PNAS QNAS

QnAs with Linda Hsieh-Wilson

Farooq Ahmed

Since the 1890s, neuroscientists have known that the space between neurons, the extracellular matrix, plays a crucial role in brain function. Early brain anatomists uncovered specialized substructures of the brain's extracellular matrix called perineuronal nets, which can constrain neuronal activity. However, it was not until decades later that the molecular composition of these structures was unraveled. The structures were found to be primarily composed of a mix of proteins and carbohydrates, called proteoglycans. California Institute of Technology biochemist Linda Hsieh-Wilson investigates how posttranslational modifications to proteoglycans affect brain function. Her group has developed chemical and informatics tools that demonstrate that specific sulfation pathways contribute to neuronal functions such as social memory. They have also linked these pathways to neurological and neuropsychiatric disorders such as Alzheimer's disease, schizophrenia, and depression. Hsieh-Wilson was elected to the National Academy of Sciences in 2022. PNAS recently spoke to her about her current research.

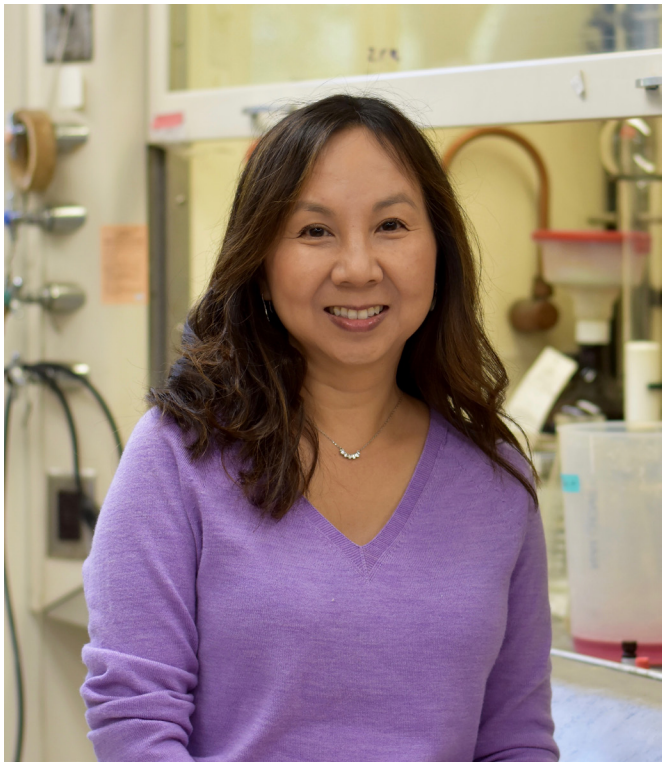
PNAS: Your Inaugural Article (IA) examines changes in the brain's perineuronal nets (1). Why are these structures important?

Hsieh-Wilson: Historically, much of the attention in neurobiology has focused on neurons and, to a lesser extent, glial cells in the brain. The extracellular matrix (ECM) is relatively unchartered, and its precise roles are only beginning to be understood. The ECM occupies a large proportion of the brain, about 20% by volume, and it's a dense network of diverse chemical signals, proteins, and essential biomolecules like proteoglycans and their carbohydrate or glycan constituents.

Perineuronal nets (PNNs) are lattice-like structures that enwrap specific neural cell types and modulate brain plasticity. Their assembly marks the end of critical periods of development when neural circuits lose plasticity and are less readily shaped by experience and sensory stimuli. Remarkably, PNNs are dynamic structures that can be modulated to enhance plasticity and even restore juvenile-like plasticity to adult animals.

PNNs also have a not-very-well-understood role in disease processes. Pathological alterations have been noted in the perineuronal nets of patients with Alzheimer's disease, bipolar disorder, schizophrenia, and autism spectrum disorder. If you remove or prune PNNs away, you can reverse memory deficits in mouse models of Alzheimer's disease. Pruning can also erase fear memories and drug memories. Thus, manipulating perineuronal nets may be a novel way to enhance plasticity and could potentially aid in the treatment of neurodegenerative and psychiatric disorders.

PNAS: How did you become interested in perineuronal nets?



Linda Hsieh-Wilson. Image credit: Paolina Martinez (California Institute of Technology, Pasadena, CA).

Hsieh-Wilson: In the early 2000s, seminal papers demonstrated the many roles that PNNs play in modulating plasticity, stabilizing neural connectivity, and constraining brain development. Since then, a core group of researchers, including my lab, has been interested in these structures.

As a chemist by training, I approached PNNs from the perspective of studying their molecular components—specifically, proteoglycans and their associated glycosaminoglycans, which are sulfated polysaccharides that are very abundant in these brain structures. We've studied glycosaminoglycans in the context of axon regeneration after neuronal injury, and we're trying to understand how these molecules modulate the functions of PNNs and neural plasticity from a structural and molecular perspective.

The sulfation patterns of glycosaminoglycans are spatially and temporally regulated in the brain. We believe that sulfated polysaccharides form a type of encoded alphabet that can alter brain activity. Changes in the "letters" of this alphabet—in the sulfation pattern—regulate the density of PNNs and

This is a QnAs with a member of the National Academy of Sciences to accompany the member's Inaugural Article, e2301312120, in vol. 120, issue 24.

Published June 5, 2023.

alter the ratio of excitatory-to-inhibitory synapses. Imbalances in this ratio have been observed in many neuropsychiatric disorders.

Our group has created knockout mice with deletions in specific sulfation pathways to eliminate certain letters of the alphabet. We have observed dramatic changes in PNNs within a small region of the hippocampus, the CA2 region, of these animals. The CA2 region is important for social memory—the ability, for example, to remember someone you have met before.

PNAS: What did your IA reveal about how glycosaminoglycan sulfation patterns affect the CA2 region of the brain's hippocampus?

Hsieh-Wilson: We began by studying the deletion of a specific sulfation pattern, 4-O sulfation, on a ubiquitous glycosaminoglycan called chondroitin sulfate in adult mice that seem to have developed normally. Knockout animals showed a striking increase in PNN density surrounding neurons in the CA2 region of the hippocampus.

We also observed an increase in the density of inhibitory synapses and decreases in the density of excitatory synapses as well as mature dendritic spines in these mice. Dendritic spines respond mainly to input signals from axons, and so, taken together, this work is consistent with the proposed inhibitory role that PNNs play in brain development and plasticity. A small-molecule inhibitor of 4-O sulfation that our lab developed also recapitulated the effects we observed from the knockout model.

We then looked at whether we could rescue the effects of the chondroitin 4-O sulfation knockouts. We pruned back PNNs enzymatically or added natural polysaccharides enriched in specific sulfated motifs to affected neurons. Both methods restored wild-type levels of excitatory synapses.

We were interested in what might cause these synaptic defects and thought that it could involve the transcription factor CREB, which is known to play a role in plasticity and memory and has been implicated in the progression of Alzheimer's disease as well as in drug addiction. Sure enough, we observed a specific decrease in the phosphorylation of CREB, which activates the transcription factor, in the PNNs of the CA2 hippocampus in chondroitin 4-O sulfation knockout mice. Pruning back PNNs in the affected neurons restored wild-type levels of phosphorylated CREB, as did the addition of 4-O-sulfated chondroitin polysaccharides.

PNAS: How was social memory affected in the knockout animals?

Hsieh-Wilson: The social memory of chondroitin 4-O sulfation knockout mice was disrupted when compared with control animals. The knockouts did not recognize familiar mice that they had been introduced to previously. They also experienced increased levels of anxiety.

Pruning back PNNs in the knockout animals restored wild-type behaviors. But this technique also disrupted social memory in control animals, which suggests that a fine balance in PNN density is required for CA2 neurons to function effectively. We were able to recapitulate these findings by selectively deleting chondroitin 4-O sulfation in only the CA2 region of the hippocampus in adult mice, as opposed to deleting it from the whole brain of adult animals. This research establishes a strong link between chondroitin 4-O sulfation in the PNNs of the hippocampus' CA2 region, phospho-CREB levels, and neurological defects.

I'm particularly excited about further investigations into the molecular contributors of neuropsychiatric and neurodegenerative diseases and understanding how PNNs participate in these various disorders. PNNs and glycosaminoglycans present attractive targets to study neuroplasticity and to address conditions associated with social cognitive dysfunction.

PNAS: How does this research fit into the broader context of your work?

Hsieh-Wilson: I've been interested in the sulfation code of glycosaminoglycans like chondroitin and heparan sulfate and how the patterns contribute to important functions. We have linked a specific sulfation motif, for example, to axon regeneration after spinal cord injury, and, now, chondroitin 4-O sulfation to social memory and anxiety disorders. My group also studies other forms of glycosylation and carbohydrates from a similar perspective, relating chemical structures to function and developing chemical tools that enable us to elucidate biological mechanisms that underlie complex cellular processes.

1. H. Huang *et al*., Chondroitin 4-O-sulfation regulates hippocampal perineuronal nets and social memory. *Proc. Natl. Acad. Sci* **120**, e2301312120 (2023).