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Researchers survey protein family that helps the brain form synapses

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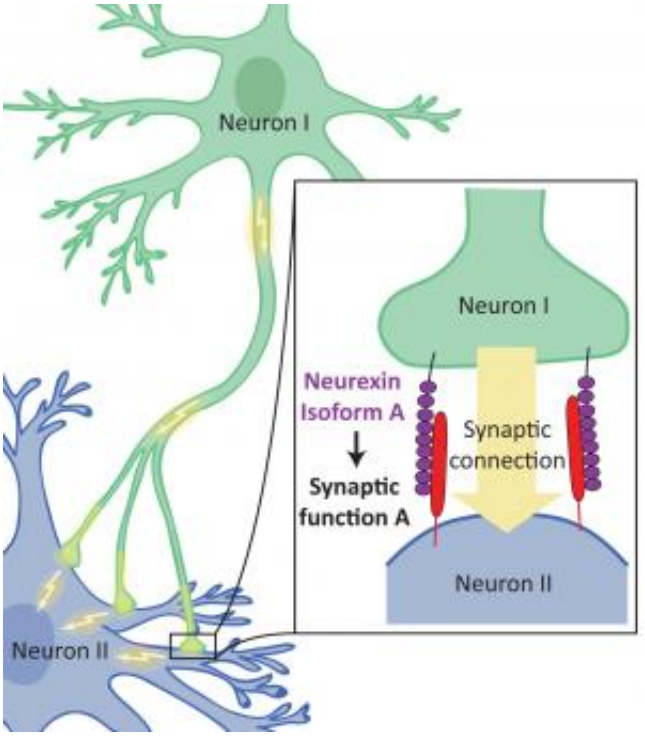

This simplified diagram shows how the neurons in our brain form connections known as synapses. A family of proteins called neurexins, shown here as blue beaded structures, help to form the synaptic connections that allow the brain to do different tasks. Defects in neurexins were found to be associated with neurological conditions like autism. Neuroscientists think that slightly different forms, or isoforms, of neurexins may create different types of synaptic connections, and thus enable our brains to perform so many different tasks. In the first comprehensive survey of this important protein family, Stanford researchers observed more than 450 variants of neurexins, and estimate that this protein family may have 2,500 or more isoforms. Credit: Barbara Treutlein, Quake Lab, Stanford

Neuroscientists and bioengineers at Stanford are working together to solve a mystery: how does nature construct the different types of synapses that connect neurons—the brain cells that monitor nerve impulses, control muscles and form thoughts.

In a paper published in the *Proceedings of the National Academy of Sciences*, Thomas C. Südhof, M.D., a professor of molecular and cellular physiology, and Stephen R. Quake, a professor of bioengineering, describe the diversity of the neurexin family of proteins.

Neurexins help to create the synapses that connect neurons. Think of synapses as switchboards or control panels that connect specific neurons when these brain cells must work together to perform a given task.

Neurexins play a key role in the formation and functioning of synaptic connections. Past human genetics studies have linked neurexins to a variety of cognitive disorders, such as autism and schizophrenia.

Südhof, the Avram Goldstein Professor in the School of Medicine and a winner of the 2013 Nobel Prize in Medicine, has spent years studying the many different forms, or isoforms, of neurexin proteins. He has postulated that different isoforms of neurexins may help to create different types of synaptic connections with distinct properties and functions, and thus enable neurons to do so many complex tasks.

But Südhof had no way to know exactly how many isoforms of neurexins existed until he sat down last year with Quake, the Lee Otterson Professor in the School of Engineering. Quake has pioneered new ways to sequence DNA – the master blueprint that nature follows when making proteins.

The study being published in PNAS represents the results of a year-long collaboration between neuroscientists and bioengineers to better understand how different neurexin proteins affect the behavior of synapses and, ultimately, normal brain functions and neurological conditions such as autism.

Though this will not be the last word on the subject, the findings help illuminate how the brain works and improve our understanding of neurological disorders.

Subhead: Understanding the experiment

Inside cells, a molecular machine unzips a double-stranded DNA molecule to create an RNA molecule. The RNA molecule is a copy of all the genetic instructions encoded into the DNA. But only specific regions of this RNA molecule contain instructions for making a specific protein. The cell has ways to remove the unnecessary regions and splice the protein-coding regions into a shorter RNA molecule called messenger RNA or mRNA. Thus, each mRNA contains the full instructions for making a specific protein.

To begin this experiment, Ozgun Gokce, a postdoctoral scholar in molecular and cellular physiology in Südhof's lab, and Barbara Treutlein, a postdoctoral scholar in Quake's lab, extracted brain cells from the prefrontal cortex of a mouse, and then isolated the RNA contained in this tissue.

From this large pool of RNAs they then identified the mRNAs for neurexins. They ran those messenger molecules through equipment designed to read the entire long sequence of chemical instructions for making a specific isoform in the neurexin family of protein.

Quake's lab is adept at using new instruments that allow researchers to

read the long sequence of chemicals in an mRNA strand, allowing them to ascertain exactly what directions this messenger is carrying to the cell's protein-making machinery.

"This experiment couldn't have been done even a few years ago," Treutlein explained.

The mRNAs for neurexins are very long chains of nucleotides – the chemicals that encode genetic information. Only recently have instruments been capable of reading the exact sequence of such long nucleotide chains.

The ability to read the entire sequence of each mRNA was essential because neurexins have 25 constituent parts. But not all of these parts are used each time neurons produce a copy of the protein. Isoforms of neurexin have different combinations of these 25 possible parts. This experiment was designed to discover how many isoforms of neurexin existed, and how prevalent each of these isoforms was.

The researchers analyzed more than 25,000 full-length neurexin mRNAs. They found 450 variants. Each variant omitted one or more of the 25 possible components. Most of these isoforms occurred infrequently. A handful comprised the predominant isoforms.

Although the Stanford scientists sequenced 25,000 mRNAs to discover 450 variants, they believe that if they were to sequence even more mRNAs they would discover more isoforms – their estimate is that at least 2,500 isoforms of the neurexin family exist.

"The fact that we see so many isoforms supports the theory that these protein variants contribute to the huge diversity of synaptic connections that neuroscientists have observed," Treutlein said.

The experiment raises many questions for future study. For instance, what functions are performed by the predominant isoforms versus the rare variants; how does the inclusion or exclusion of components affect that isoform and the synapse in which it works?

"This experiment was like a flight over the terrain," Gokce said. "Now we have to go down and look at the details."

More information: Cartography of neurexin alternative splicing mapped by single-molecule long-read mRNA sequencing, www.pnas.org/cgi/doi/10.1073/pnas.1403244111

Provided by Stanford University