A WHOLE NEW TOOLBOX FOR IMAGING BRAIN FUNCTION

Researchers in Japan are using clever methods to map out THE BIG PICTURE OF NEURONAL NETWORKS.

If you want to understand an electronic device, you can simply review its circuit diagram. But even if such diagrams existed for the brain, they would be of limited use in unraveling this organ's bewildering complexity. This is because the physical connections between its billions of neurons tell just a small part of the story. The real key is understanding how these cells communicate in real time, and how these patterns synchronize in the service of different behaviours.

Critical gaps exist in the toolbox for addressing such questions. "Electrode-based measurements can provide sensitive and precise measurements of neuronal activity," says Atsushi Miyawaki, team leader of the Cell Function Dynamics lab at the RIKEN Center for Brain Science (CBS) in Wako, Japan. "But they can only measure very limited numbers of neurons simultaneously."

In contrast, functional magnetic resonance imaging (fMRI) can map activity throughout the brain, but lacks the resolution to dissect individual circuits. In between lie a range of microscopic methods that do not quite bridge the gap separating these extremes.

Armed with support from a ten-year Japanese research initiative known as Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS), Miyawaki and his CBS collaborator Masanori Murayama are making critical headway in building out the toolbox for imaging brain functions. Although this work is preliminary, these methods could enable researchers to begin interpreting the functional consequences of complex neuronal activity across large swaths of brain tissue.

THE LAB HAS PIONEERED THE DEVELOPMENT OF NUMEROUS POWERFUL IMAGING TOOLS.

A SMALL WORLD AFTER ALL

The cerebral cortex is the central hub for coordinating behaviour and cognition in the brain. As the head of the Haptic Perception and Cognitive Physiology lab at CBS, Murayama is interested in understanding how this process unfolds at both the single-cell and network level.

Two-photon microscopy (2PM) is among the most powerful methods for studying neuronal activity in live animals. This technique uses rapid laser pulses to selectively illuminate fluorescently-labeled 'reporter' proteins, while inflicting minimal damage, making relatively long-term observations possible.

But most 2PM systems have only a tiny field of view — the area of tissue that can be observed at any instant. Attempts to broaden this field of view have created trade-offs between image resolution and the frequency at which images can be collected.

"Those microscopes at the time had just one frame per one or two seconds, and that's too slow," says Murayama. "Indeed, most neurons fire at frequencies of more than one burst per second."

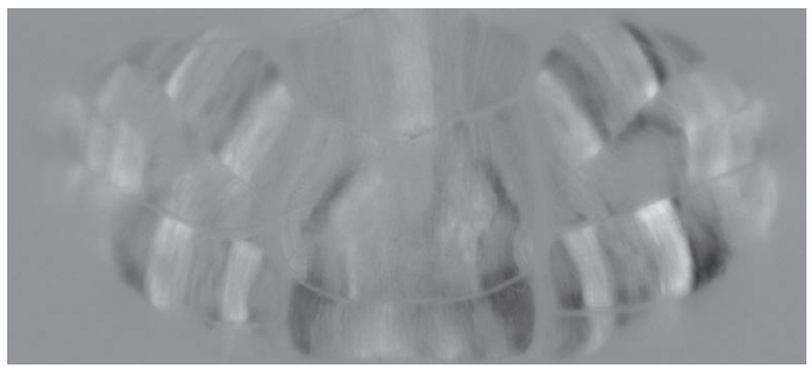
Fast, high-resolution imaging with a broad field of view requires a microscope equipped with an extra-large, specially-designed lens — and over the course of nearly a decade, Murayama's team was able to develop such a system. In 2021, they used their novel FASHIO-2PM platform to visualize more than 16,000 neurons across 9 square millimetres of cerebral cortex tissue in live mice at more than 7.5 to 15 frames per second[1].

Their analysis revealed that this cortical region has a 'small world' architecture, in which any given neuron is generally only separated from another by relatively few degrees of separation. Most have small numbers of connections, but Murayama also noted a subpopulation of 'hub' neurons. "These are very active, with more than 100 neurons together, including long-distance pairs," he says.

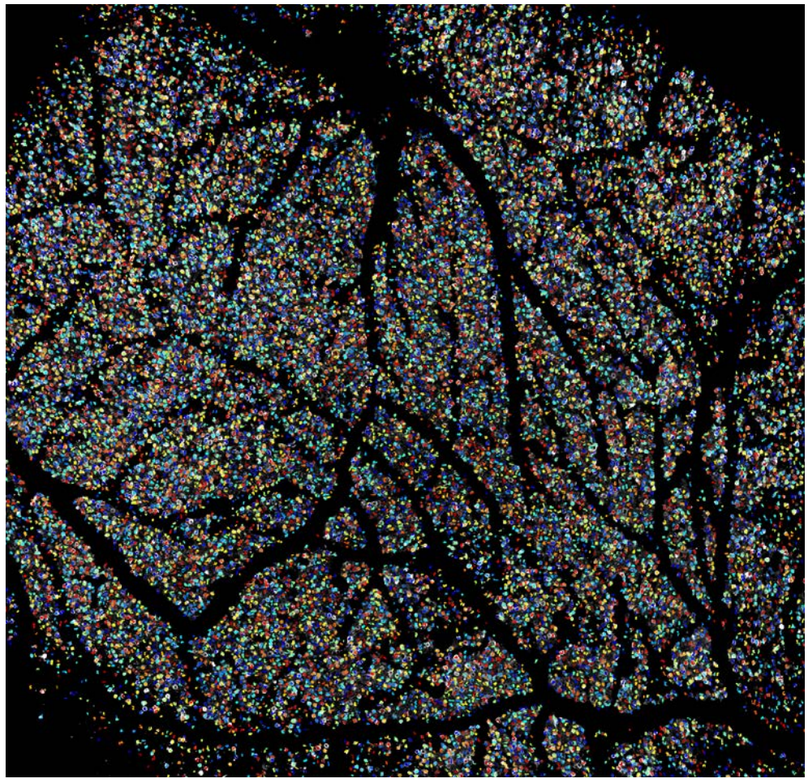
Preliminary functional analysis of these hubs has already revealed distinct changes in their activity between sleep and waking states in the brain.

Murayama's team is continuing to explore network function in the healthy brain, but also plans to look at how these patterns are derailed in cognitive disorders like Alzheimer's disease. For example, fMRI studies of Alzheimer's patients have indicated abnormal activity in the default mode network, a brain system that plays a central role in self-awareness and introspection as well as behavioural planning.

FASHIO-2PM could offer a cellular-scale view of these changes — and Murayama notes that his team's microscope is also suitable for so-called optogenetics experiments, where lasers are used to modulate brain activity via specially engineered light-responsive proteins.



▲ A fluorescence microscopy image showing the clustering of activated Purkinje cells in the whole mouse cerebellum.



▲ The two-photon microscopy (2PM) imaging technique FASHIO-2PM monitors more than 16,000 cortical neurons in an awake mouse.

MOTOR FUNCTION FOCUS

Recently, Miyawaki's lab has been focusing on another brain region, the cerebellum, which coordinates physical movement. Any time we extend our arm or open our mouth, the cerebellum is issuing instructions to direct that action.

Neuroscientists have long debated whether this region of the brain exhibits a property called somatotopy. This describes the concept wherein every part of the body can be directly mapped onto the cerebellum, such that one specific region of neurons controls the left leg, for example, whereas another governs the right leg.

In principle, this can be assessed by measuring the distinctive firing patterns, known as complex spikes, generated by Purkinje cells — the neurons that generate the output of the cerebellum. But in practice, existing imaging methods are ill-suited for achieving this task at scale.

"Consequently, the somatotopic organization of the cerebellum is still controversial," says Miyawaki. "We needed a new method for wide and high-spatiotemporal-resolution mapping of complex spikes."

Miyawaki's lab has pioneered the development of numerous powerful imaging tools, including a fluorescent reporter protein called cameleon that reveals changing cellular levels of calcium ions associated with neuronal spikes. A few years ago, they managed to label every Purkinje cell (large neurons with numerous branching extensions) in the mouse cerebellum with the cameleon reporter. In parallel, they devised a custom-built 'macroscope' instrument that could survey the entire cerebellar surface through a surgical window into the skull.

ORGANIZATIONAL PRINCIPLE

In a 2021, Miyawaki's group succeeded in clustering Purkinje cells into roughly 200 'segments', each containing 50 to 100 Purkinje cells[2]. They subsequently examined how these segments responded after stimulating muscles in each of the mouse's four limbs, and were surprised to learn that the activity profiles were indistinguishable at first glance. But more extensive mathematical analysis revealed that each stimulus was actually eliciting a subtle but distinctive spatial and temporal pattern of response in multiple segments throughout the cerebellum.

This offers compelling evidence against somatotopy, and Miyawaki believes this organizational principle makes good evolutionary sense. He draws parallels to computer servers, which use arrays of redundant hard drives. "The whole system is composed of 16 hard drives and is based on distributed processing, and any two hard drives can be broken at any time," he says. This same strategy could preserve motor function in the aftermath of localized injury at a particular site in the brain.

Miyawaki's lab now aims to take a deeper dive into these segments — perhaps using the Murayama lab's FASHIO-2PM platform. "By using 2PM, we could maybe zoom in on a specific segment to visualize how Purkinje cells in the segment synchronize their firing," he says. "That's one of our next goals." ■

REFERENCES

1. Ota, K. *et al. Neuron* **109,** 1810–1824 (2021).
2. Michikawa, T. *et al. Cell Rep.* **37,** 109966 (2021).

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