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Study finds brain cell regulator is volume control, not on/off switch

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UC Davis researchers have discovered that proteins that regulate brain-cell activity by controlling the flow of potassium ions behave more like volume controls on stereos rather than on/off power switches. The research, which appears in the 19 August issue of *Science*, provides a new model for the behavior of critical gatekeeper proteins found in neuronal membranes.

"We've shown that brains cells regulate activity in an incremental way, with thousands of different possible levels of activity," explained James Trimmer, senior author of the paper and professor of medical pharmacology and toxicology at UC Davis School of Medicine. He and his colleagues studied an ion channel that controls neuronal activity called Kv2.1, a type of voltage-gated potassium channel that is found in every neuron of the nervous system.

"Our work showed that this channel can exist in millions of different functional states, giving the cell the ability to dial its activity up or down depending on the what's going on in the external environment," said Trimmer. This regulatory phenomenon is called 'homeostatic plasticity' and it refers, in this case, to the channel protein's ability to change its function in order to maintain optimal electrical activity in the neuron in the face of large changes within the brain or the animal's environment. "It's an elegant feedback system," he added.

For years, scientists have attempted to study how neurons regulate the function of potassium ion channels -- pore-like openings in cell

membranes that control the flow of potassium ions into the cell -- with limited success. The current study is the first to combine mass spectrometry-based proteomics and ion channel biophysics to the study of living brain cells. "This is an important biological question that couldn't have been answered any other way," Trimmer said.

Most cells in the body can get by with on/off-like switches, allowing them grow and proliferate when needed. In fact, examples of these 'switches' include the well-studied products of oncogenes, proteins that get stuck in the 'on' position and cause cancer. Brain cells, however, must multi-task, receiving and processing signals from various sources, both inside and outside the body. "This ability to deal with a variety of signals involves some fairly sophisticated and subtle regulation of neuronal activity," Trimmer said.

Scientists have long known that potassium channels are crucial to the normal workings of brain cells. Neurons respond to stimuli, such as noise from the environment or chemical messengers from different parts of the body, by conducting weak electric currents across their membranes. This is possible because of an unequal distribution of charged ions, or atoms, on either side of the nerve cell membrane. Voltage-gated potassium channels regulate the passing of potassium ions across these membranes in response to changes in electric signal.

Brain cell activity is diminished when potassium channels are open. Closed channels lead to an increase in neuron excitability. Certain kinds of snake venom exploit this mechanism by blocking potassium channels and causing seizures. Likewise, defects in potassium channels have been associated with epilepsy and reduced brain development, as well as neurodegenerative disorders similar to Alzheimer's and Parkinson's diseases.

The type of potassium ion channel examined in the current study, Kv2.1,

has been shown in studies by assistant research scientist Hiroaki Misonou to be highly regulated in response to epileptic seizures, stroke, and anesthesia.

Trimmer and his colleagues are the first to use a mass spectrometry technique called SILAC (stable isotope labeling with amino acids in cell culture) to study ion channels in brain cells. The problem for researchers has been that while mass spectrometry gives incredibly accurate measures of mass, quantifying amounts of a protein in different samples can be difficult. SILAC allows scientists to add additional atomic weight to one sample so that two different samples can be analyzed in a given run, allowing for precise measurements of quantity. The 'mass tag' separates the two samples--the experimental and control--on the mass spectrometry read out.

Using this technique, postdoctoral fellow Kang-Sik Park revealed 16 sites where the protein is modified by the cell by via addition of a phosphate group. Further study--in which each of the sites is removed to reveal its role in modulation-- followed by careful biophysical analyses of channel function by postdoctoral fellow Durga Mohapatra, revealed that seven of these sites were involved in the regulation of neuronal activity. Since each site can be regulated independently on the four channel subunits, the neuron can generate a huge (>1018) number of possible forms of the channel.

Using this mechanism, Kv2.1 channels are quickly modified, even mimicking the activity of other potassium ion channels. "The beauty of doing it with a single protein is that it is already there and can change in a matter of minutes. It would take hours for the cell to produce an entirely different potassium channel," Trimmer explained.

Based on these results, Trimmer and his colleagues hypothesize that parts of the Kv2.1 channel protein interact in ways that make it either

easier or harder for it to change from closed to open. The protein, they believe, can exist in either loose states that require low amounts of energy, or voltage, to change from one state to another or a locked-down state that requires lots of energy (high voltage) to open or close. The number and position of phosphate molecules are what determine the amount of voltage required to open the channel.

The next step will be to determine how brain neurons regulate the addition and removal of phosphates at individual sites on the Kv2.1 protein during normal animal behavior. This involves proteomic analysis of Kv2.1 from different brain regions after stimulation with light, sound and with different learning paradigms. Trimmer and colleagues will also explore the pharmacological modulation of Kv2.1 phosphorylation in therapeutic intervention for neurological and psychiatric disorders.

Source: University of California, Davis