Application Note
#2006

Webinar Recap: Imaging Hippocampal Microcircuitry Using Glass Microperiscopes

Multiphoton microscopy (2P) overcomes many of the limitations that conventional microscopy faces by being able to image deeply, quickly, and in 3D — providing compelling and useful data in a variety of disciplines. A combination of ultra-fast pulsed lasers, lenses with high numerical apertures, and highly sensitive detectors provide fast Z imaging and makes Bruker's 2P technology a go-to technique for revealing dynamic biological processes deep within living tissues. This webinar recap summarizes the webinar content we presented with Michael Goard, PhD., an Associate Professor for the Department of Molecular, Cellular & Developmental Biology at UC Santa Barbara. His lab investigates the neural circuitry underlying perceptual, spatial, and cognitive abilities in the mouse cortex and hippocampus by using two-photon microscopy, microprisms, and microperiscopes.

Why Use Microprisms and Microperiscopes

Microperiscopes are glass devices chronically implanted into different brain regions to provide novel access to neural circuitry. By imaging tissue transverse to the plan of the objective, users can image otherwise inaccessible regions at a relatively high resolution and in vivo. Now a relatively well-studied concept, microperiscopes have a similar principle to the periscopes that you may see on a submarine. However, the periscopes designed to let researchers unravel neurological processes and structures are flipped upside down and implanted into the brain. An important consideration is that this form of imaging does cause tissue damage and users must be conscious that by implanting this device, they are severing surrounding pathways. However, using these technologies for functional imaging damages a smaller volume of the cortex and, with actions directed at reducing detrimental effects, combined with 2P microscopygives a new approach to accessing deep structures in the brain.

ABOUT THE RESEARCHER

Michael Goard, Ph.D.,
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Imaging the Transverse Plane of the Hippocampus

2P technology has the ability to access deep structures and to flip optical access, which Michael Goard and his lab used to investigate and image neural activity in the transverse hippocampus in mice. This project led by Will Redman, a DYNS graduate student, with significant contributions made by Nora Wolcott, a MCDB graduate student, looked at the behavior of the hippocampal microcircuit subfields CA1, CA3, and the dentate gyrus (DG). To begin, they designed a microperiscope large enough to achieve a field-of-view that encompassed the entire hippocampus. After a sensitive surgery to implant the prism into the hippocampus, they created a nice optical cross-section (see Figure 1).

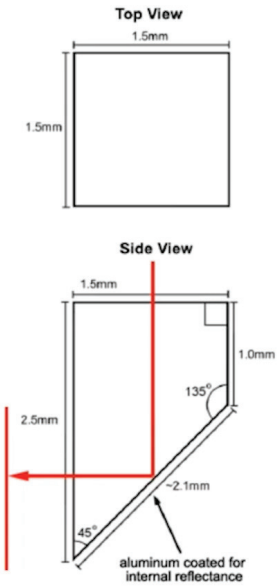

FIGURE 1.

The UC Santa Barbara microperiscope was designed to have a large field-of-view that encompassed the entire mouse hippocampus. Top view shows the footprint, and the side view shows the dimensions of the prism that was implanted.

Using Bruker's Ultima Investigator Plus, which Goard described as a great workhorse system for two-photon imaging, they found that there was a sparse expression of neurons in CA1 and CA3 with nice structural resolution but a dense expression in the DG (see Figure 2). The implant for this experiment causes localized damage to the cortex and hippocampus but was found to be stable over months and, therefore, was utilized for long-term imaging of these neurons (see Figure 3).

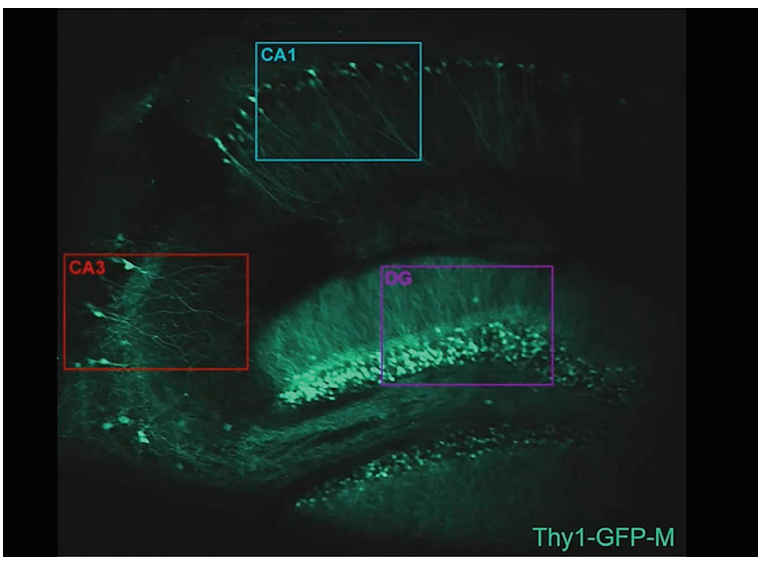

FIGURE 2.

Tiled average projection of the transverse imaging plane using the v2HPC microperiscope implant in a Thy1-GFP-M transgenic mouse.

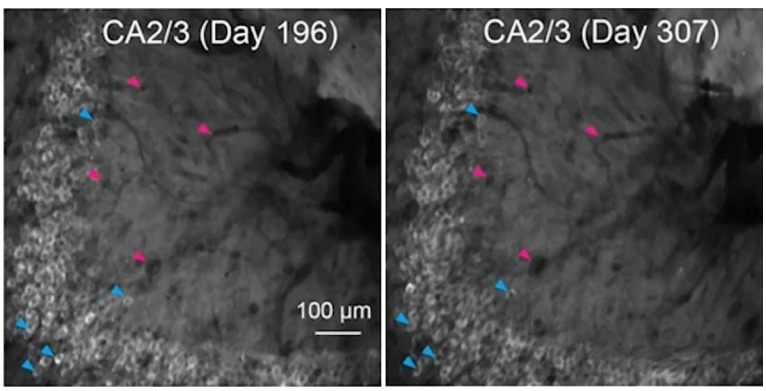

FIGURE 3.

Example average projection of CA2/3 imaging plane 196 days post-implantation (left) and 111 days later (right). Image was aligned using non-rigid registration to account for small tissue movements. Magenta arrowheads mark example vasculature and blue arrowheads mark example neurons that are visible in both images.

During this study, they found that imaging through microperiscopes reduces axial resolution, but still achieves single-cell resolution sufficient for imaging subcellular compartments. Specifically, sparsely expressing GFP revealed individual spines in the CA1-CA3 subfields that they were then able to define as stubby, thin, mushroom, or filopodium. Next, they determined the average number of spines and their turnover rates with filopodia turning over faster than other spine types – which was an expected result (see Figure 4). Lastly, they imaged functional activity across the hippocampal circuit. By putting mice in an ecological "floating environment," they were able to behave under their own control while the researchers measured functional responses, such as place fields. They found that responses weren't super reliable, but studies in other labs also revealed less robust responses when subjects were not given rewards. However, they were able to measure place cells in CA1, CA3, and DG and found that each region had different proportions of place cells and speed cells (see Figure 5). For example, CA1 has a higher proportion of place cells than CA3 or DG, and DG had lots of cells that were speed modulated but fewer that had nice spatial responses.

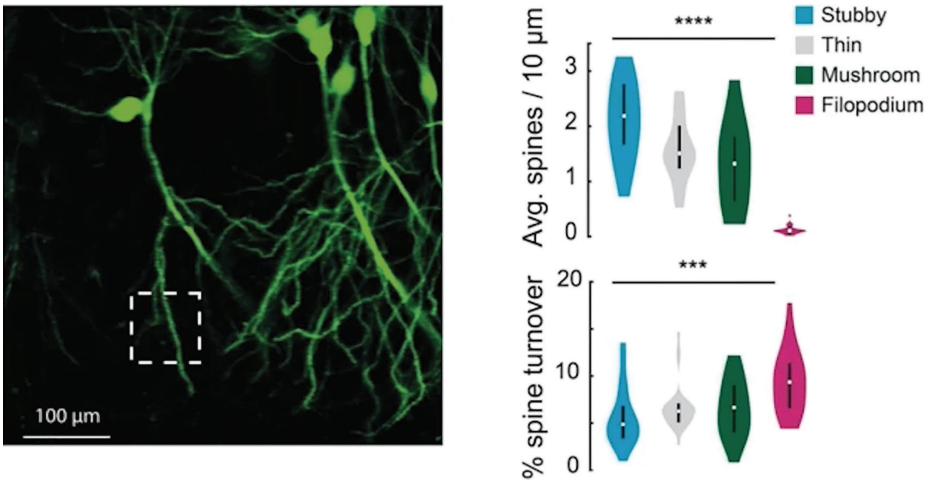

FIGURE 4.

Left: Average projection of CA1 neurons sparsely expressing a GFP reporter (Thy1-GFP-M) imaged through the v1CA1 microperiscope.
Right: (Top): Average number of spines per 10 µm section of dendrite for each of the four classes of spine. (Bottom): Percent spine turnover across days in each spine type.

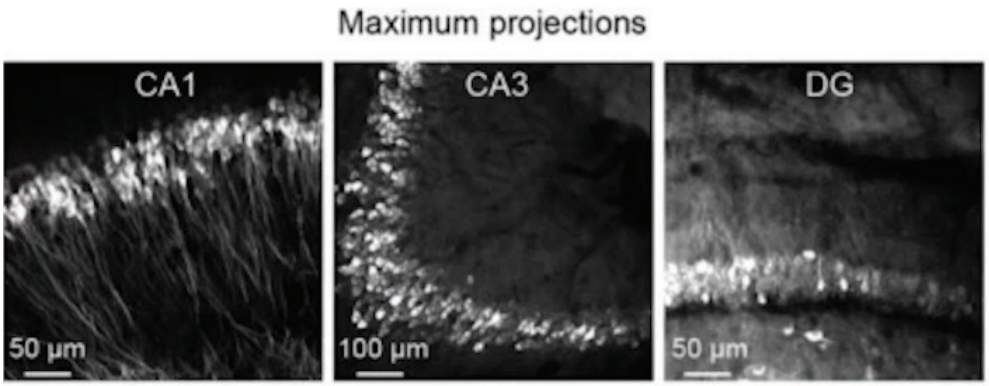

FIGURE 5.

Example maximum projections of GCaMP6s-expressing neurons in each subfield.

Considerations and Solutions

Microprisms and microperiscopes are unique technologies that are creating previously unattainable images and producing groundbreaking data. Importantly, Michael Goard talks in our webinar about some of the key challenges associated with these techniques and how to overcome them to create valuable data:.

1. Damage is limited to 100-150 microns – the acute damage is limited, and researchers can plan their experiment around the location of the brain region they want to observe;
2. Implanting the prism can give a nice optical cross-section but damages the targeted structure – can maximize information collected by conducting these manipulations near the end of the experiment;
3. Reflecting your image (as is the nature of microprisms) causes modest reductions in image quality – imaging fluorescent microspheres smaller than the diffraction limit of your imaging system creates point spread functions with good resolutions;

4. Using an incision approach, rather than a typical 3 millimeter circle removal, requires an experienced surgeon – an incision has a high success rate for keeping targeted structures intact.

Ongoing studies with microprisms and microperiscopes

Within the Goard lab and in collaboration with other research labs, there are various questions being explored by using 2P technology such as:

Tracking dendritic morphology across days

How do hippocampal neural structure and function change throughout the mouse estrous cycle?

Comparing responses across and within subregions

Regarding social coding in Ca2 neurons, how do different subfields of the hippocampus respond differently during tasks and what are the resulting dendritic responses?

Imaging dendritic responses

How do dendritic plateau potentials and dendritic events play a role in place field formation and synaptic plasticity in the hippocampus?

Optogenetic dissection of circuit function

After using two-photon excitation to stimulate cells in particular subregions, what can we learn by probing the hippocampal circuitry?

Conclusion

2P technology is offering novel approaches to studying pressing questions in neuroscience research. Michael Goard and his lab utilize microprisms, microperiscopes, and multiphoton microscopy to study the microcircuitry of the hippocampus, specifically the subfields CA1, CA3, and DG, which are critical for memory formation. By developing an approach for two-photon imaging of the transverse hippocampal plane in awake mice via implanted glass microperiscopes, they tracked dendritic morphology over repeated sessions while measuring functional responses using genetically encoded calcium indicators. Bruker's suite of multiphoton solutions continues to offer the imaging speed and single-cell resolution needed to create compelling images of live processes deep within the brain.

Resources

To view the webinar "Imaging Hippocampal Microcircuitry Using Glass Microperiscopes" visit here.

Read Michael Goard's most recent publication "Long-term transverse imaging of the hippocampus with glass microperiscopes" here

To learn more about Bruker's impressive suit of multiphoton solutions visit here

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