New function of crucial brain protein described

On the Right Track

By Lois Baker

University at Buffalo biophysicists have revealed a new function of a brain protein, called the NMDA receptor, which is crucial for learning and memory. A better understanding of how NMDA receptors work will help neuroscientists learn more about neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's, and may provide novel therapies for stroke and schizophrenia.

The study, which was published in the August 12, 2004 issue of *Nature,* was led by Gabriela Popescu, PhD, research assistant professor of anesthesiology and physiology and biophysics in the UB School of Medicine and Biomedical Sciences.

What Popescu and her colleagues discovered is that the NMDA receptor can function as a "frequency discriminator," translating stimulation frequency into current amplitude and possibly deciding whether the neuron will learn to become more or less receptive to future experiences. They were able to uncover this phenomenon using advanced techniques that allow them to monitor one receptor at a time and by employing advanced computer software developed at UB, to analyze the complex behavior of individual proteins.

"At most synapses, both long-term potentiation (LTP) and long-term depression (LTD) require the activation of NMDA receptors," explains Popescu, who is affiliated with UB's Center for Single Molecule Biophysics. "Unfortunately, these receptors exhibit notoriously complex behaviors and have resisted the solution of their activation mechanism for more than 10 years.

"In our past work we were able to make some headway in understanding the pathway by which these receptors become active. Our current research gives us confidence that we are on the right track."

NMDA receptors, which respond to the neurotransmitter glutamate, have been the focus of intense neuroscience research

Gabriela Popescu, PhD, left, and Anthony Auerbach, PhD.

for good reason, Popescu says. "Ninety percent of all excitatory neuronal signaling is mediated by glutamate, and half of it occurs through NMDA receptors. We need these proteins for the correct wiring of our brains and throughout life to form and retain memories, to learn new skills and behaviors. We cannot live without them."

Underactivity of NMDA receptors may be the cause of schizophrenia, while overly active NMDA receptors kill neurons, causing devastating brain damage following a stroke. NMDA receptors also are involved with pernicious illnesses such as Alzheimer's, Parkinson's and Huntington's, and a better understanding of how these proteins work holds great hope for addressing these diseases.

Of particular importance to the function of these receptors, and often to their malfunction, is the pathway by which these molecules change shape after binding the neurotransmitter glutamate to allow ions to rush into the postsynaptic neuron and send the signal onward. Popescu and colleagues Anthony Auerbach, PhD, professor of physiology and biophysics in the UB Center for Single Molecule Biophysics, along with Antoine Robert, MD, PhD, and James R. Howe, PhD, of the Yale University School of Medicine, report in *Nature* that only about half of the pool of receptors bombarded with glutamate is recruited to respond by each stimulus, while the remaining half stays ready to take "orders" from the next signal.

"The interplay between how fast the receptor decides to open and how fast it loses the bound glutamate may, in fact, be a mechanism to tell the cell about the rate at which signals are coming in," explains Popescu. "The NMDA receptor may therefore be a device that translates the stimulation frequency into current amplitude: a bona-fide frequency discriminator."

Stimulus frequency carries information of particular interest to synaptic plasticity, widely believed to be the cellular basis of memory, says Popescu. "Often, stimuli that arrive at high frequency (100 or more per second) will cause a synapse to become more efficient or potentiated. Conversely, stimuli that arrive at a slower pace (about 10 per second) will cause the same synapse to become less responsive to subsequent stimulation or depressed. Scientists study the phenomena of long-term potentiation (LTP) and long-term depression (LTD) with great hope of understanding how we learn and how we form and recall memories. These processes are impaired in many neurological diseases.

"This work vividly demonstrates the value of learning about mechanisms," she adds. "Once we understand how things work, we often discover novel, unsuspected phenomena, which open new avenues for inquiry that, in turn, could lead to fresh strategies to treat or prevent malfunctions with devastating consequences." BP

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Frederick Sachs, PhD

Tarantula Venom Peptide

Mirror image shows promise as a drug

By Lois Baker

In a paper that was published in the July 8, 2004, issue of *Nature,* University at Buffalo biophysicists have shown that a tarantula venom peptide, GsMTx4, known to affect many organs, can be manipulated to withstand destruction in the stomach, making it a promising candidate for drugs that could treat cardiac arrhythmias, muscular dystrophy and many other conditions.

Moreover, the peptide, which is amphiphilic, i.e., fat-soluble on one side and water-soluble on the other, affects mechanically sensitive ion channels in membranes in a manner totally different than the standard "lock-and-key" binding mechanism.

The peptide is the only agent known to specifically block stretch-sensitive channels. Unlike other membrane channels that are sensitive to electrical potential or the binding of hormones and neurotransmitters, stretch-sensitive channels are activated by changes in membrane tension.

"Stretch-sensitive channels can play a key role in many normal tissue functions," says Tom Suchyna, PhD, research associate in the UB Center for Single Molecule Biophysics and first author on the paper. "These channels are involved in hollow-organ filling such as the bladder, in heart and circulatory-system responses to changes in blood pressure, proprioception—knowing where your limbs and head are in space and time—and fluid balance.

"They also are involved in abnormal tissue functions such as cardiac arrhythmias, congestive heart failure, elevated calcium levels in muscular dystrophy, and angiogenesis-supported tumor growth."

Earlier research by the UB group had shown that the novel peptide inhibits stretch-sensitive channels, but the researchers didn't know how. To gain more information on the peptide's possible receptor, Philip Gottlieb, PhD, a co-investigator from the UB Department of Physiology and Biophysics and the Center for Single Molecule Biophysics, created a mirror image of the molecule, referred to as "right-handed," to observe the peptide-membrane interaction.

Since almost all proteins in nature are "left-handed," right-handed proteins won't fit into a left-handed receptor, even if they have the same amino acid sequence. "It's like putting your right foot into your left shoe," says Suchyna.

In this case, however, they found that both proteins inhibited stretch-sensitive channels. "If the right-handed GsMTx4 works as well as the left-handed, it must be interacting with the stretch-activated channel by changing the tension that the channel senses in the membrane, rather than locking onto the channel," he says.

"This leads us to believe that there is something unique about the mem-

brane that surrounds stretch-sensitive channels, and that this special membrane environment attracts GsMTx4. That would explain why this peptide blocks only this type of channel."

In addition to providing valuable information on how the peptide works, the finding that both versions blocked the channels makes the peptide an attractive drug candidate. "This was an awesome tool to find," says Frederick Sachs, PhD, UB professor of biophysics in the Center for Single Molecule Biophysics and senior author on the study.

"Peptides usually don't make good drug candidates. They can't be given by mouth because the stomach enzymes digest them, and they can cause an immune response. But because this peptide works in its right-handed form, and the normal left-handed digestive enzymes and left-handed antibodies don't recognize it, oral administration is a definite possibility. It may be more than a lead compound for drug development. It may work just as it is.

"If this prognosis proves correct, the peptide could be an effective treatment for atrial fibrillation, incontinence, muscular dystrophy, high blood pressure and other conditions governed by stress-sensitive channels," says Sachs.

Suchyna says the next steps will be to investigate the environment surrounding the channels, to study the role of stretch-activated channels in cardiac arrhythmias and to mutate the peptide to make it specific for different tissues.

Studies of these peptides on a model ion channel called gramicidin, reconstituted in artificial lipid membranes, were carried out by Sonya E. Tape, a graduate student, and Olaf S. Anderson, MD, both from the Weill Medical College of Cornell University, and Roger E. Koeppe II, PhD, from the University of Arkansas. BP

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Fiber-Optic Network Completed

By Ellen Goldbaum

Boosts transmission 1,000-fold between UB and research partners

Approximately 12 miles of new fiber-optic cable has been constructed by UB, enhancing high-speed data links between UB's campuses and its affiliated research institutions, an essential step toward the creation of a life-sciences economy for the region.

The Bioinformatics Network Initiative, as the UB effort is called, increases 1,000-fold the speed with which massive amounts of research data are transmitted between UB and the Buffalo Niagara Medical Campus.

It also allows UB's affiliated research institutions—Hauptman-Woodward Medical Research Institute (HWI), Roswell Park Cancer Institute, UB's Research Institute on Addictions, and the New York State Center of Excellence in Bioinformatics and Life Sciences—to boost by a factor of 1,000 the speed with which they can send data to collaborating scientific teams around the world.

"The lighting of the fiber-optic cable is truly a momentous occasion for UB's New York State Center for Excellence in Bioinformatics and Life Sciences and for our research partners at the Roswell Park Cancer Institute and the Hauptman-Woodward Medical Research Institute," says UB President John B. Simpson. "This latest vital link in our ongoing chain of progress is deeply significant as we continue to move forward with the groundbreaking, collaborative biomedical research that will help to foster a strong life sciences economy in the Buffalo-Niagara region.

"In marking the official activation of this fiber-optic connection, we also celebrate the nexus of innovative research partnerships that link UB with the region's other leading biomedical research institutions, as well as the outstanding network of governmental, community and regional support that has made this progress possible."

At Hauptman-Woodward Medical Research Institute, for example, scientists can now provide results of experiments to investigators around the world in minutes rather than weeks.

"This network will have a huge impact on HWI," says George DeTitta, PhD, executive director of the institute and UB professor of structural biology.

DeTitta explains that HWI works with more than 600 scientific investigators—at institutions around the globe, as well as in Western New York—who use techniques developed at HWI to study the molecular structures of proteins involved in a broad range of diseases.

Each of these investigators routinely sends proteins to HWI, where laboratory staff execute 1,536 crystal growth experiments per protein and then use digital photomicroscopy to follow the results of these experiments.

Typically, each protein produces more than 12,000 photomicrographs that have to be transmitted back to the investigators.

"We used to have to wait a month to accumulate all the results, put the images on a CD and send them back to the investigators," notes DeTitta, "but now we can make them available within minutes of their completion."

By building its own fiber-optic infrastructure, UB will be able to increase capacity whenever it needs to, explained Voldemar A. Innus, vice president and chief information officer.

"This network positions us to respond to increasing demand in a much shorter timeframe, while at the same time avoiding significant cost increases," he says.

Innus adds that this is the first example in the state where an academic institution has built its own fiber-optic network that will be used collaboratively with its research partners, providing a vital link for the life sciences initiative.

The network extends from UB's North Campus to its South Campus, and to the Buffalo Niagara Medical Campus running in the Niagara Frontier Transportation Authority Metro Rail tunnel and in above-ground and underground locations approved by the City of Buffalo. BP