Molecular movements of neural transporters unveiled

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A team of scientists from Columbia University College of Physicians and Surgeons and Weill Cornell Medical College has shed light on the molecular workings of transporter proteins, molecular machines embedded in the cell membranes of neurons that modulate the transfer of signals between cells and recycle neurotransmitters.

The research, published today in the journal *Nature*, reveals with unprecedented detail how the molecule performs its task, says one of the senior authors, Dr. Jonathan Javitch, the Lieber Professor of Experimental Therapeutics in Psychiatry and professor of pharmacology in the Center for Molecular Recognition at Columbia University Medical Center. "This level of understanding may ultimately lead to improved treatments for psychiatric disorders and increase our understanding of how drugs such as cocaine work."

In the brain, one neuron communicates to another by releasing chemicals called neurotransmitters into the gap between them, called the synapse. To stop the signal, specialized transporters must remove the released neurotransmitter from the synapse by pumping it back into the releasing cell. In the treatment of some diseases it is beneficial, however, to allow the neurotransmitters to build up in the synapses. Antidepressants make this possible by interfering with particular transporters, as do stimulant drugs like cocaine and amphetamines.

Neuronal cells have different transporters. One family of transporters, known as neurotransmitter/sodium symporters (NSSs) are specialized for

the uptake of certain neurotransmitters, including dopamine, noradrenaline and serotonin. They are named symporters because the transport process requires energy to concentrate neurotransmitter inside neurons -- the energy required is provided by the imbalance of sodium ions across the cell membrane. Thus, sodium ions flow down their concentration gradient into the neurons through the NSSs, thereby allowing neurotransmitters to move back into the cell where their concentration is higher than outside. But until now, exactly how these transporters function has been a mystery.

"The transporters themselves are of enormous interest both medically and specifically to the National Institute on Drug Abuse because, fundamentally, they are essential for signaling," says one senior author, Dr. Harel Weinstein, chairman and Maxwell M. Upson Professor of Physiology and Biophysics, and director of the Institute for Computational Biomedicine at Weill Cornell Medical College. "The better we understand neuronal signaling, the better we understand brain function, disease and drug addiction."

To figure out how transporters work, it is first necessary to study their molecular structure, Dr. Weinstein says. Because these membrane proteins are so flexible and prefer lipid-rich surroundings, it is more difficult to obtain their crystal structures than those of soluble proteins or DNA. But in 2005, scientists characterized the structure of a bacterial equivalent of NSSs called the leucine transporter (LeuT). This protein is easier to analyze structurally, as it is available in large quantities and is stable because it is found in heat-loving bacteria that live in extreme environments (proteins have to be very stable and rigid to withstand high temperatures). Although LeuT transports amino acids such as leucine and alanine, rather than neurotransmitters, it closely resembles mammalian NSSs in both structure and function.

But structural analyses alone provide only snapshots of the transporter

molecule. To elucidate the entire molecular sequence of LeuT action, the team performed imaging studies using single-molecule fluorescence resonance energy transfer (smFRET) under the leadership of the other senior author, Dr. Scott Blanchard, associate professor of physiology and biophysics at Weill Cornell Medical College. Unlike traditional biochemical approaches, this method does not simply generate information about the average movements of a collection of proteins. "Applying single-molecule imaging to the transporter gave us a unique view of the dynamics enabling the function of the transporters, because it allowed us to look at individual molecules and watch their movements in real time rather than time and ensemble averages," Dr. Blanchard says.

Last year, the researchers used smFRET to collect the first such single-molecule data for a membrane protein, and these results were also published in Nature. In their most recent experiments, they used the technique to monitor changes in LeuT conformation and dynamics by labeling moving parts of the protein with fluorescent dyes that emit distinct amounts of light when the distance separating them changes. As the transporter protein moves during function, time-dependent changes in distance between the fluorophores could be directly imaged to extract the first quantitative insights into the motions underpinning the transport mechanism.

Using powerful computational simulations, the researchers had predicted such movements through previous studies aimed at understanding how the transported molecule changes the conformation of LeuT. The new experiments demonstrated that alanine binding to LeuT increased the rate of the transporter's flickering between two conformations: facing outward, as if ready to accept substrates from outside the cell ("inward-closed"), and facing inward, as if releasing its contents into the cell ("inward-open"). How the presence of sodium affects the transporter's response to the binding of the transported substrate, alanine, was also

revealed from these experiments: Sodium was essential for the alanine-enhanced dynamics. Surprisingly, alanine did not alter the total amount of time spent in either the open or closed state.

By contrast, the binding of sodium ions alone, without alanine, was found to decrease the transition rate between open and closed states and stabilized the closed state. The antidepressant clomipramine was shown to block the measured effects of alanine and to constrain the transporter in its inward-closed state, thus inhibiting transport. These findings contrast with the traditional view that substrate binding simply changes the conformation from one state to another in a single smooth transition, Dr. Weinstein says. "Unless we understand the dynamics, we can't really understand how the drug molecules work," he explains.

The researchers also report how LeuT utilizes two binding sites on the outward-facing side to enable its function, consistent with their previous findings. Their latest evidence may help to settle a controversy about the number of binding sites in this transporter, Dr. Weinstein says. Thus, they found that the two binding sites must work cooperatively to transport molecules. When either site was mutated, alanine was incapable of causing the transporter to flicker between open and closed states. Therefore, substrate binding to both sites is necessary for altering transporter dynamics and recycling molecules.

"These results may lead to key insights into which binding sites mediate the specific effects of various drugs," says Dr. Javitch. Using computer simulations, the researchers described the molecular events that link substrate binding to changes in transporter conformation. In brief, binding at one site induces structural changes that propagate to the other site, causing the transporter to release its contents into the cell. "We're looking at an unprecedented molecular level at the mechanics of this protein and how the binding of the substrates causes conformational changes," Dr. Javitch says. "We think that our observations have broad

relevance to how other sodium-dependent transport processes work."

The results will likely translate to mammals, including the transporters in human nerve cells, as bacterial and mammalian transporters are nearly identical, Dr. Weinstein says. In the future, the team plans to investigate how drugs induce conformational change in human proteins.

The study's equally contributing lead authors are Dr. Yongfang Zhao of the Center for Molecular Recognition at Columbia University; Daniel Terry, a graduate student in the Blanchard and Weinstein labs, enrolled in the Tri-Institutional Program in Computational Biology and Medicine at Weill Cornell Medical College; and Dr. Lei Shi, assistant professor of physiology and biophysics and of computational biophysics at Weill Cornell Medical College. The study is also co-authored by Dr. Matthias Quick, assistant professor of clinical neurobiology in psychiatry and in the Center for Molecular Recognition at Columbia University Medical Center.

Provided by Weill Cornell Medical College