

## **Three-Dimensional Analysis of Spinule-Bearing Boutons Within Inhibitory Synapses in CA1 Hippocampus**

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A crucial synapse type found in the brain is the inhibitory synapse, which regulates the timing of neuronal activity and whose dysfunction can result in neurological disorders such as epilepsy, and schizophrenia. Although inhibitory synapses are critical to normal brain function, a key part of their anatomy, called synaptic spinules, remains unexplored. Synaptic spinules are thin, finger-like projections produced by one neuron that embed themselves into another neuron's presynaptic bouton (neurotransmitter-releasing end of a synapse). Work on excitatory synapses suggests that spinules may represent a new form of neuronal communication and/or regulate the synaptic strength and stability, yet there is no published data on spinules within inhibitory synapses. In order to study spinules within inhibitory spinule-bearing boutons (SBBs) in detail, we quantified the prevalence and characteristics of spinules within inhibitory boutons in an electron microscopic image volume from CA1 hippocampus (memory formation center) of an adult male mouse. To this end, we three-dimensionally reconstructed 38 boutons, 22 spinules, and 58 postsynaptic densities (synaptic regions) and calculated their surface areas and volumes. We discovered that 46% of perisomatic inhibitory boutons in our volume were SBBs, and that SBBs were 41% larger than non-SBBs and contained significantly larger mitochondria. These findings demonstrate that synaptic spinules are ubiquitous structures within CA1 inhibitory boutons, that inhibitory SBBs represent a subpopulation of larger and likely stronger boutons, and that somatic spinules may allow for novel forms of excitatory to inhibitory communication.