Analysis and 3D Reconstruction of Perisomatic Inhibitory Boutons in CA1 Hippocampus

Elizabeth Turner and Marc Nahmani
Division of Sciences & Mathematics, University of Washington Tacoma

Abstract

- Inhibitory boutons function to regulate timing of neuron activity and are critical to normal brain function
- Neurites (like axons) and cell body of the neuron (soma) produce projections that are enveloped into another neuron's bouton (neurotransmitter releasing side of the synapse)
- Spinule prevalence and characteristics have been studied in excitatory synapses but not explored in inhibitory synapses
- Spinules have been hypothesized to play roles in stabilizing mature synapses and acting as communication between neurons
- Electron microscope image volume of CA1 hippocampus from an adult male mouse was analyzed for inhibitory synapses (n = 60) from one pyramidal soma
- Found that 48% of inhibitory boutons were spinule bearing boutons (SBBs) suggesting they are common structures with inhibitory synapses in the hippocampus
- 22 inhibitory synapses were randomly selected and were three dimensionally reconstructed with spinules (if present), post synaptic densities (i.e., synapse size), and mitochondria (if present)
- SBBs tended to be larger in volume (115 %) and in surface area (130 %), an indicator of synaptic physiological strength
- Trends observed helps to support the idea that spinules help strengthen synapses due to SBBs larger volume and synapse size, indicating spinules as markers of stronger and most matured inhibitory synapses in CA1 hippocampus

Methods

- **Focused Ion Beam Scanning Electron Microscopy Image Volume:** This study used a large previously published focused ion beam scanning electron microscopy (FIB-SEM) image volume from stratum pyramidale of CA1 hippocampus from an adult male mouse, provided by Dr. Shu-Hsien Sheu (Sheu et al., 2022, Cell 185, 3390–3407).

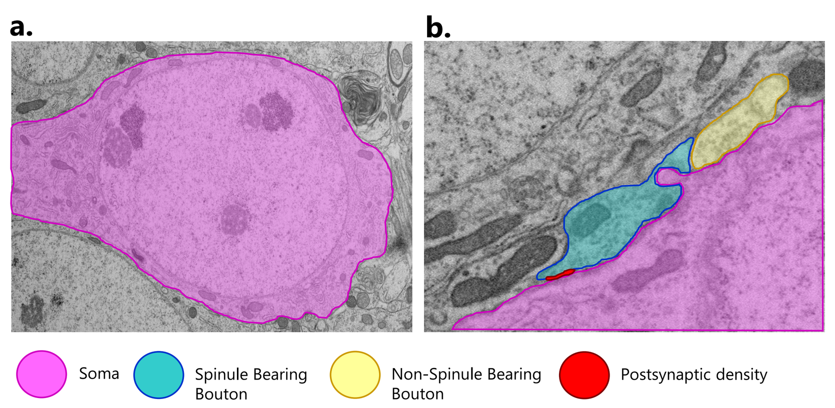
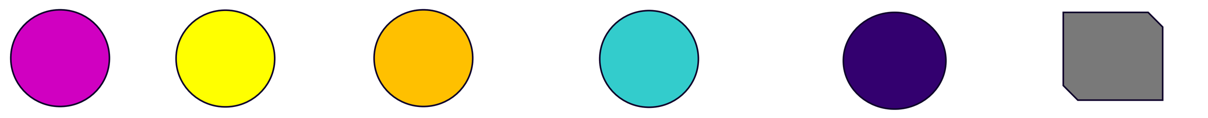


Figure 1a. Using ImageJ (FIJI), we were able to analyze one soma for inhibitory synapses.

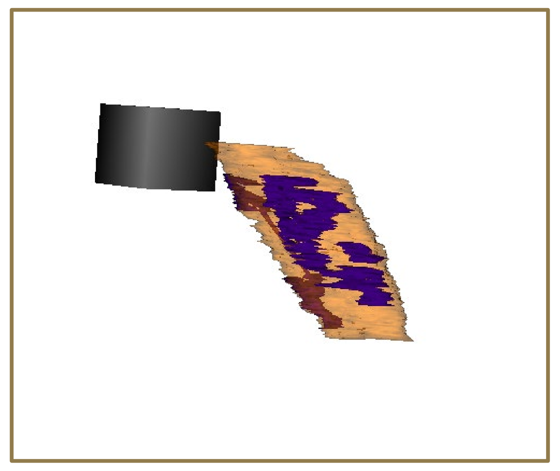
Figure 1b. Inhibitory synapses were identified by the PSD and the boutons were named, described, and recorded (n = 60). After analyses, 12 SBBs and 10 non-SBBs were 3D modeled in a software called Reconstruct by outlining each bouton, PSD, and spinule. The models were measured for volume and surface area of the boutons, spinules, PSDs, and mitochondria.

Reconstructions

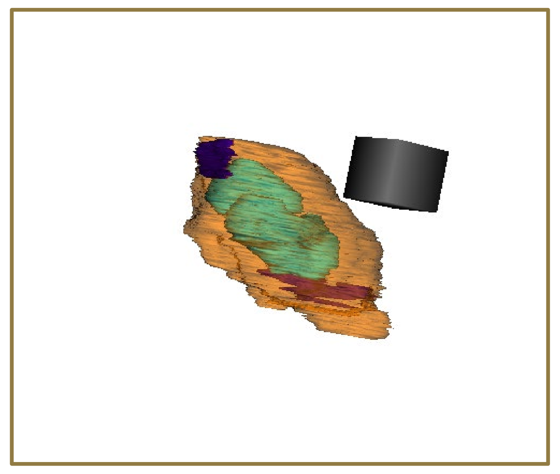
PSD: Postsynaptic density
SBB: Spinule bearing bouton
Non-SBB: Non-spinule bearing bouton



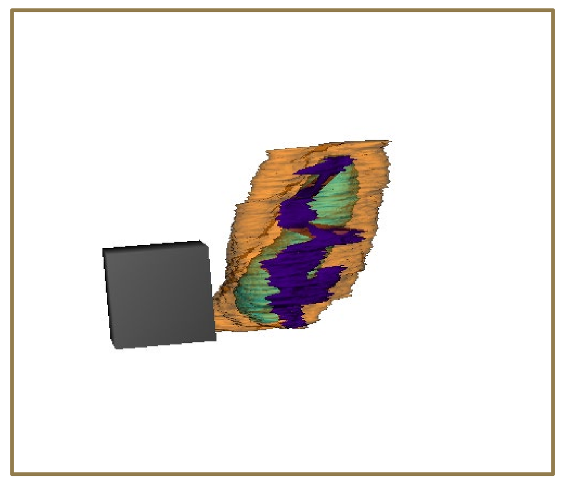
Non-SBBs



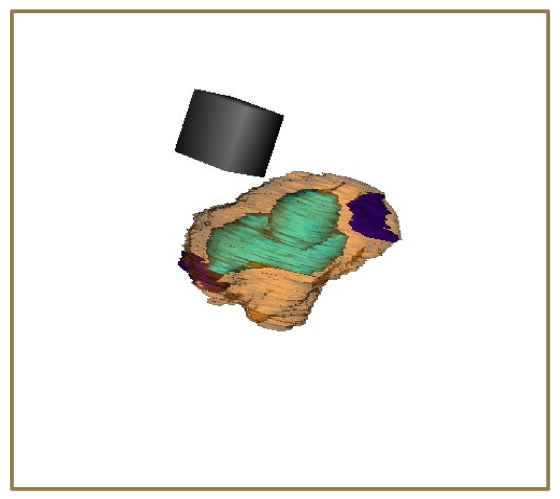
Non-spinule bearing bouton with two PSDs demonstrating the smaller volume of non-SBBs



Non-spinule bearing bouton with two PSDs

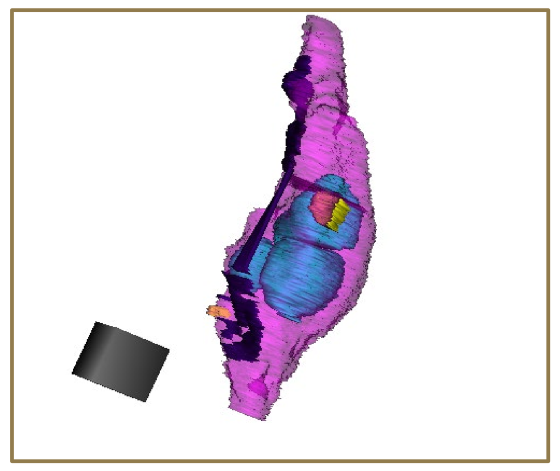


Non-spinule bearing bouton with one PSD

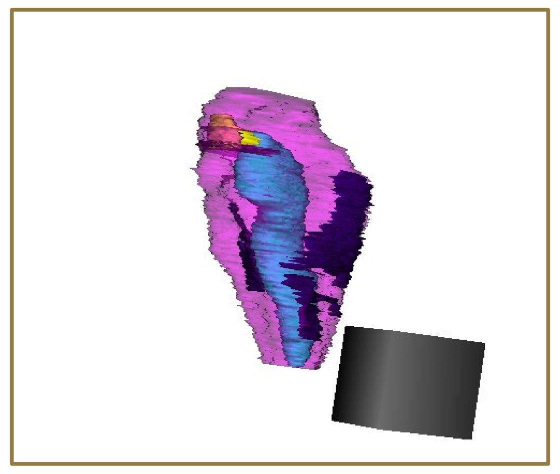


Non-spinule bearing bouton with one PSD

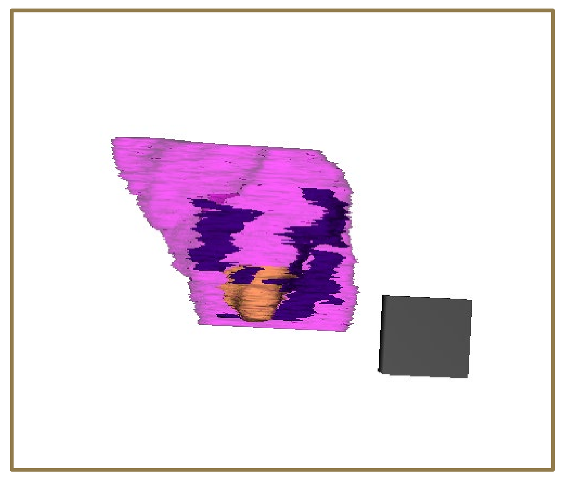
SBBs



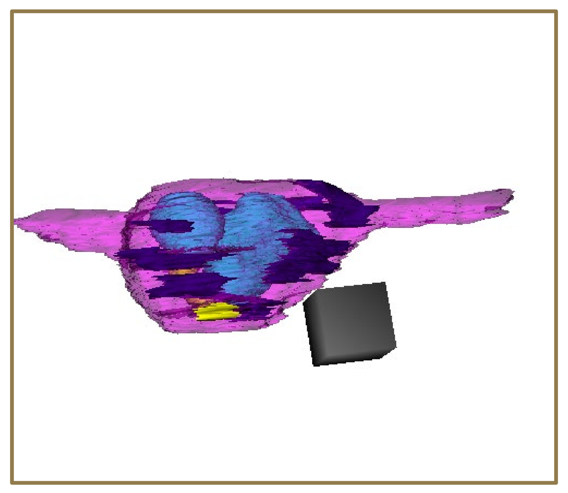
Spinule bearing bouton with one PSD and spinules from a soma and an antspinule from bouton into soma demonstrating larger volume of SBB



Spinule bearing bouton with two PSDs and a spinule from a soma



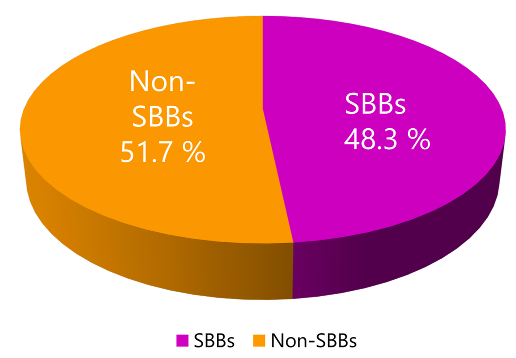
Spinule bearing bouton with one PSD and an antspinule into a soma



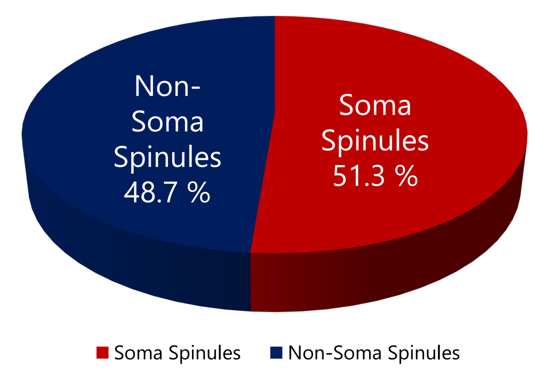
Spinule bearing bouton with two PSDs and three spinules

Results

Prevalence of SBBs and Non-SBBs in CA1 Hippocampus



SBBs with soma spinules compared to non-soma spinules



a. Comparative Volumes between SBBs vs non-SBBs

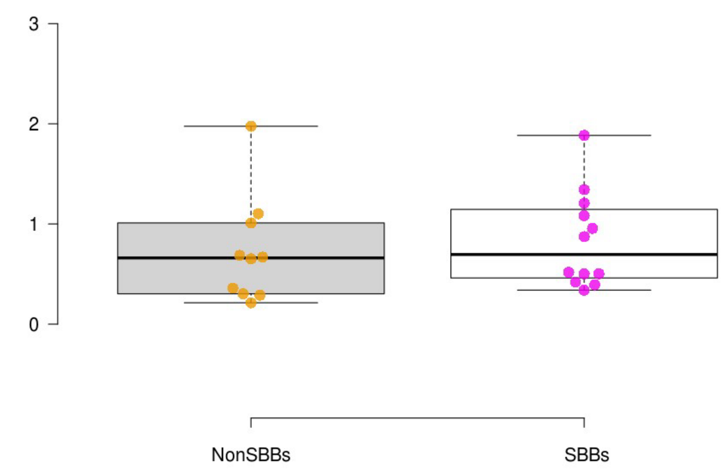


Figure 2a. Volume differences between SBBs and non-SBBs mapped along a Box-whisker plot (or a frequency histogram) which demonstrates there is no significant difference.

Data collected from **12** SBBs and **10** non-SBBs.

b. Comparative Surface Areas Between SBBs vs non-SBBs

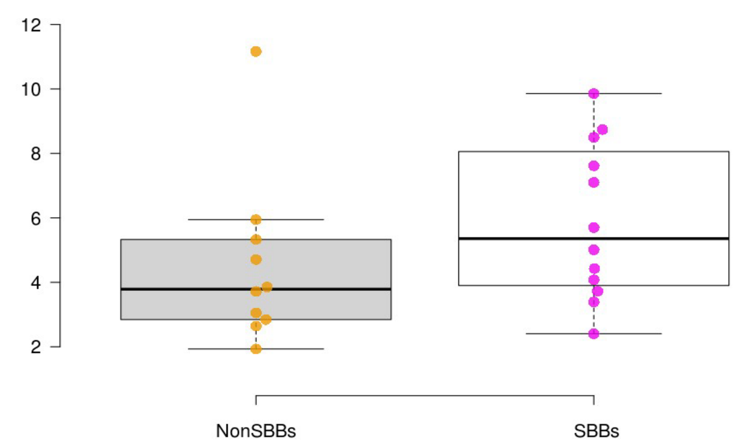


Figure 2b. Surface area differences between SBBs and non-SBBs mapped along a Box-whisker plot (or a frequency histogram) which demonstrates there is no significant difference.

Data collected from **12** SBBs and **10** non-SBBs.

c. Comparative Surface Areas of PSDs Between SBBs vs non-SBBs

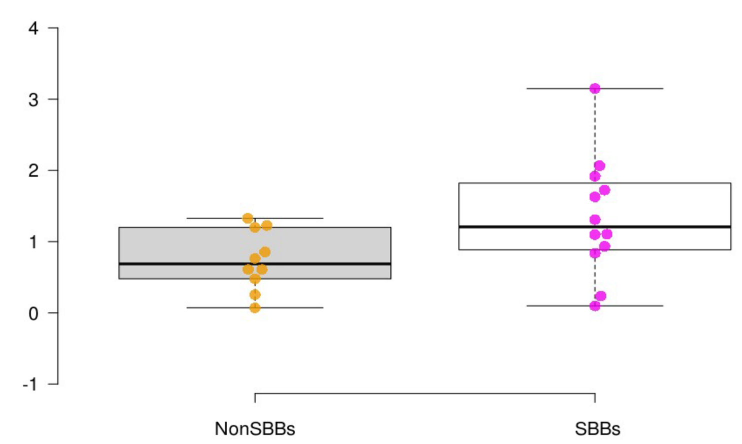


Figure 2c. Surface area differences of SBB PSDs and non-SBBs PSDs mapped along a Box-whisker plot (or a frequency histogram) which demonstrates there is no significant difference.

Data collected from **18** SBB PSDs and **12** non-SBB PSDs.

d. Comparative Volume of Mito Sum Between SBBs vs non-SBBs

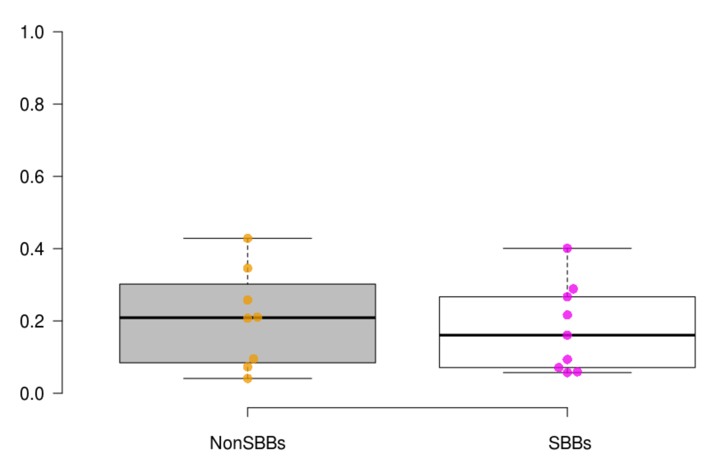


Figure 2d. Volume differences between SBB and non-SBB mitochondria sums mapped along a Box-whisker plot (or a frequency histogram) which demonstrates there is no significant difference.

Data collected from **12** SBB mitochondria and **17** non-SBB mitochondria.

Conclusion

- **48%** of inhibitory boutons in the CA1 hippocampus contain spinules, indicating that they are important and common structures within this region of the brain.
- SBBs are **115%** larger in volume than non-SBBs, indicating the correlation between bouton volume and spinule presence.
- The data shows a trend toward larger PSD surface area in SBBs which may indicate that SBBs have physiologically stronger synapses.
- **52%** of SBBs bear spinules from a soma, indicating spinules role in an unexplored form of communication between somas and boutons as well as spinule role in stabilizing mature synapses in the CA1 hippocampus.

Future Directions

- Evaluate two different subpopulations of inhibitory boutons (regular and fast spiking) based on mitochondrial size, in order to understand how spinules relate to functionally distinct sets of boutons
- What is the cause for spinule formation?
- How do spinules affect synaptic strength?
- What roles do spinules play in neuronal communication?
- What is the influence of spinule characteristics (e.g., size) on function?
- What is the role of spinules in bouton growth or are large, mature boutons involved in bouton formation?

Acknowledgements

- Focused ion beam scanning electron microscopy volume from CA1 hippocampus of an adult mouse generously provided by Dr. Shu-Hsien Sheu (Sheu et al., 2022, Cell 185, 3390-3407).
- A special thank you to Dr. Nahmani for the opportunity to do this research and the guidance and support throughout this research