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RESEARCH NEWS

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The Brain Is Broadly Wired for Reproduction

Howard Hughes Medical Institute researchers have discovered a vast network of neurons in the brain of mice that governs reproduction and controls the effects of reproductive status on other brain functions.

In their studies, the researchers found neural circuits that coordinate a complex interplay between neurons that control reproduction and brain areas that carry the neural signals triggered by odorant molecules and those triggered by pheromones, chemical signals produced by animals. The researchers characterize their findings as an initial step in understanding the far-reaching influence that odors and pheromones may have on reproduction and other behaviors.

The research team, which was led by HHMI investigator Linda B. Buck at the Fred Hutchinson Cancer Research Center, included first author Ulrich Boehm and Zhihua Zou, who did the work as postdoctoral fellows while in Buck's lab. The researchers published their studies in an immediate early publication on November 10, 2005, in the journal *Cell*. Related studies by HHMI investigator Catherine Dulac are published in the same issue.

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- Linda B. Buck

The scientists began their studies by focusing on tracing the neural pathways leading to and from neurons that produce gonadotropin releasing hormone (GnRH), which is also known as luteinizing hormone releasing hormone (LHRH). These neurons regulate sexual physiology—including onset of puberty, ovulation, and the menstrual cycle in females and testosterone production in males—by regulating the release of hormones from the pituitary gland. Interestingly, GnRH neurons also appear to be involved in the control of sexual behaviors.

“Consistent with the idea that GnRH neurons might have additional functions beyond controlling the pituitary, other investigators have shown that GnRH axons can be found in many different areas of the brain,” said Buck. “Those findings suggested that GnRH neurons were sending signals to other neurons, but the neurons that received the signals were unknown. Even more importantly for us, though certain brain areas had been implicated in pheromone signaling, specific neurons that transmit pheromone signals to GnRH neurons had not been identified.”

To map the neural circuits involving GnRH neurons, the researchers used a genetic tracing method that they previously developed for charting neural pathways. They first engineered mice in which GnRH neurons produce barley lectin (BL), a tracer molecule that travels upstream and downstream to connected neurons, and green fluorescent protein (GFP), to mark the producing cells. By visualizing the locations of BL, GFP, and GnRH neurons and their axons, the researchers were able to identify neurons directly connected to GnRH neurons. They also determined which neurons sent signals to GnRH neurons and which neurons received signals from GnRH neurons.

These studies revealed that connections go in both directions between GnRH neurons and relay stations in the brain that process signals from both the olfactory and vomeronasal systems, said Buck.

In mice and other mammals, the olfactory and vomeronasal systems are distinct pathways for sensing chemicals in the environment. While the main olfactory system that begins in the nose processes odors, the vomeronasal (accessory) system receives signals—triggered by pheromones—from the vomeronasal organ (VNO) in the nasal septum. However, the systems are not entirely parallel. Buck and her colleagues have shown that the VNO can detect some odorants. And conversely, there is evidence that some pheromone signals require input from the nose in addition to the VNO.

“Our findings suggest that both odor and pheromone relay areas in the brain are sending pheromone signals to GnRH neurons. Moreover, GnRH neurons, in turn, are sending information back to those relay areas,” said Buck. “This surprising finding suggests that the GnRH neurons are influencing the processing of odor and pheromone signals in the brain. It may be the brain's way of saying whether or not it wants to receive particular sensory information—depending on the reproductive circumstances, such as the stage of the female's estrus cycle.”

The researchers also sought to determine whether pheromones could trigger olfactory pathways to activate GnRH neurons. To investigate this, they exposed male and female mice, respectively, to female or male sex-related pheromones and measured how neurons that are connected to GnRH neurons reacted. They also exposed the males to clean bedding, which was thought to be a neutral stimulus.

They found that pheromones triggered responses in neurons upstream of GnRH neurons in both odor and pheromone relay areas. “This suggests that there is a redundancy in pheromone detection, with at least some pheromone information being conveyed by both the main and accessory systems,” said Buck. “This redundancy is not too surprising, if you consider how important it is to the animal to be able to sense pheromones. The redundancy might guard against the loss of a pheromone receptor from either the VNO or the olfactory epithelium causing a devastating loss of pheromone detection.”

The studies showed that the odor of clean bedding also activated some neurons upstream of GnRH neurons. “This suggests that the animal's environment could also influence GnRH neurons, perhaps signaling whether the animal is in the optimal environment for mating.” Buck said.

In tracing the connections between GnRH neurons and neurons throughout the brain, Boehm and his colleagues soon found that they had undertaken an enormous task to figure out these connections. “We were really shocked with what we found,” Buck said. “We found that, although the GnRH neurons number only about eight hundred in mice, they connect directly with about fifty thousand other neurons. And these neurons are in brain areas involved in a wide array of functions—for example, appetite, feeding, reward, arousal, and the relay of information to higher brain areas that control cognitive function. I don't think anyone ever suspected this complexity. It reveals that GnRH neurons are master integrators of information about the external environment, as well as the internal state of the animal.”

The studies also suggest that GnRH neurons influence a wide array of brain functions, possibly coordinating those functions with neuroendocrine status in order to optimize reproductive success, according to Buck.

Almost all the GnRH neuron-connected areas were identical in male and female mouse brains. However, there were some telltale sex differences in the circuitry, which offer important new pathways for investigating differences in male and female reproductive physiology and behavior, Buck said.

Although it is still early, the researchers suspect that the findings in mice could have implications for humans. “Because humans don't have a vomeronasal system, many have speculated that they may not detect pheromones,” Buck said. “But these studies clearly indicate that the main olfactory system, which humans do have, is capable of transmitting pheromone signals. Therefore, if there are human pheromones—although no one has yet identified one—they would presumably transmit their signals through the main olfactory pathway.”

Although the findings are considered a first step in exploring the GnRH reproduction-related circuitry in the brain, Buck acknowledges that they have already learned a great deal just by defining the circuits. “These findings now set the stage for studies in which the neurons in those circuits can be analyzed to determine the genes they selectively express. Then those genes can be used—for example in gene knockout studies—to determine what role the neurons play in reproduction and behavior,” Buck said.

“Understanding how the brain's neural circuitry controls behavior has been largely a black box,” she said. “I think that through studies like these we and others are going to make substantial progress in understanding the neural circuits that underlie behavior.”