studied by Richard Palmiter, a neurobiologist at the University of Washington in Seattle. Palmiter was drawn to the neural circuitry controlling eating when his student made a mouse lacking the gene needed to produce neuropeptide Y (NPY), one of the appetite stimulators produced by AgRP neurons. When NPY is injected into rodent brains, the animals eat voraciously. Palmiter expected mice without NPY to ignore food and lose weight, yet the NPY knockout mice fed normally and followed a normal growth curve¹. Researchers at Merck Research Laboratories in Rahway, New Jersey, found the same thing when they knocked out both NPY and another appetite stimulator, Agouti-related peptide (AgRP), also produced by AgRP neurons².

Were AgRP neurons unimportant in appetite regulation? Or did they secrete so many appetite-stimulating neurotransmitters that knocking them out one at a time had no effect? To find out, Palmiter destroyed the AgRP neurons entirely. Over six days, the mice ate less and lost 20% of their body weight. "We were shocked," says Palmiter. "These neurons were vital. If we got rid of them the animals would starve to death." It seems the AgRP neurons blocked a neural pathway that otherwise suppressed appetite and mediated anorexia.

NEUROSCIENCE

Dissecting appetite

A slew of new technologies are helping to map the neural circuits that control when, and how much, we eat.

BY BIJAL P. TRIVEDI

Many people think that overeating results from flagging willpower. But feeding behaviour is actually under intense biological control. Bradford Lowell, a neuroscientist now at Beth Israel Deaconess Medical Center in Boston, Massachusetts, realized this in the late 1970s when, as an undergraduate at the University of Massachusetts, he made tiny nicks in the brains of rats to see which

regions might be responsible for the obesity observed in earlier brain lesion studies. As the animals began to overeat and became massively obese, Lowell "was amazed that such tiny lesions could have such an enormous effect."

Lowell had cut neurons near the paraventricular nucleus of the hypothalamus — the nerve centre of feeding behaviour. The hypothalamus comprises at least a dozen different nuclei (clusters of many different types of neuron), and receives and emits a variety of signals

Apart from NPY and AgRP, what other appetite-promoting signals did these neurons produce? Palmiter had a hunch that the missing piece of the puzzle was GABA, which the AgRP neurons also secrete. He destroyed the AgRP neurons again, but this time he also stimulated GABA receptors in the parabrachial nucleus — a relay station that receives sensory inputs, including those from the tongue and gut, and sends signals to other brain regions. After 10 days of GABA signalling, the mice began to eat again, even without the AgRP neurons. Mammals seemed to have evolved a mechanism to prevent starvation when AgRP neurons were destroyed³.

CIRCUITS AND SWITCHES

To control feeding, the brain needs to constantly monitor the body's nutritional status and energy needs — and the neurons in the ARC initiate this behaviour. Scott Sternson, a neuroscientist at the Howard Hughes Medical Institute's Janelia Farm campus in Ashburn, Virginia, has been studying the AgRP and POMC neurons, which promote and suppress eating, respectively. Using optogenetics, he showed that activating only 800 AgRP neurons gave the mice a voracious appetite. "What fascinates me," says Sternson, "is that we can activate this tiny group of AgRP neurons and trigger very complex behaviour — not just chewing and swallowing, but obsessive searching and other complex behaviours to get food."

Controlling feeding behaviour is like using the accelerator in a car, explains Sternson. The more AgRP neurons are excited, the stronger the desire to eat. That probably suggests that as we get hungry, more nutritional signals circulate in the blood and activate increasing numbers of AgRP neurons.

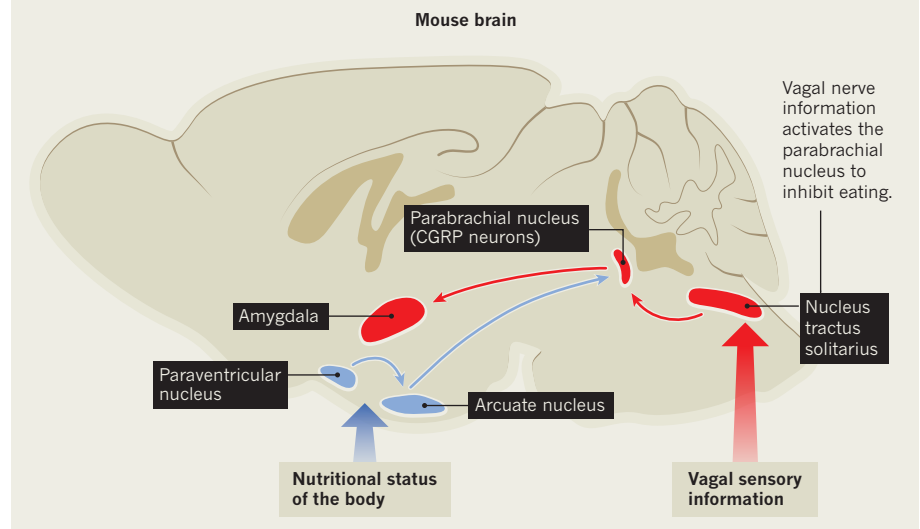
Sternson's team activated POMC neurons at the beginning of the dark cycle when mice typically feed, but this didn't initially affect food intake. Over the next 24 hours, however, the mice ate dramatically less food and lost 7% of their body weight. This suggests that AgRP and POMC neurons together regulate feeding behaviour and body weight over longer timescales, from hours to days⁴.

Lowell used chemogenetics to investigate the reverse scenario — activating AgRP neurons during the day, when mice do not normally feed. These mice searched for food as if they were starving⁵. When neurons were activated twice a day, the mice gained weight; when the neurons were no longer activated, the mice stopped overeating and returned to their normal weight. "The AgRP neurons greatly increase the reward value of food — causing the seeking and consumption of food," says Lowell.

But neurons do not work in isolation. The next step was to identify which neurons triggered the AgRP neurons. By using a rabies virus expressing a reporter gene that makes active upstream neurons glow green, Lowell traced the AgRP neural circuit back to the hypothalamic paraventricular nucleus⁶ — the region associated with satiety that Lowell had cut decades earlier.

THE HUNGER CIRCUITS

The neural circuits and chemicals that make us seek out food and eat, and those that stop us, are being charted in the brains of mice. Neuroscientists expect there will be analogous circuits in the human brain that could potentially be manipulated with drugs to treat obesity or loss of appetite.



Lowell showed that activating a tiny subset of neurons in this part of the hypothalamus — those that express the proteins TRH and PACAP — would activate the AgRP neurons and drive well-fed mice to eat voraciously. Conversely, if these neurons were turned off, starving mice would barely eat.

From his experiments with AgRP, NPY and GABA, Palmiter knew that without signals from the AgRP neurons, the parabrachial nucleus would inhibit feeding. But he didn't know which neurons were responsible for the loss of appetite. He thought that without input from AgRP neurons, calcitonin gene-related protein (CGRP) neurons in the parabrachial nucleus would become overactive, leading to starvation.

To test this hypothesis, Palmiter created mice with CGRP neurons that responded to blue light, and illuminated these neurons with blue laser light from optical fibres in the skull. Turning on the laser activated the CGRP neurons — mice that should have been hungry after sleeping most of the day lost all interest in food. It was as if they got a signal that said "I'm not hungry after all," says Palmiter. "Flicking off the laser made the mice hungry again." When these neurons were repeatedly stimulated at 12-hour intervals for several days, the mice ate less and lost weight, indicating that stimulating CGRP neurons is sufficient to cause starvation⁷.

To map neurons downstream of the CGRP neurons, Palmiter used a fluorescent protein to light up the axons. This revealed that they projected into the amygdala — a brain structure involved in memory and emotion. Palmiter says this could explain why we lose our appetites when we are nauseous or in pain — when eating might be harmful — or when we have an emotional experience or suffer food poisoning.

All this research — figuring out the appetite

circuits and signalling molecules — offers tantalizing clues that it may be possible to design drugs to tackle eating disorders. For example, oxytocin neurons (OXT) in the hypothalamus are a downstream target of the AgRP neurons. The loss of these OXT neurons is seen in Prader-Willi syndrome, a rare genetic disorder characterized by poor growth, intellectual impairment and a chronic, insatiable appetite that often leads to obesity. But stimulating both AgRP neurons and OXT neurons at the same time blocks AgRP-driven feeding, suggesting that the AgRP–OXT neural circuit might be a good mouse model for studying Prader-Willi⁸. Sternson adds that molecules that can regulate AgRP, OXT or POMC neurons would be promising candidates for treating overeating and anorexia.

Intervening too early in one of these pathways to inhibit eating and promote weight loss, or to combat loss of appetite, could have undesirable side effects, however. So finding the last neural link that inhibits or promotes eating is vital. But the neural maps confirm that eating is hardwired and tightly governed by biochemical and physiological mechanisms — a sign that it should be possible to design targeted obesity and anorexia drugs. ■

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1. Erickson, J. C., Clegg, K. E. & Palmiter, R. D. *Nature* **381**, 415–418 (1996).
2. Ollmann, M. M. *et al. Science* **278**, 135–138 (1997).
3. Wu, Q., Boyle, M. P. & Palmiter, R. D. *Cell* **137**, 1225–1234 (2009).
4. Aponte, Y., Atasoy D. & Sternson, S. M. *Nature Neurosci.* **14**, 351–355 (2011).
5. Krashes, M. J. *et al. J. Clin. Invest.* **121**, 1424–1428 (2011).
6. Krashes, M. J. *et al. Nature* **507**, 238–242 (2014).
7. Carter, M. E. *et al. Nature* **503**, 111–114 (2013).
8. Atasoy, D. *et al. Nature* **488**, 172–177 (2012).