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Without Dopamine, Neurons Continue to Fire Normally

Researchers are learning whether normal neuron behavior depends on the ability to produce an essential neurotransmitter. Recent studies in living mice indicate that dopamine-producing neurons are capable of triggering nerve impulses even when they are deprived of dopamine.

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— **Richard D. Palmiter**

According to the study's senior author, Howard Hughes Medical Institute researcher Richard Palmiter, at the University of Washington, Seattle, these kinds of basic questions are important to ask because dopamine-producing neurons are affected in a number of disorders, including Parkinson's disease, attention deficit hyperactivity disorder, schizophrenia, and Tourette's syndrome. Their activity is also implicated in most forms of drug abuse.

The results of the experiments, performed by Siobhan Robinson in Palmiter's lab, are published in the September 7, 2004, issue of the *Proceedings of the National Academy of Sciences*.

Dopamine is a neurotransmitter, a specialized chemical messenger that plays a specific role in the brain. When neurons release neurotransmitters in bursts, they trigger nerve impulses in neighboring neurons. The finding that dopamine-deprived neurons continue to fire normally, including in bursts, even in the absence of dopamine suggests that neuronal inputs from other neurons play a major role in influencing the firing pattern of dopamine neurons.

“Among the unanswered questions in neurobiology is whether a neuron is controlled by some sort of feedback mechanism that regulates how often it fires,” Palmiter said. “We were asking a relatively simple question: Can a neuron fire properly in the absence of its own neurotransmitter?”

To address that question, Palmiter and his colleagues turned to a mouse that they had genetically engineered to lack an enzyme known as tyrosine hydroxylase, which converts the amino acid tyrosine to L-DOPA, which is then converted into dopamine. “Past experiments had involved making dopamine-deficient mice by killing the neurons, but obviously you can't study the properties of a neuron once it is dead,” said Palmiter. “In our mouse, though, the neurons seem perfectly healthy, but they are in essence ‘firing blanks.’ They don't have any dopamine in their synaptic vesicles that can be released to activate other neurons.”

Mice lacking dopamine manifest symptoms of a severe form of Parkinson's disease - they move very little and fail to eat adequately, said Palmiter. However, when the animals are injected with L-DOPA, thereby bypassing the need for the enzyme that was missing in their bodies, the animals behave normally for a few hours until the dopamine produced in their brains is degraded.

The scientists recorded the behavior of the neurons in awake, behaving animals, which meant that the experimental conditions were more natural than some previous experiments, which used anesthetized mice. When Robinson measured the activity of the neurons after the mice had received L-DOPA, she was surprised to find that even though this treatment stimulated the animals to eat and move about, their dopaminergic neurons were substantially inhibited. “We realized this was part of the feedback system that regulates these neurons. Lacking dopamine, the neurons had become hypersensitive to the neurotransmitter. So, when dopamine is restored, they become overly excited and respond by inhibiting the dopaminergic neurons, to achieve a normal level of reactivity,” said Palmiter. Thus, these dopamine neurons fire normally in the absence of dopamine but are sensitive to feedback inhibition when dopamine signaling is restored.

Two possible feedback systems might be regulating the dopaminergic neurons. The “short-loop” pathway involves feedback control by receptors on the dopamine neurons themselves. The “long-loop” pathway involves dopamine-responsive brain circuitry. According to Palmiter, both pathways likely contribute to the feedback control, but the long-loop pathway is likely to be more critical.

An important observation emerged when the researchers subjected the dopamine-deficient mice to anesthesia. They found that the firing rate of the dopamine neurons is greatly reduced in the dopamine-depleted animals, whereas is relatively unaffected in control mice. These findings underscore the importance of measuring the activity of dopamine neurons in awake animals, Palmiter said. Furthermore, they help explain why earlier studies with anesthetized mice led to the erroneous conclusion that the dopamine

neurons were inactive in the absence of dopamine.

According to Palmiter, further studies will aim to discover the neural inputs that regulate the activity of dopamine neurons. At the moment, the simplest idea is that dopamine-producing neurons receive lots of inputs from other circuitry in the brain, and those circuits may be unaffected by the absence of dopamine," said Palmiter. "Those neurons don't know that there isn't any dopamine, and they may continue to influence the firing pattern of the dopaminergic neurons."