Development of a new version of miniature two-photon microscopes for functional network studies of the medial entorhinal cortex

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The medial entorhinal cortex (MEC) uses multiple spatially modulated cell types such as grid cells, head direction cells, border cells, and object-vector cells to generate a dynamic map of space. How this map is generated is less well understood, although the potential for progress on this question has grown considerably with the introduction of methods for large-scale population recordings. One reasonable starting point for understanding neural systems dynamics in MEC is to describe the anatomical organization and functional connectivity of MEC neurons with known response profiles at single-cell resolution. Such functional mapping can be performed powerfully with cellular-resolution two-photon (2P) imaging of fluorescent calcium indicators [1]. However, the majority of current 2P imaging protocols require the animal to be head-fixed and to navigate in virtual environments, which can impede the analysis of spatially tuned cell types [2]. A miniature, portable two-photon microscope has recently been developed to address this limitation [MINI2P; 3]. This microscope has high spatial resolution, light weight and good stability, but still faces problems that prevent its direct application to large-scale MEC imaging in mice exploring extended open field arenas (MINI2P Version 1, [3], 2017 and Version 2, paper under review).

Here we report progress on developing a new version of miniature two-photon microscopes for large-population MEC imaging in freely moving mice. To be able to simultaneously record many hundreds of neurons, we re-designed the optics of the 2017 microscope to extend the FOV from ~130x130 μm^2 to ~420x420 μm^2. To further increase recorded cell numbers and to cover continuous 3D anatomical space, we are now developing miniature, focus tunable modules, which enable rapid sampling from multiple imaging planes. To adapt to various chronic implants such as cranial windows, GRIN lenses and prisms that support recordings in different regions and depths, we have customized several interchangeable objectives, which feature different magnifications, media and working distances. To further promote naturalistic behavior in large open fields, we decreased the scope body weight by using lighter materials, and we improved the flexibility of the collection fiber bundle with a new compound design. Finally, to quantitatively analyze the data, a complete data processing and analysis pipeline has been developed [4]. With the help of this new version of miniature two-photon microscope, we are now able, in freely moving mice, to monitor activity in volumes containing many hundreds of cells, including all known spatially modulated cell types. We are currently investigating how these cells are topographically organized and functionally connected within and across layers in superficial MEC.

[1] Gu, Y., et al., *Cell*, **175**, 736–750 (2018)
[2] Minderer, M., et al. *Nature* **533**, 324–325 (2016)
[3] Zong, W., et al., *Nat Methods* **14**, 713–719 (2017)
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