Study unveils the distinct activation patterns of glutamatergic projection neurons in the cortex of living mice

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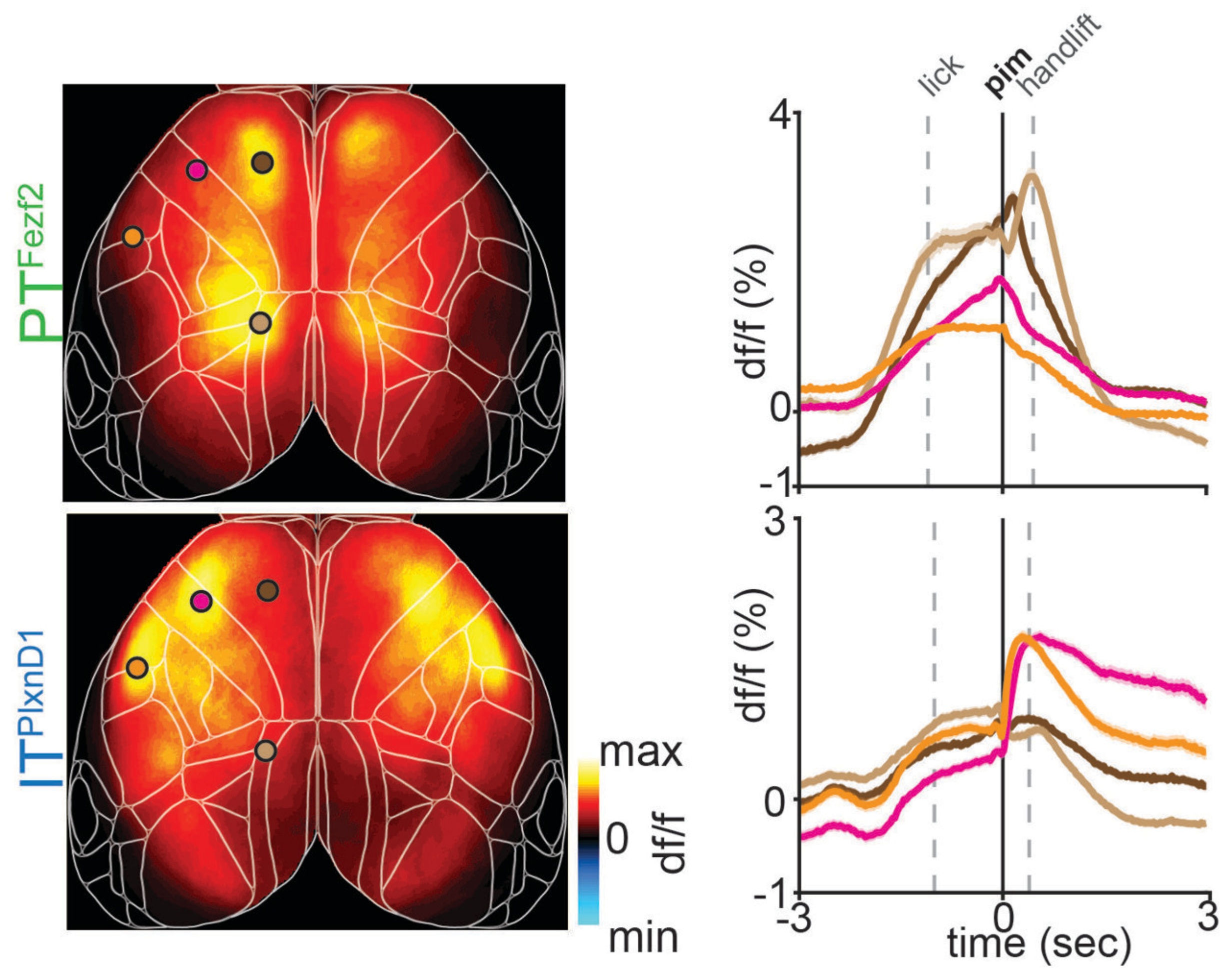


Credit: Mohan et al

The cerebral cortex, the outer layer of the mammalian brain, is known to play a key role in numerous higher-level processes, including language, memory and decision-making. While countless studies have explored its structure and function, imaging its neuronal dynamics with the identity of its cell types in behaving animals has so far proved very difficult.

Researchers at Duke University Medical Center and Cold Spring Harbor Laboratory have recently carried out a study aimed at monitoring and better understanding neural circuit dynamics in the cerebral cortex of behaving mice. Their findings, published in *Nature Neuroscience*, showed that while mice engaged in different behaviors, distinct glutamatergic projection neurons types in the cerebral cortex exhibited different activation patterns.

"The main objective of our study was to understand the neural circuit mechanism underpinning brain function, particularly the function of the cerebral cortex," Josh Huang, one of the researchers who carried out the study, told Medical Xpress. "When we ask this question, the obvious next question is what cell type resolution do we capture, as cells are basic elements of neural circuits that form connections and have different functions."

So far, many studies examining the cerebral cortex used functional magnetic resonance imaging (fMRI), a technique that detects and measures small changes in blood flow associated with brain activity. Despite its value in some settings, fMRI captures brain activity with poor spatial and temporal resolution. Thus it is not ideal for examining neural circuit dynamics in depth.

Recently, neuroscientists started using wide field imaging to study activity in the brain. This is a promising imaging method that relies on genetically encoded sensors to detect changes in brain activity with greater precision.

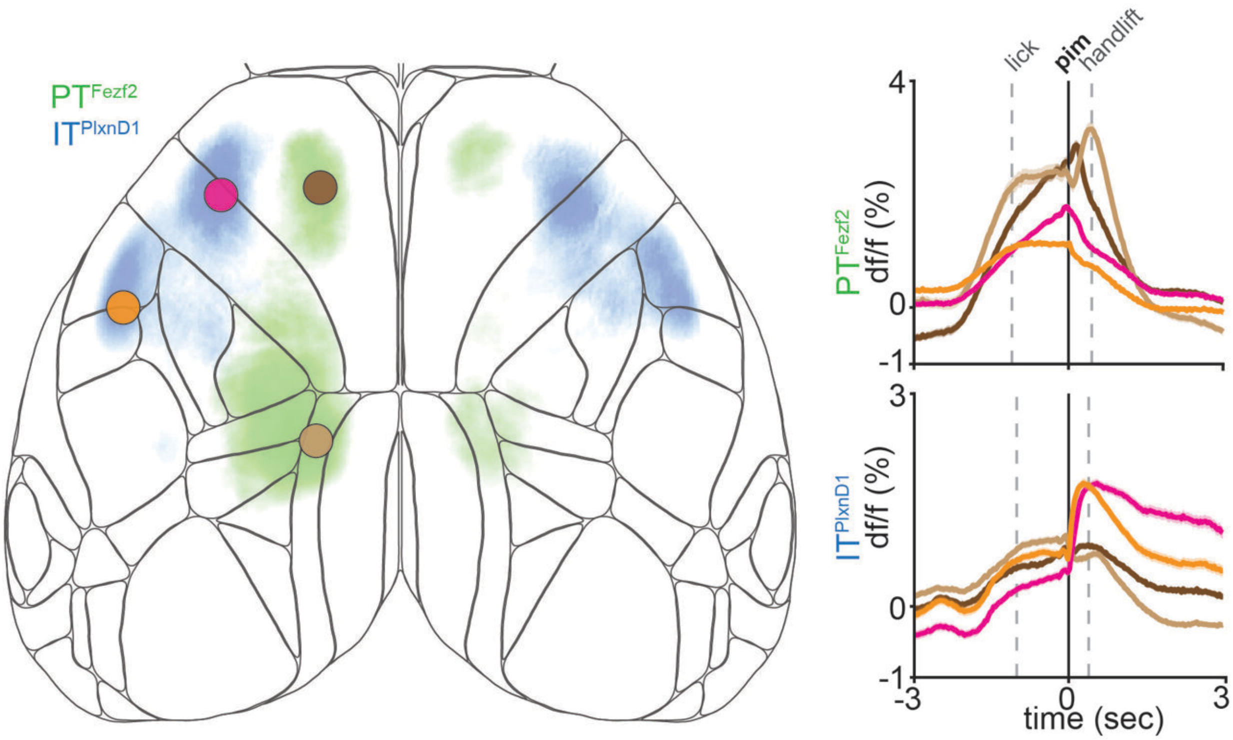
"While wide field imaging brought major progress, it usually can only be used to image all neural populations," Huang explained. "So again, it does not resolve these basic elements of neuronal cell type, especially the projection neuron types. The motivation behind our study was to study the cerebral cortex using a systematic set of genetic tools that we built recently, which can resolve different classes and types of glutamatergic projection neurons with cell type resolution."

In their experiments, Huang and colleagues measured activity of neurons in the cerebral cortex of mice using a series of genetic and imaging techniques. Firstly, they used genetic engineering to ensure that they could specifically monitor the activity of different projection neuron types.

"We did this relatively systematically to target projection neurons that project within the cortex, outside of the cortex to subcortical targets and to the thalamus," Huang said. "The key here is to not just look at one cell type, but to look at multiple, in a way to screen across cell types. We combine this with wide field imaging to not just look at one area, assuming that area is what is involved in behavior, but to look at many areas simultaneously."

A key achievement of this study is that the team was able to apply wide-field imaging techniques to study different projection neuron types in awake behaving mice as they engaged in different activities. Compared to previous studies, however, they did this systematically and with better resolution, allowing them to distinguish cell type function apart.

"What we were able to achieve, which I think has not been achieved before is to look at the basic elements of neuron circuits, projection neuron types and their dynamics in real-time and in behaving animals across the global cortical network," Huang said. "This allowed us to uncover the cell type basis of cortical network dynamics, enhancing our understanding of how the cortex is organized."



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The cerebral cortex has so far known to be comprised of different areas with distinct functions. These areas were found to have a similar neuronal circuit organization, as they include so-called "canonical" cortical microcircuits, vertical sets of neurons that are organized in similar ways. Past findings suggest that these circuits process information and communicate with other brain regions with similar circuit organization.

"These columns of neurons across different cortical layers are considered structural and, in some cases, functional units," Huang said.

"This view, based on both anatomical and functional evidence collected in the past, has been very influential for a long time. What we find here, to our surprise, is that when you look at projection neuron types along the vertical depth, these types of neurons have rather distinct dynamic patterns."

Essentially, Huang and colleagues identified projection neuron types that do not always function as a single unit, specifically the intratelencephalic (IT) and pyramidal track (PT) population.

IT neurons project to other cortical areas within the cerebral cortex. PT neurons, on the other hand, send information outside of the cerebral cortex, to subcortical brain regions.

"In many cases, although not always, these two broad types of neurons seem to act in distinct spatial and temporal patterns," Huang said. "These observations significantly deviate from the more traditional historical concept of columnar neuronal organization and suggest that these cell types also form separate processing and output networks."

The findings gathered by Huang and colleagues show that IT and PT projection neuron populations sometimes operate independently from each other and sometimes together as a unit. Their 'mode of operation' could potentially depend on what mice or other mammals are doing at the time, as well as the associated brain states and behavioral demands.

"Looking at particular functions of cortical dynamics with cell type resolution will really allow us to discover principles at finer granularities," Huang explained. "Different cortical areas are involved in behavior function and because these cell types also have distinct inputs and outputs that we can now measure, our techniques could really help us examine neural circuits at a finer level."

This current study offers new insights about the neural dynamics governing cerebral cortex function. In the future, these genetic techniques they used could be applied by other teams to further examine these dynamics, potentially leading to exciting new discoveries.

"In the current paper, we mainly examined two broad cell types, namely the ones that project within the cortex and the ones that project outside of the cortex," Huang said. "In reality, there is far more granularity as there are different cell types that project to distinct subsets of cortical and subcortical areas. We are now experimenting with other genetic tools that will allow us to look at finer resolution and hopefully piece together a cortical network view that is not just at the broad population, but at a finer population level including anatomical connectivity combined with functional activity."

In their next studies, the researchers plan to also apply wide-field imaging techniques to study neuronal circuits in other areas of the brain including more cell types. Moreover, they would like to experiment with methods that can image two cell population in the same living animal.

"In our recent study, we inferred our conclusion by looking at one population in one mouse, another population in another mouse, and then integrated our findings together," Huang added. "In our next work we plan to label two cell population in the same animal, using different spectrum: one with a green light and the other a red light-based calcium sensor. This will allow us to look at two cell populations that are interacting and examine them simultaneously, which should give us even greater precision with new insight."

More information: Hemanth Mohan et al, Cortical glutamatergic projection neuron types contribute to distinct functional subnetworks, *Nature Neuroscience* (2023). DOI: 10.1038/s41593-022-01244-w