Imaging long-range neuronal connections in the mouse brain by light sheet fluorescence expansion microscopy

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Abstract

Sensory perception is modulated in a top-down fashion by higher brain regions to regulate the adaptation of behavioral responses. This process is called olfactory habituation and functionally allows to ignore repetitive, irrelevant stimuli, but remain responsive to a novel, eventually more significant stimuli. In olfactory perception, the horizontal limb of the diagonal band of Broca (HDB) embedded in the basal forebrain modulates olfactory information processing by shaping excitatory olfactory bulb (OB) output and thus regulates olfactory-mediated behaviors. HDB to OB top-down projections constitute a central pathway for olfactory-mediated behaviors. The brain circuits involved in olfactory habituation are currently only partially characterized. Trans-synaptic tracer viruses have also been successfully used to determine synaptic connections over long distances and, notably, can be used to unambiguously determine a synaptic connection (1). By using genetically encoded fluorescent indicators, which specifically label synaptic connections between OB interneurons and HDB projection neurons, we elucidated the network architecture underlying top-down olfactory information processing.

To this end thick sagittal mouse brain slices (~2 mm) were treated with an optimized light-sheet fluorescence expansion microscopy (LSFEM) protocol (2,3), which allows the preservation of even the smallest autofluorescence clusters present in the presynaptic terminals, thus avoiding the use of antibodies.

This optimized LSFEM technique (4) enables zooming from a mesoscopic perspective into super-resolution within a single imaging session using the same sample, thus revealing synaptic connections between OB interneurons and HDB projection neurons.

Here we present this optimized technique for expanding, cleaning, and investigating especially large samples of neuronal tissue with a thickness of up to 2 millimeters for expansion factors of 1.5 to 4-fold, employing LSFEM, and present our results concerning the aforementioned neuronal connections.

References

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