MATRIEX imaging: Simultaneously seeing neurons in action in multiple regions of the brain

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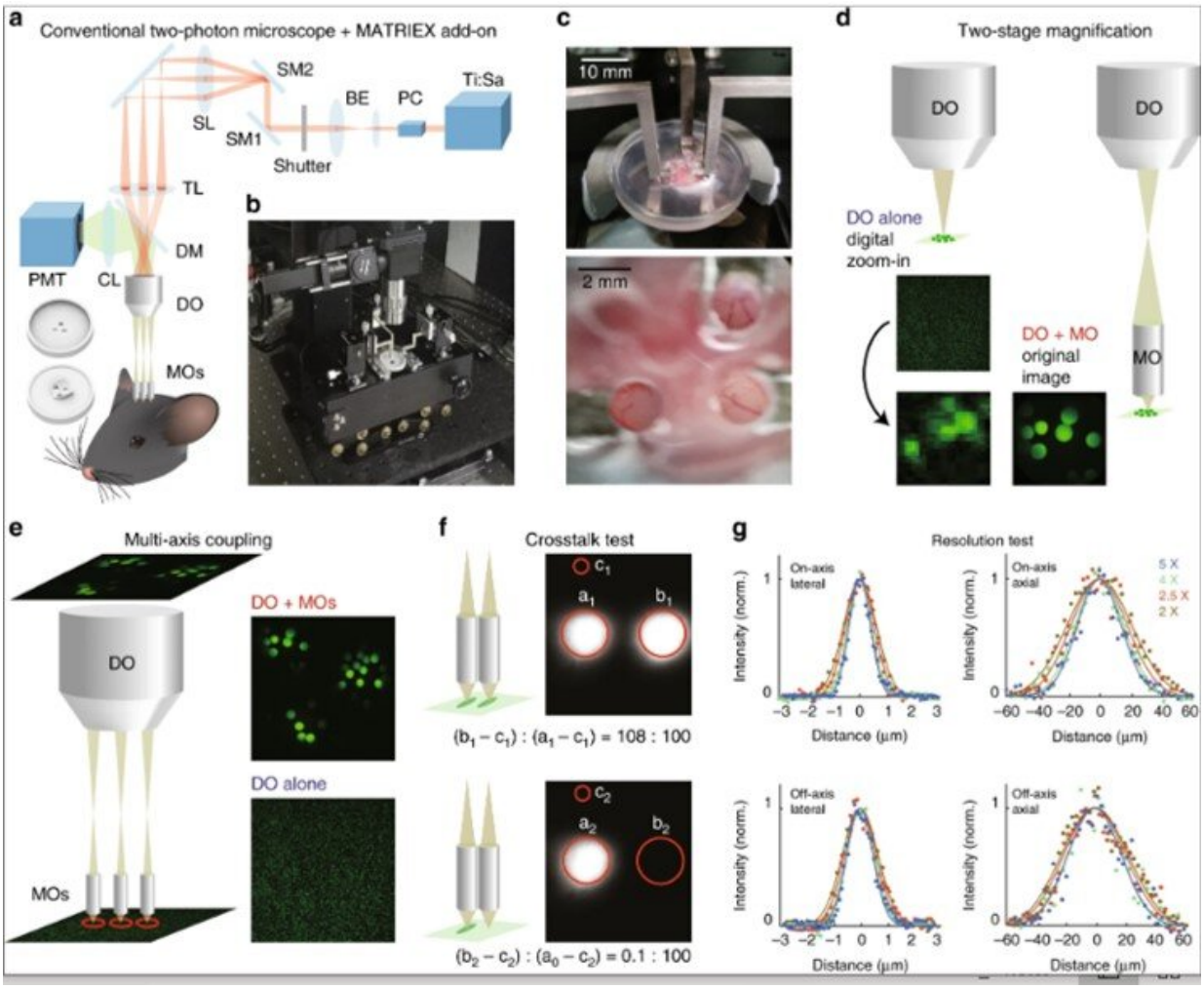


Design and implementation of MATRIEX imaging: (a) Experimental diagram of the MATRIEX imaging system. The two round 3D objects in the lower-left

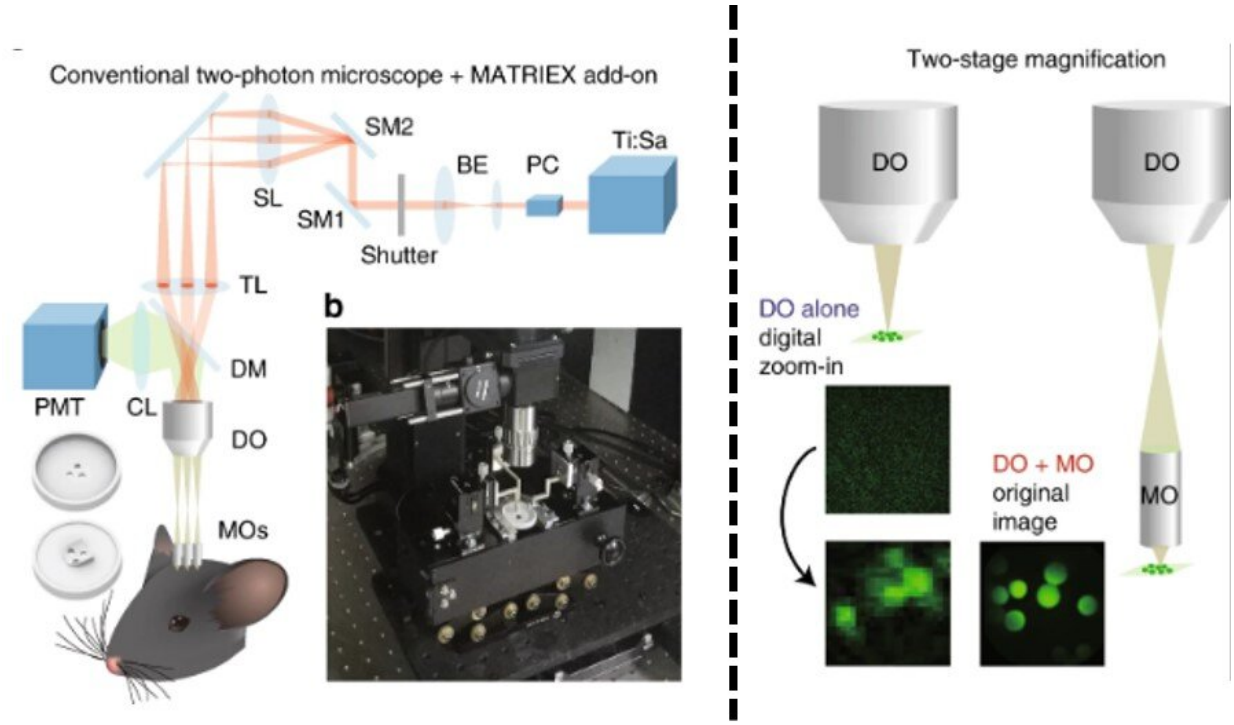
corner are the top and bottom views of the mouse head chamber used for in vivo imaging. (Ti:Sa): Ti:Sapphire ultrafast pulsing laser; PC: Pockels cell; BE: beam expander; SM1 and SM2: x–y scanning mirrors; SL: scan lens; TL: tube lens; DM: dichroic mirror; CL: collection lens; PMT: photomultiplier tube; DO: dry objective; MOs: miniaturized objectives. (b) Photograph showing an oblique overview of the actual MATRIEX imaging system. (c) The photograph in the upper image shows a zoomed in view of the three MOs attached to the manipulating bars over the head chamber; the lower photograph was taken directly above the MOs with a smartphone camera. All MOs used in this figure are of the same model: 'standard version.' (d, e) Illustrations of the two-stage magnification and multiaxis coupling. The square images are actual two-photon images taken of 20-μm beads. Each red circle indicates one FOV. The model of DO used in panels (d-f) is the Olympus MPlan ×4/0.1, and all MOs in this figure are of the same customized model. (f) Illustration showing the absence of inter-FOV crosstalk under adjacent MOs. The images were taken on a uniform fluorescent plate. The red circles indicate the areas of analysis used to compare the image contrast between two conditions; the left-side condition shows the fluorescent plate under both MOs, and the right-side condition shows the fluorescence plate under only one MO. (g) Testing the optical resolution of the compound assembly with 0.51-μm beads. Curves: Gaussian fittings of raw data points. The on-axis or off-axis fluorescence intensity profiles were measured when the axis of the MO was aligned with the axis of the DO or apart from the axis of the DO (2 mm for the DO of ×4 or ×5, 3 mm for the DO of ×2.5, and 4 mm for the DO of ×2), respectively. Credit: Light: Science & Applications, doi: 10.1038/s41377-019-0219-x

Two-photon laser scanning microscopy imaging is commonly applied to study neuronal activity at cellular and subcellular resolutions in mammalian brains. Such studies are yet confined to a single functional region of the brain. In a recent report, Mengke Yang and colleagues at the Brain Research Instrument Innovation Center, Institute of Neuroscience, Center for Systems Neuroscience and Optical System Advanced Manufacturing Technology in China, Germany and the U.K.

developed a new technique named the multiarea two-photon real-time in vitro explorer (MATRIEX). The method allowed the user to target multiple regions of the functional brain with a field of view (FOV) approximating 200 μm in diameter to perform two-photon Ca^{2+} imaging with single-cell resolution simultaneously across all regions.

Yang et al. conducted real-time functional imaging of single-neuron activities in the primary visual cortex, primary motor cortex and hippocampal CA1 region during anesthetized and awake states in mice. The MATRIEX technique can uniquely configure multiple microscopic FOVs using a single laser scanning device. As a result, the technique can be implemented as an add-on optical module within existing conventional single-beam-scanning, two-photon microscopes without additional modifications. The MATRIEX can be applied to explore multiarea neuronal activity in vivo for brain-wide neural circuit function with single-cell resolution.

Two-photon laser microscopy originated in the 1990s to become popular among neuroscientists interested in studying neural structures and functions in vivo. A major advantage of two-photon and three-photon imaging for living brains include the optical resolution achieved across densely labelled brain tissues that strongly scatter light, during which optically sectioned image pixels can be scanned and acquired with minimal crosstalk. However, the advantages also caused significant drawbacks to the method by preventing the simultaneous view of two objects within a specific distance. Researchers had previously implemented many strategies to extend the limits, but the methods were difficult to implement in neuroscience research labs. Nevertheless, an increasingly high demand exists in neuroscience to investigate brain-wide neuronal functions with single-cell resolution in vivo.

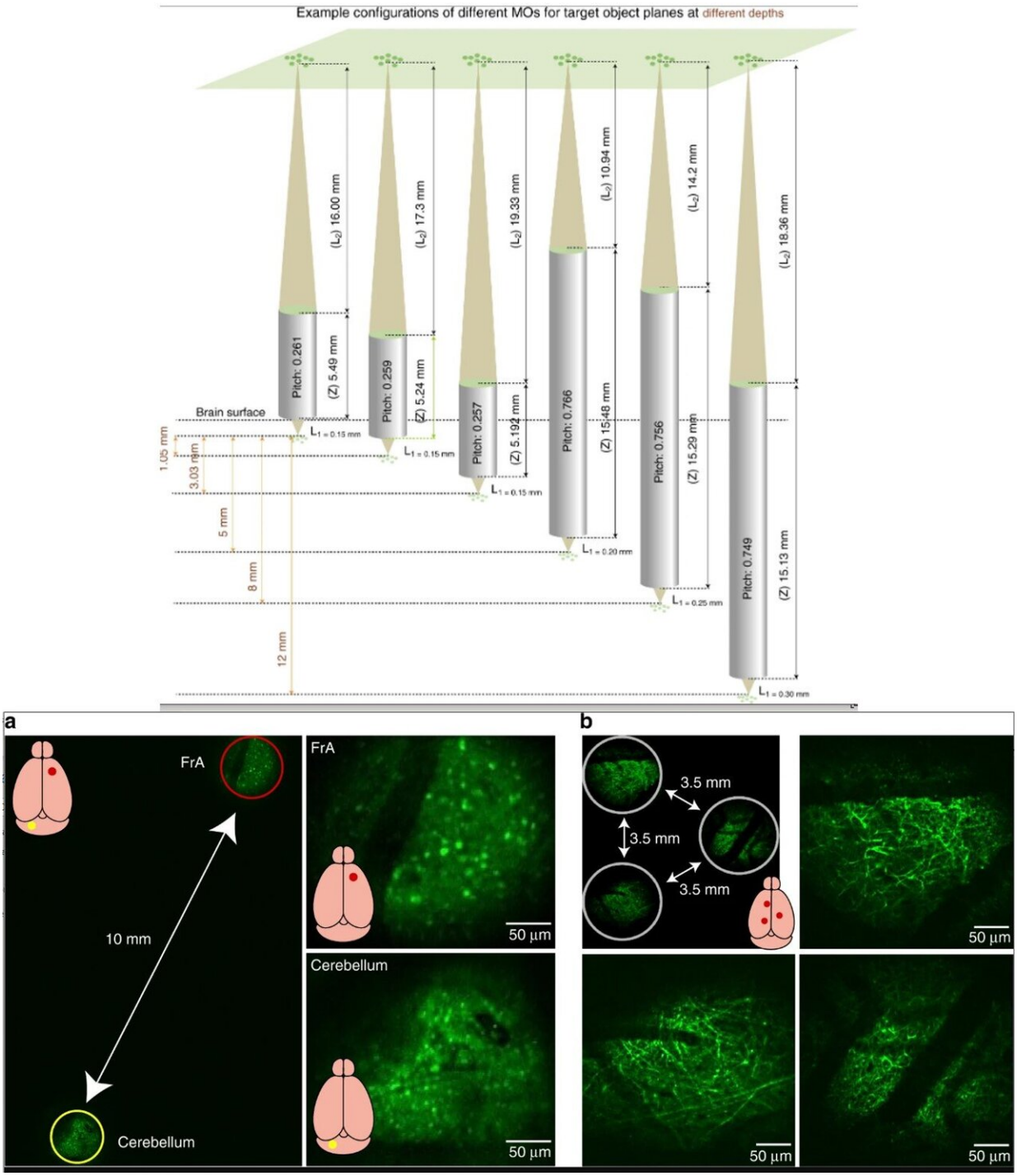


LEFT: Experimental diagram of the MATRIEX imaging system. The two round 3D objects in the lower-left corner are the top and bottom views of the mouse head chamber used for in vivo imaging. (Ti:Sa): Ti:Sapphire ultrafast pulsing laser; PC: Pockels cell; BE: beam expander; SM1 and SM2: x–y scanning mirrors; SL: scan lens; TL: tube lens; DM: dichroic mirror; CL: collection lens; PMT: photomultiplier tube; DO: dry objective; MOs: miniaturized objectives. RIGHT: Illustrations of the two-stage magnification and multiaxis coupling. The square images are actual two-photon images taken of 20-μm beads. Each red circle indicates one FOV. Credit: Light: Science & Applications, doi: 10.1038/s41377-019-0219-x

In a straightforward approach, scientists can place two microscopes above the same animal brain to image the cortex and cerebellum simultaneously. But such efforts can lead to substantial increases in complexity and cost. The existing high expectations for performance and feasibility therefore pose a highly challenging engineering question on

how a single imaging system can simultaneously obtain live microscopic images from multiple brain regions in vivo. To address the question, Yang et al. introduced a new method that combined two-stage magnification and multi-axis optical coupling.

They realized the method using a low-magnification dry objective (DO), with multiple water-immersed, miniaturized objectives (MOs) under the dry objective. The scientists placed each of the MOs at the desired target position and depth in the brain tissue. The team used the new compound object assembly similarly to the original water-immersed microscope objective without additional modifications to the image scanning and acquisition subsystem.



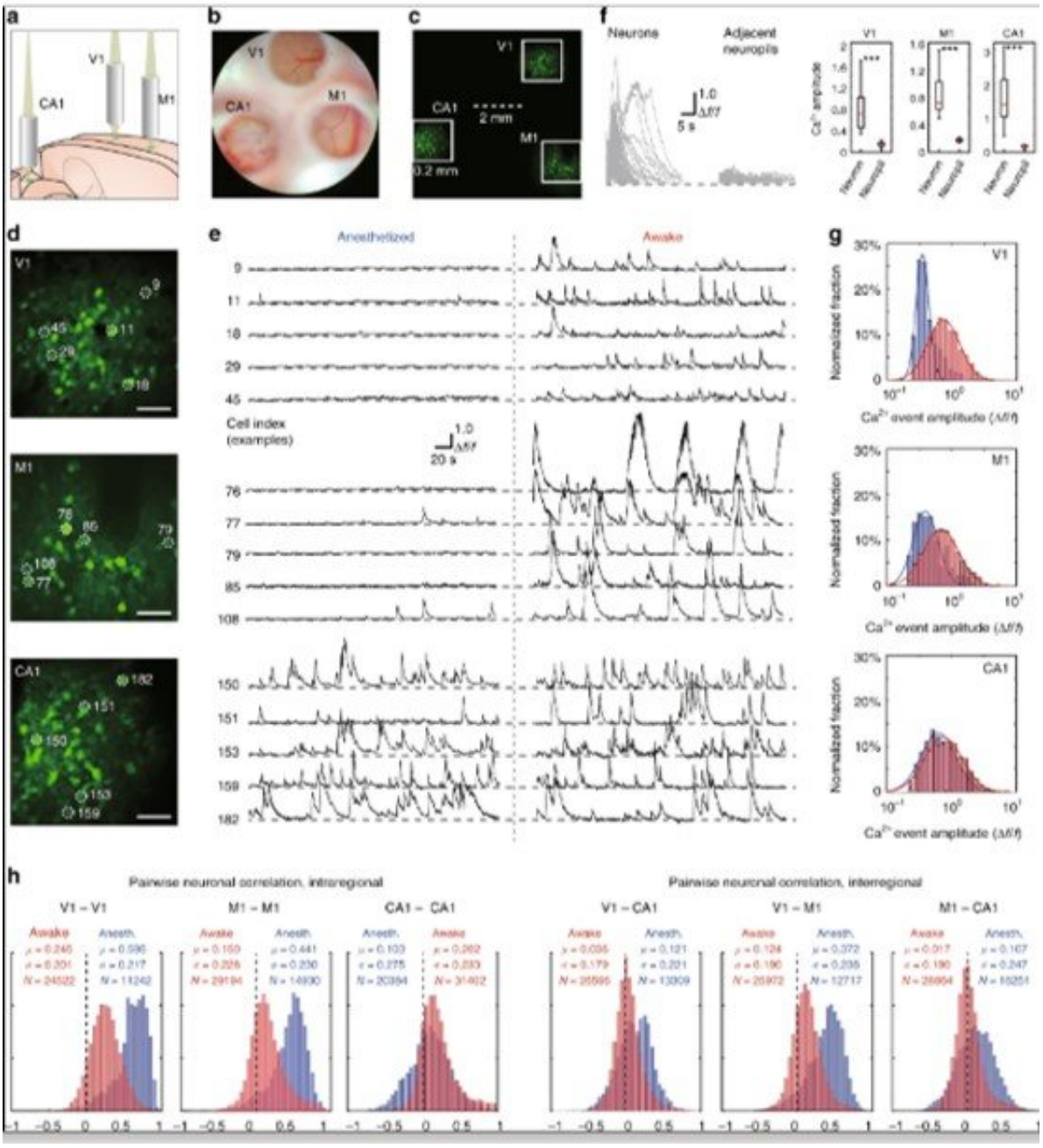
TOP: Configuring the MOs with different parameters to target object planes at different depths to then be conjugated on the same image plane. Each gray cylinder represents one lens with a pitch value, front working distance (L1), back working distance (L2) and length (Z). BOTTOM: Demonstration of MATRIEX imaging: structural imaging in multiple brain areas in vivo. a Left image: a full-

frame image including two FOVs in the frontal association cortex (FrA) and the cerebellum. The red and yellow circles indicate two FOVs that are digitally enlarged and shown in the upper-right and lower-right images. A GAD67-GFP transgenic mouse (with the interneurons labeled brain-wide) was used. Two MOs ('standard version') were placed at the same depth under a DO (Mitutoyo ×2/0.055). b Example configuration of three FOVs in the cortex of a Thy1-GFP transgenic mouse (with layer 5 cortical neurons specifically labeled and with tuft dendrites visible near the cortical surface). Three MOs ('standard version') were placed at the same depth under a DO (Olympus ×4/0.1). Credit: Light: Science & Applications, doi: 10.1038/s41377-019-0219-x

The research team first assembled the MATRIEX compound objective. For this, they replaced the conventional water-immersion microscope objective with a customized compound objective assembly, inside a two-photon laser scanning microscope equipped with a conventional single-beam raster scanning device. The compound assembly contained multiple MOs (miniaturized objectives) inserted through multiple craniotomies during which the scientists glued a 3-D printed plastic chamber to the skull of the mouse model. The chamber roughly aligned the MOs with the same space to adjust lateral position and depth. Yang et al. precisely manipulated the individual MOs to view the objects under all MOs simultaneously in the same image plane.

They implemented the MATRIEX method using two principles; two-stage magnification and multiaxis coupling. For example, using two-stage magnification with the dry objective (DO) alone, they observed 20 µm beads as tiny blurry dots while observing crisp, round circles through the compound assembly. During multiaxis coupling, the scientists coupled a single DO with multiple MOs on the same image plane. Using a simple raster scan in a single rectangular frame, the research team acquired a rectangular image containing multiple circular FOVs (Field of Views) – where each FOV corresponded to one MO with minimal inter-

FOV pixel crosstalk.



Demonstration of MATRIEX imaging: simultaneously acquiring live neuronal activity patterns in V1, M1, and hippocampal CA1 in mice in the anesthetized state or awake state. The neurons were labeled by a genetically encoded fluorescent Ca2+ indicator, GCaMP6f (a) Illustration showing the positioning of three MOs over the V1, M1 and hippocampal CA1 regions in a model mouse

brain. (b) A camera photograph taken through the microscope ocular lens under white light bright-field illumination, in which three FOVs are readily visible. The upper region is V1, the lower-left region is CA1, and the lower-right region is M1. (c) A two-photon image, which is an average of 100 frames, acquired by simple full-frame raster scanning with a two-photon microscope. The solid white boxes show the three parts of the image that are enlarged in panel (d). (d) Digitally enlarged individual FOVs showing neurons in V1, M1, and CA1, from top to bottom. Scale bar: 40 μm. (e) Time-lapse Ca2+ signal traces of five example cells from each region, with each labeled by the cell index. Recordings of the same cell in the same animal in the anesthetized state (left side) and in the awake state (right side) are shown. (f) Left: traces showing individual Ca2+ signal events (split from each onset time and overlaid) from randomly selected example cells. Middle: Ca2+ signal traces of each of the neuropil zones that are directly adjacent to each of the example cells. Right: three box plots comparing the neuronal Ca2+ signal event amplitude to the neuron's adjacent neuropil Ca2+ signal amplitude; paired Wilcoxon rank sum test, ***P

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