



Donut-like Topology of Synaptic Vesicles in the Adult Calyx of Held

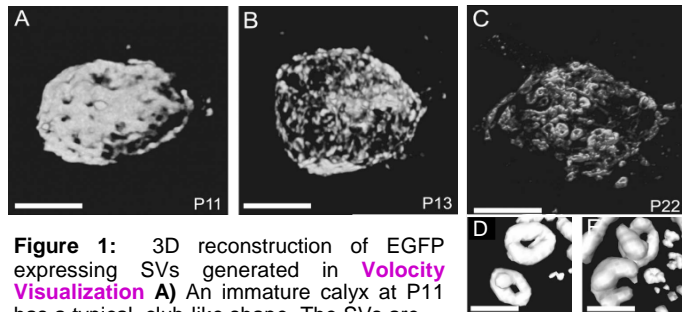


Figure 1: 3D reconstruction of EGFP expressing SVs generated in **Volocity Visualization** **A)** An immature calyx at P11 has a typical club-like shape. The SVs are almost homogeneously distributed with a slight tendency to form aggregates. **B)** With the onset of hearing (P12-P14), when the calyx has developed a more digitated morphology, ring-like local accumulations of SVs became apparent. **C)** At around P21 most SVs were arranged in clusters resembling donuts. **D, E)** The detailed structure of donuts. Scale bars **A-C:** 5 μ m, **D, E** 1 μ m

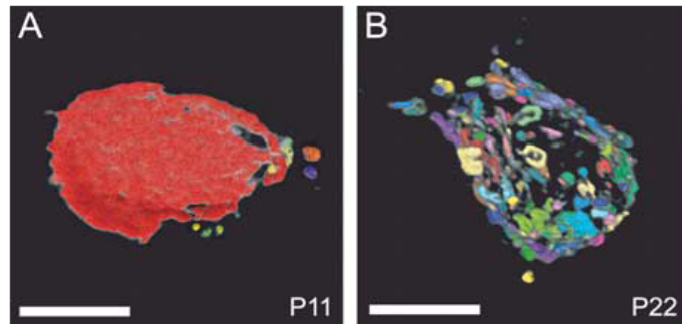


Figure 2: **Volocity Classification** was used to identify independent volume units within each confocal stack rendered in 3D. Each volume unit is represented in a different colour **A)** P11 **B)** P22. Scale bars, 5 μ m

Dr. Kuner and colleagues from the department of Cell Physiology at the MPI in Heidelberg study structural and functional properties of synapses. For their research, they use the giant nerve terminal of the rat auditory brain stem known as the calyx of Held, one of the largest nerve terminals in the CNS of mammals.

The synapse formed by the calyx with its postsynaptic neuron, the principal cell of the medial nucleus of the trapezoid body (MNTB), is highly specialized to meet the demanding functional requirements needed for processing of sound. Whereas a typical central nerve terminal contains a single active zone (AZ), the location of vesicular neurotransmitter release, the calyx contains 500-800 AZs functioning in parallel. This high number of AZs can only be accommodated by an increase in size. These specializations of the calyx typically emerge during the first three postnatal weeks. Of special interest is the functional organization of synaptic vesicles (SVs), because a continuous replenishment of SVs at the AZs is required to sustain this high-frequency neurotransmission.

In this study the researchers investigate SVs at different postnatal stages of maturation by genetically labeling vesicles with synaptophysin-enhanced green fluorescent protein (EGFP). Image stacks of fixed brain slices were acquired with a laser scanning confocal microscope and 3D reconstructions were generated using **Volocity Visualization**. **Fig. 1** shows the arrangement of SVs at different postnatal stages of maturation (see also the QTVR movie file at <http://www.jneurosci.org/cgi/content/full/26/1/109/DC1> created in **Volocity**). The number and volume of SV clusters was determined in immature and mature calyces using object detection and measurements functions in **Volocity Classification** (now know as **Volocity Quantitation**). **Fig. 2** shows 3D rendered images of distinct contiguous volumes in P11 and P22 terminals. Analysis showed that the number of donut like SV clusters increases whereas the total vesicle volume remains constant during postnatal maturation.

This study describes a new topological property of a central presynaptic nerve terminal in the adult nervous system of the rat: within distinct membrane protrusions, SVs form donut-like assemblies housing a central cluster of interconnected mitochondria. These structural units had not been discovered previously, since most studies have focused on immature calyces and have not used 3D reconstructions to analyze immunohistochemical data. 3D reconstructions generated in **Volocity** helped Dr. Kuner and colleagues to identify the donut-like topology and contribute to the understanding of the adult auditory system.