

Exploring Olfactory Bulb Glomeruli with Serial Section Electron Microscopy

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Serial section electron microscopy (ssEM) is a powerful tool for analyzing complex structures in the brain. Cutting, collecting, imaging, and analyzing series of 100 – 200 ultrathin (~50 nm) sections of various brain regions can provide information about synaptic connectivity, subcellular organization, and surrounding cellular composition that would be more challenging to glean from lower resolution imaging techniques. In particular, areas where multiple cell types converge to process sensory information such as olfactory bulb glomeruli benefit from the use of ssEM. Receptors expressed on olfactory sensory neurons (OSNs) in the nasal epithelium bind to odorants and those OSNs expressing the same receptor all converge onto the same glomerulus in the olfactory bulb. Within a glomerulus, OSNs form excitatory synapses onto the dendrites of a variety of cell types, including the principal excitatory neurons, mitral and tufted cells that project to higher cortical areas. Rather than a simple relay station, physiology experiments have revealed that odor signals undergo complex processing within glomeruli from an assortment of inhibitory and neuromodulatory inputs [1]. However, the anatomical correlates of this signal processing have been difficult to decipher due to the intermingling of excitatory and inhibitory neuronal cell types and compartments (axons and dendrites) that can be indistinguishable on single sections. Analyses are further complicated by the presence of dendrodendritic synapses and gap junctions in addition to the typical axodendritic synapses [2]. Here we combined the selective labeling of mitral cells (MCs) and a subtype of tufted cells, external tufted cells (eTCs) with an electron dense substrate and followed their labeled dendrites through serial sections to determine their respective synaptic connectivity. In addition, we were able to identify unlabeled cell types based on ultrastructural characteristics that could be confirmed across multiple sections and determined their relationship to the labeled dendrites within the same series.

We prepared olfactory bulb slices from three young Sprague Dawley rats (P8-14) and labeled MCs and eTCs with biocytin and then created an electron dense substrate within their dendrites using an avidin-biotin complex and 3,3'-diaminobenzidine (DAB). The slices were fixed and processed for electron microscopy [3]. Serial sections (50 nm thickness) of glomeruli containing DAB-labeled MC or eTC dendrites were cut and imaged either on an FEI Tecnai G2 transmission electron microscope at 80 kV with a Gatan UltraScan 1000 digital camera or a Zeiss SUPRA 40 field-emission scanning electron microscope (FE-SEM) equipped with an integrated module called ATLAS (automated large area scanning; software version 3.5.2.385; [4]). The serial section images were aligned and dendrites were traced in RECONSTRUCT software [5].

Although the DAB precipitate obscured postsynaptic densities necessary to determine whether synapses onto the labeled MC and eTC dendrites were asymmetric (excitatory) or symmetric (inhibitory), we were able to use other ultrastructural criteria including the appearance of round, clear vesicles (excitatory, Figure 1) or flattened, pleiomorphic vesicles (inhibitory) docked at the active zone. In addition, we followed the presynaptic processes to where they formed other synaptic contacts and confirmed whether they were asymmetric or symmetric. Using this approach, we found that MCs and eTCs have similar densities of excitatory and inhibitory synaptic inputs [3]. On unlabeled dendritic processes, we were able

to identify putative MCs based on whether they formed excitatory dendrodendritic synaptic contacts and if they had a gap junction, identified as dark, dense-staining plaques between adjacent dendritic membranes. Three dimensional reconstructions of these dendrites allowed us to determine how synaptic inputs, dendrodendritic release sites, and gap junctions were organized relative to one another. Finally, we drew a 2x2 micron grid around dendrodendritic release sites of the labeled MCs and eTCs and analyzed the composition of the surrounding neuropil for the two cell types including the distribution of astroglial processes that could be important for regulating the spillover of glutamate (Figure 2). The use of ssEM and 3-D reconstructions will provide further insight on the organization of olfactory bulb glomeruli and help us determine how the ultrastructure facilitates sensory processing.

References:

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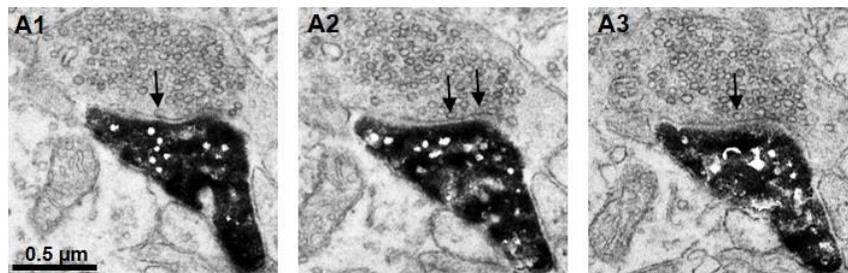


Figure 1. Serial sections of an excitatory OSN synapse (A1-A3) onto a DAB-labeled mitral cell dendritic process. Docked synaptic vesicles are indicated by arrows.

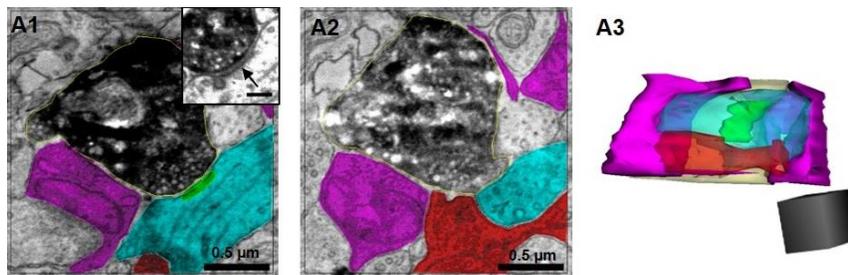


Figure 2. Sequential electron micrographs (A1 and A2) and three dimensional reconstruction (A3) of a DAB-labeled eTC dendrite (light yellow in the reconstruction) forming a synapse (green) onto an inhibitory dendrite (blue). A putative MC dendrite (red) and glial processes (purple) are in close proximity. Insert in A1 shows the same image as A1 but without the colors so that the synapse (arrow) can be seen more clearly. Scale = 0.1 micron in insert. Scale cube = $0.5 \mu\text{m}^3$.