Complex brain cell connections in the cerebellum more common than believed

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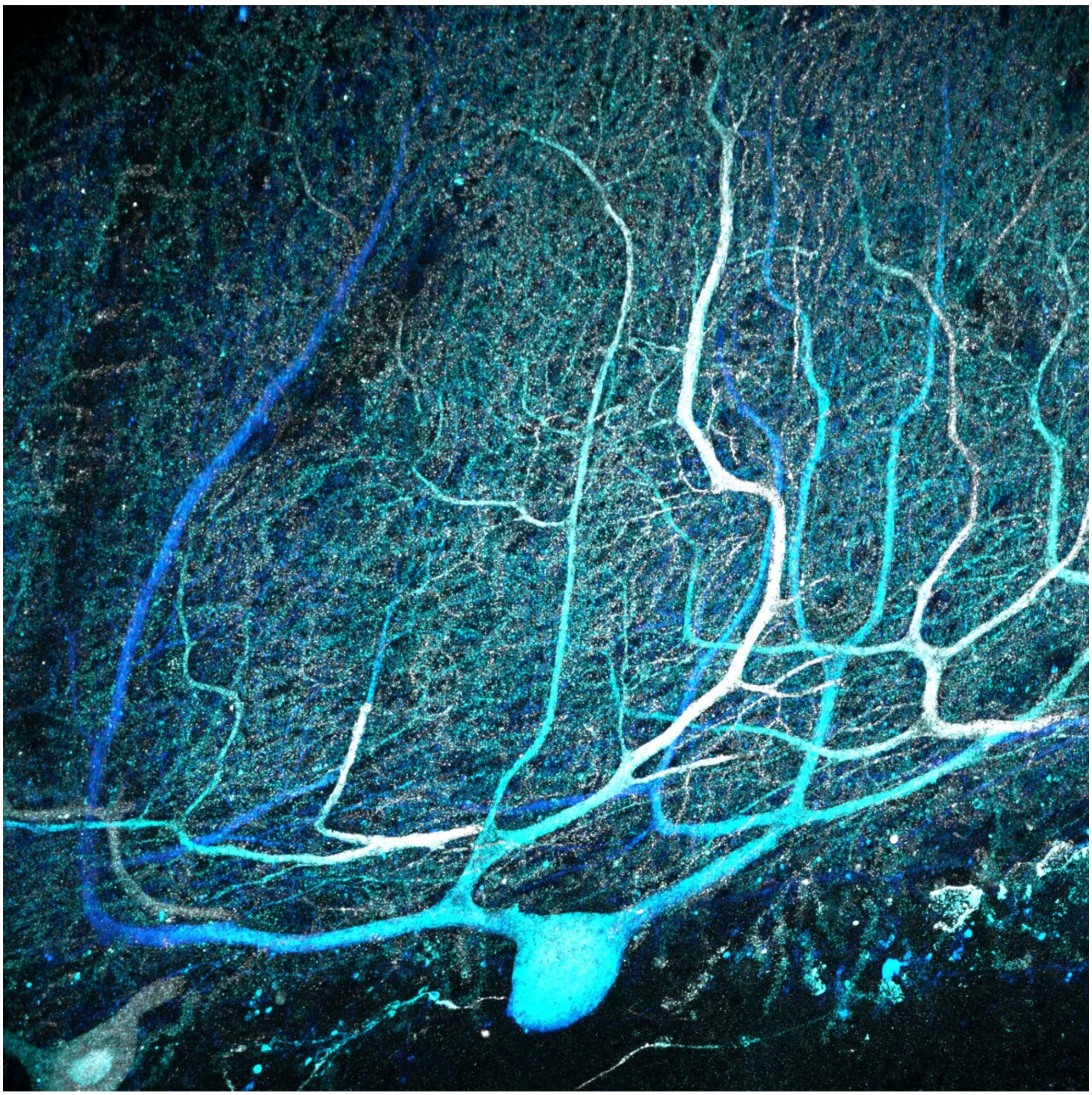


Image of a human Purkinje cell. Nearly all Purkinje cells in the human cerebellum have multiple primary dendrites sprouting from the cell body and splitting into beautiful, leaf-like patterns. Credit: Silas Busch, University of Chicago

In 1906, Spanish scientist Santiago Ramón y Cajal won the Nobel Prize for his pioneering studies of the microscopic structures of the brain. His famous drawings of Purkinje cells in the cerebellum show a forest of neuron structures, with multiple large branches sprouting from the cell body and splitting into beautiful, leaf-like patterns.

Although these early depictions captured multiple dendrites extending from the cell body, the prevailing wisdom among neuroscientists to this day is that Purkinje cells have just one primary dendrite that connects with a single climbing fiber from the brain stem. New research titled, "Climbing fiber multi-innervation of mouse Purkinje dendrites with arborization common to human," from the University of Chicago published in *Science* shows that Cajal's drawings were accurate all along, however—nearly all Purkinje cells in the human cerebellum have multiple primary dendrites.

Further studies in mice showed that about 50% of their Purkinje cells have this more complex structure too, and of these cells, 25% receive input from multiple climbing fibers that connect with different primary dendrite branches. Experiments recording cell activity in live mice also revealed that the primary branches can be activated independently, responding to different stimuli from the environment.

"The more you work with a certain prototype of a cell in your mind, the more you accept it," said Christian Hansel, Ph.D., Professor of Neurobiology at UChicago and senior author of the study, referring to the canonical model that Purkinje cells have one primary dendrite that connects with one climbing fiber.

"These drawings by Cajal have been around since the 1900s, so we definitely had enough time to pay attention, but only now with this quantitative analysis do we see that it's almost universal that human cells have multiple full dendrites each, and we can see that it makes a qualitative difference too."

Rewriting a textbook idea

The cerebellum sits at the base of the cranium, just above where the spinal cord connects. Ever since French physician Jean Pierre Flourens first described the cerebellum's function in 1824, scientists believed that its sole job was coordinating movement and muscular activity, but advances in technology have shown that the cerebellum also plays a significant role in processing input about the body's internal and external environment, including sensations of proprioception and balance.

Cerebellar Purkinje cells are like large antennae receiving thousands of inputs conveying a spectrum of contextual information from the rest of the body. These signals are then integrated with a prediction-error signal, indicating a mismatch between the context and the brain's expectation. This error signal is provided by nerve fibers that climb up from the brain stem and connect with their target Purkinje dendrite structures. Quite appropriately, these nerves are called "climbing fibers."

The standard understanding of these connections has been that each Purkinje cell has one primary dendrite that branches from the cell body and connects with one climbing fiber, forming a single computational unit. Belief in this one-to-one relationship between climbing fibers and Purkinje cells, a central dogma in the field that can be found in every neuroscience textbook, largely comes from studies on rodents, which do primarily have the single dendrite configuration.

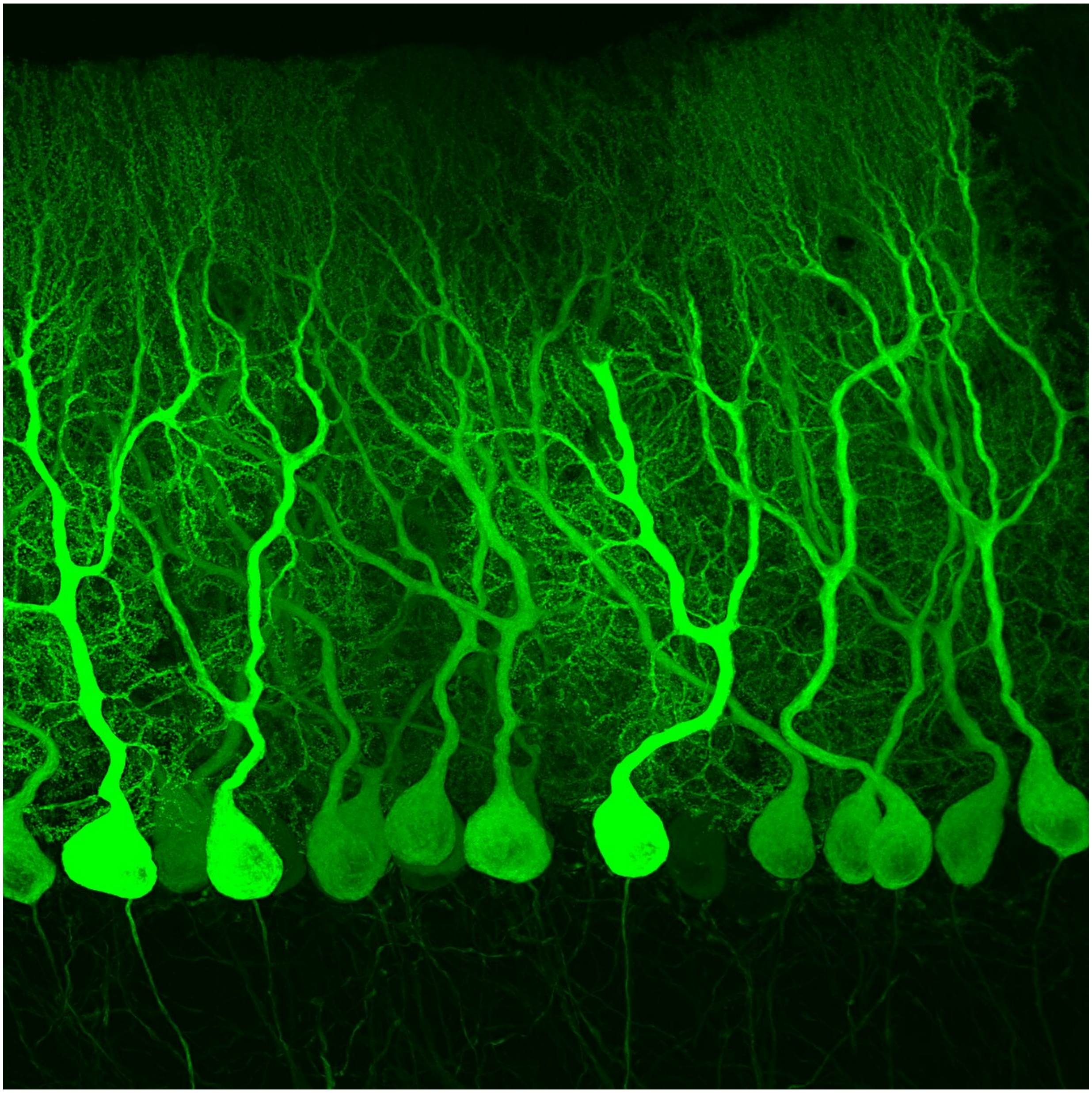
Many studies of these structures in the past have focused on small numbers of cells though, so for this new research, Silas Busch, a Ph.D. student in Hansel's lab and first author on the paper, started by looking at thousands of cells from both human and mouse tissue. He used a targeted, antibody-based staining technique, known as immunohistochemistry, to selectively label Purkinje cells in thin slices of cerebellum.

He then categorized the structure of all the cells he could observe and found that more than 95% of human Purkinje cells had multiple primary dendrites, while in mice that figure was much closer to half.

"You get a sense for how much this was a prevailing idea in the field because anatomically, they are referred to as the 'primary' dendrite of a cell," Busch said. "So, even the description of the structure of these cells is based on that mouse prototype that has one dendrite you can call a primary dendrite."

This remarkable species difference, in one of the most evolutionary conserved brain areas shared across mammals and even other vertebrates, led Busch and Hansel to ask if there might be a functional consequence to having multiple primary dendrites instead of just one. The climbing fiber, with an auspicious one-to-one relationship and intimate entanglement of the primary dendrite, was their first suspect.

Using sections of mouse cerebellum that contained still living cells, Busch injected the cells with dye to see their branches and then stimulated climbing fiber inputs. He observed that 25% of cells with multiple primary dendrites received multiple climbing fibers, rewriting a textbook idea that each and every Purkinje cell gets only one climbing fiber input, while cells with a single primary dendrite did not.



Mouse Purkinje cells. Although 50% of mouse Purkinje cells have a single primary dendrite, the other half have multiple dendrites much like human cells. Credit: Silas Busch, University of Chicago.

Walking mice and wiggling whiskers

Encouraged by this finding that a sizable portion—albeit a minority—of Purkinje cells with multiple primary dendrites also received input from multiple climbing fibers, Busch conducted a series of experiments in living mice to see if it led to functional differences in the live mouse.

First, he injected a fluorescent calcium indicator dye into the cerebellum and implanted a small glass window so he could later observe the flow of calcium into the Purkinje cell dendrites. By restraining the mouse's head under a microscope while it ran on a treadmill, he could measure calcium flow that indicated when a climbing fiber is providing a strong input to the cell.

In cells with one primary dendrite, high-resolution images showed that the activity signal was uniform across its structure; in cells with multiple primary dendrites, he could detect activity on each side occurring at different times, meaning that one dendrite could be activated by its climbing fiber while the other dendrite in the same cell was not.

Next, Busch wanted to see if he could tease out individual climbing fiber activity by using a more precise stimulus: the mouse's whiskers. For this experiment though, Busch had to sedate the mice ("I don't know if you've ever tried to stimulate individual whiskers in an awake mouse, but it's really hard," he said).

With the mice asleep, Busch threaded individual whiskers into a small glass tube and wiggled them back and forth. Here, he could also see activity in distinct dendritic branches of the Purkinje cells, suggesting that individual climbing fibers were signaling the input from individual whiskers to individual dendrites.

Finally, for a more real-world scenario, Busch also tested awake mice with several stimuli, like flashes of light, sounds, or air puffs on the whisker pad. Again, he saw differences across the Purkinje cells. In some, one branch would differentially favor one stimulus, so it might be particularly responsive to light but not sound. Then the other branch might be preferentially responsive to sound, but not light.

"This happened in a minority of cells since there are fewer with multiple branches in mice, and not all of them get multiple climbing fibers, but still, the presence of this effect was very interesting," Busch said. "It confirmed this idea that the two climbing fiber inputs will have different functional purposes that represent different information."

The cerebellum's connectivity becomes more clear

This new evidence upends standard thinking about a brain area thought to be fairly solved anatomically and has functional consequences as well. As the climbing fibers provide input from the brain stem, the Purkinje cells aggregate and process that information. Multiple inputs connecting at multiple points on the cells provide more computational power, allowing brain circuits to adapt and respond to changes in the environment or the body that require different movements, and this non-canonical connectivity is closely tied to the structure of Purkinje cell dendrites.

There is also evidence that these connections in the cerebellum can be involved in disease. In 2013, for example, Hansel worked on a study with UChicago neurologist Christopher Gomez, MD, Ph.D., showing that Purkinje-climbing fiber connections are weaker in mouse models of cerebellar ataxia, a movement disorder.

On the other hand, Busch, Hansel, and Gomez have published work with former UChicago graduate student Dana Simmons showing these connections are stronger in genetic duplication and overexpression models of autism. Other researchers demonstrate stronger connections in certain types of tremors as well. Understanding more about the essential biological structures of these cells will hopefully provide more insight into these conditions.

"People who study other parts of the brain like the neocortex or the hippocampus always have more or less an idea of what that brain structure is doing," Hansel said. "Those of us who study the cerebellum always had this idea that it's motor coordination and adaptation, but it was also clear that it was something beyond that. Now it will be easier to grasp as the connectivity becomes clearer."

More information: Silas E. Busch et al, Climbing fiber multi-innervation of mouse Purkinje dendrites with arborization common to human, *Science* (2023). DOI: 10.1126/science.adi1024. www.science.org/doi/10.1126/science.adi1024

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