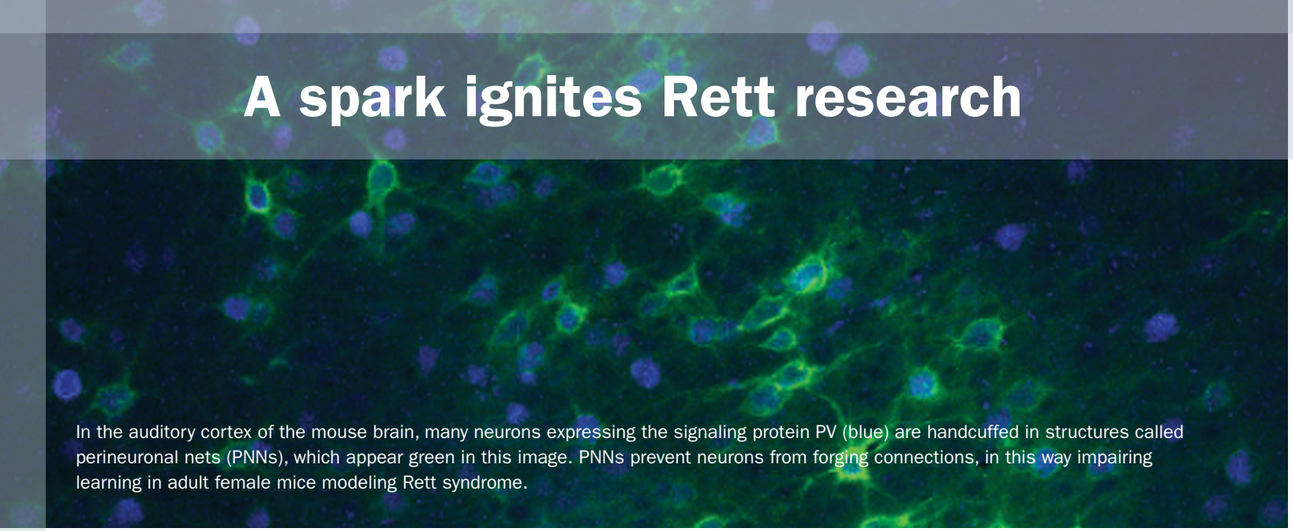
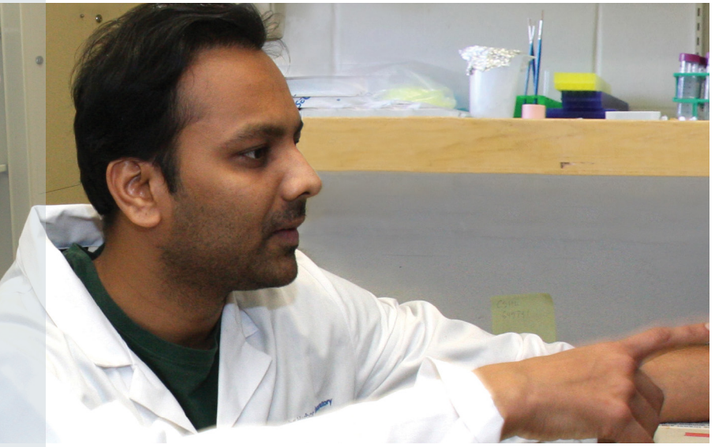
A spark ignites Rett research



In the auditory cortex of the mouse brain, many neurons expressing the signaling protein PV (blue) are handcuffed in structures called perineuronal nets (PNNs), which appear green in this image. PNNs prevent neurons from forging connections, in this way impairing learning in adult female mice modeling Rett syndrome.

Without traditional departmental silos keeping them apart, and encouraged by "everyone's-invited" lectures and an informal, non-hierarchical campus culture, the 600 research scientists at Cold Spring Harbor Laboratory are constantly trading ideas.

Often, sparks fly.



Experiments led by Dr. "Nava" Krishnan in the Tonks lab suggest some Rett symptoms may be reversible.

Case in point: In 2012, Keerthi Krishnan, a postdoc in the neuroscience labs of Professor Josh Huang and Associate Professor Stephen Shea, had a chance conversation with Navasona Krishnan, a postdoc in Professor Nick Tonks' lab. She talked about an experiment involving a mouse model of Rett syndrome—a devastating neurodevelopmental condition often grouped with the autism spectrum disorders.

Keerthi and "Nava," despite having the same family name, were unrelated. In terms of specialty, they were as distant as scientific "cousins" could be. Keerthi studied brain circuits and animal behavior; Nava was a protein biochemist focused on signaling pathways in cells.

"It's a really cool thing, being at Cold Spring Harbor, where something like this can happen," says Shea. "We have creative, intellectually curious people here, and a very free atmosphere that encourages scientists like Keerthi and Nava to find common points of interest. It's a big part of why I like being at the Lab."

In their casual conversation, Keerthi mentioned something that Nava couldn't stop thinking about. "When you delete *Mecp2* in brain cells, mice become obese. They develop resistance to leptin, the hormone that sends a signal when you've had enough to eat," Nava remembers.

Mecp2 is the mouse version of a gene that, when severely mutated or missing, causes Rett in people. Within minutes of the conversation, Tonks recalls, "Nava burst into my office and said, 'Have you ever heard of Rett syndrome and *Mecp2*? Should we look at this?'"

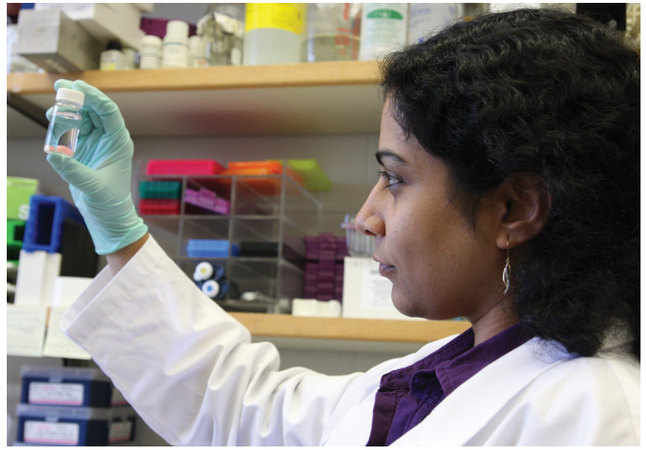
Tonks' reply—"absolutely!"—calls for some history. In 1988, he purified a protein called PTP1B. It was the first-discovered member of a "superfamily" of enzymes called PTPs (protein tyrosine phosphatases) that have the vital job of removing phosphate groups from other proteins. Adding and removing phosphates is a basic means by which signals are sent within and between cells.

Nava (now a Research Investigator in the lab) knew from Tonks that PTP1B is an important "negative regulator" of leptin, as well as insulin, the hormone that controls the way we regulate glucose. Drugs that inhibit PTP1B were identified 20 years ago, with the hope they would be next-generation treatments for obesity and diabetes.

For technical reasons those compounds were not taken further by the pharmaceutical industry (although new inhibitors are now in clinical development). In the Tonks lab, Nava had these compounds at his disposal, and soon started to test them in the "Rett" mice with which Keerthi was working. He was intrigued by the relationship between the absence of *Mecp2* and the inability of the animals to regulate their metabolism.

Keerthi and Steve Shea, meanwhile, focused on the biology behind a behavior observed in the mouse model of Rett. It seemed that mice lacking *Mecp2* could not perform a classic behavioral test that involved an adult female learning to retrieve distressed newborns.

A healthy adult female learns quickly to gather scattered pups into a compact, secure nest—even if they aren't her own and she's never cared for offspring. But females lacking *Mecp2* can't gather distressed pups, no matter how often they have the chance to learn. Why?



Dr. Keerthi Krishnan led research in the Shea lab revealing mechanisms underlying learning impairments in Rett syndrome.

Both labs' Rett projects produced exciting results, revealing (in Shea's lab) anomalies in neural circuits in the brain's auditory cortex that inhibit plasticity, and hence, the ability to learn, in the adult females lacking *Mecp2*; and (in Tonks' lab) the promise of PTP1B inhibitors in reversing symptoms of Rett syndrome.

In early experiments, Nava discovered that PTP1B inhibitors had a beneficial impact when given to frail male mice missing *Mecp2*. Males have only one copy of the gene, which is located on the X chromosome, and when it's missing they don't survive very long. (In the human disorder, 9 in 10 patients are female for this reason.) Male mice treated with PTP1B inhibitors lived nearly twice as long. Females missing *Mecp2* generally fare better; having two "Xs," they have two copies of the gene and can survive with a single working copy. Would PTP1B inhibitors ameliorate Rett-like symptoms in female mice? If so, this might be an approach to treat Rett patients.

Tests with three different PTP1B inhibitors in female "Rett" mice resulted in improvements in Rett-like impairments, including a paw-clasping behavior and the ability of the mice to remain on a rotating wheel. These effects, Nava and Tonks believe, are due to the release of a molecular "brake." Using the inhibitors to reduce PTP1B activity restores a key metabolic signaling pathway. By inhibiting PTP1B—which Nava had shown is overly abundant in mice lacking *Mecp2*—the experimenters effectively "took their foot off the brake," opening a cellular pathway through which leptin and insulin signals are normally sent. Meanwhile in the Shea lab, Keerthi (who has since joined the faculty at University of Tennessee) and postdoc Billy Lau linked the inability of female "Rett" mice to learn pup retrieval to an impairment of plasticity in neurons in the auditory cortex—cells that process the squeals made by distressed pups. They traced the pathology to neurons that release a signaling protein called parvalbumin (PV). Loss of *Mecp2* leads to elevated PV levels and the handcuffing of PV neurons within structures called perineuronal nets (PNNs). These structures prevent neurons from connecting. Forging new connections is a key part of how learning occurs in the brain.

These experiments, stemming from a casual conversation between postdocs in different fields, have led to progress in understanding Rett pathology and advancing a new treatment concept. "The critical point," observes Tonks, "is the diversity of research that's done at CSHL, that allowed Nava to listen to someone in another field talk about a problem and come away with an idea that launched a project. Cold Spring Harbor is a melting pot of people from different backgrounds, different expertise, widely different areas, and you never know where the next idea is going to come from!"

Peter Tarr