



3D Imaging of Axodendritic Contacts in the Barn Owl Auditory Space Map

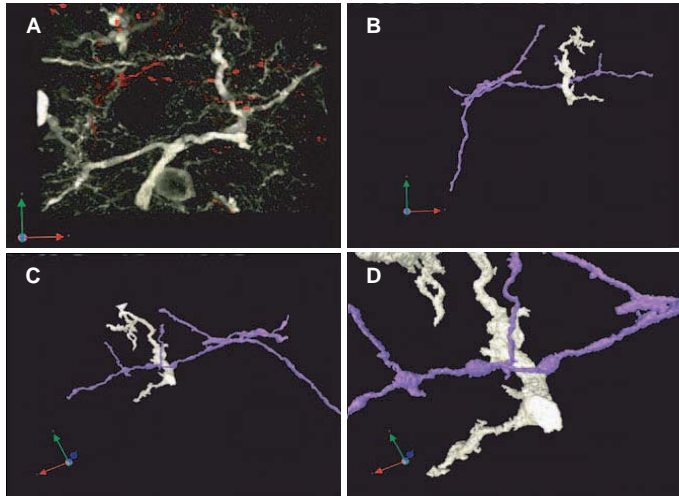


Figure shows axodendritic contacts onto CaMKIIa ICX neurons. **A)** **Volocity Restoration** deconvolved confocal image stack showing CaMKIIa cells in grayscale and ICCLs axons in red. **B)** **Volocity Visualization** rendered 3D reconstruction showing a portion of an isolated dendrite receiving a candidate contact. **C)** The 3D volume is rotated around the candidate contact (fly-through visualization, **Volocity**). **D)** Close-up of candidate contact between axonal bouton and CaMKIIa dendrite.

Dendrite is shown in white, axon is shown in purple; white scale bar = 2µm; the RGB colored vector on the bottom left of the 3D reconstructions depicts the x-axis in green, y-axis in red and z-axis in blue.

Dr DeBello and colleagues from the University of California have developed a method for high resolution confocal imaging and quantitative 3D image analysis to map out the precise spatial pattern of thousands of synaptic contacts in the Barn Owl (*Tyto alba*) auditory system. Synaptic contacts in the auditory system are physically altered during experience-dependent plasticity and therefore this study provides an ideal model for learning and plasticity in the brain.

Many neurons in the owl midbrain respond only to sounds arising from discrete directions. They are termed space-specific neurons (SSNs) and strongly express calcium/calmodulin-dependent protein Kinase type-II (CaMKIIa). These cells, mainly located in the external nucleus of the inferior colliculus (ICX), are topographically arranged to form a map of auditory space. The input to the ICX is a topographic axonal projection that originates in the lateral shell of the central nucleus of the inferior colliculus (ICCLs). The output from the ICX is also topographically organized and relayed to deep layers of the optic tectum (OT).

The aim of this study was to visualize axodendritic contacts between neurons using immunohistochemistry, antero- and retrograde tracing, high resolution confocal microscopy, three dimensional reconstruction and fly-through visualization. Deconvolution of confocal images was performed with **Volocity Restoration** using measured PSFs and an iterative maximum entropy algorithm. To search for sites of putative synaptic contact (PSC) between tracer labeled neurons and CaMKIIa SSNs, three dimensional profiles were generated from confocal stacks using the high resolution rendering mode in **Volocity Visualization**. Isolated processes were inspected by fly-through visualization, during which both viewing angle and vantage point were freely adjustable (see **figure**). Further analysis was performed with **Volocity Classification** (now known as **Quantitation**). Boutons were identified by size and diameter, total axonal length and surface area was measured using skeletal length and surface tools. This method effectively screened out a number of sites that initially appeared as candidate contacts, but under three dimensional inspection failed to meet the criteria.

This study reveals numerous sites of synaptic contact between ICCLs axonal boutons and the dendrites of CaMKIIa ICX, suggesting that these neurons are a cellular locus for computation of auditory space-specific responses. They receive monosynaptic input from ICCLs and are characterized by excitatory output to the OT. Because the ICX is the major site for learning, these results lay the groundwork for probing microanatomical rearrangements that may underlie plasticity and learning.