Three-Dimensional Analysis of Spinule-Bearing Boutons within Inhibitory Synapses in CA1 Hippocampus

Xenia Tregubov and Marc Nahmani

Division of Sciences & Mathematics, University of Washington Tacoma

Abstract

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 41 boutons, 22 spinules, and 62 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 33% 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.

Methods

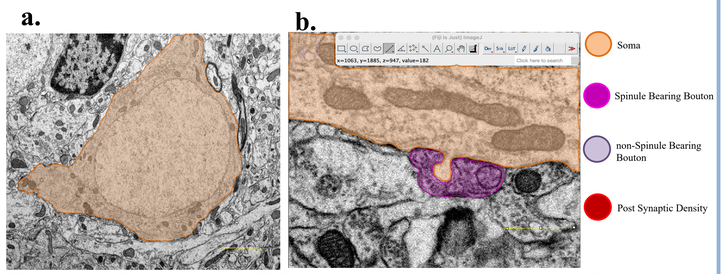


Figure 1 a, b: By using ImageJ (FIJI), we analyzed 1 individual soma in the CA1 hippocampus in the CA1 hippocampal area (a) for inhibitory synapses. Each synapse releasing bouton was named, described, and recorded (n=40) based on PSD identity, and characteristics of the spinules found (b).

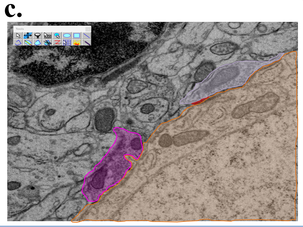
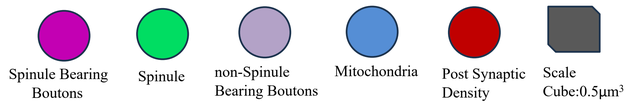
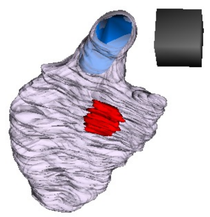


Figure 2 c: After analyzing 1 individual soma with 19 SBBs and 19 non-SBBs we used a software called Reconstruct to trace and outline each bouton, PSD (n=41), and spinule (n=22), forming 3D reconstructions (c). These models were then measured for volume and surface area in order to compare their sizes.

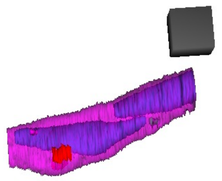
Reconstructions

PSD: Post Synaptic Density
SBB: Spinule Bearing Bouton
non-SBB: Non-Spinule Bearing Bouton

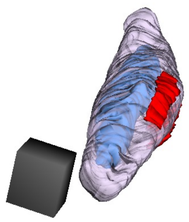




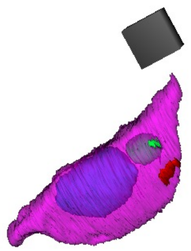
Non-Spinule Bearing Bouton with one PSD



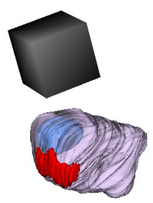
Spinule bearing bouton with two PSDs and a spinule from the soma



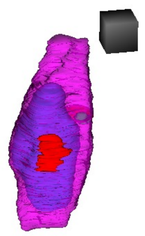
Non-spinule bearing bouton with two PSDs



Spinule bearing bouton with one PSD and a spinule from an Adjacent Axon

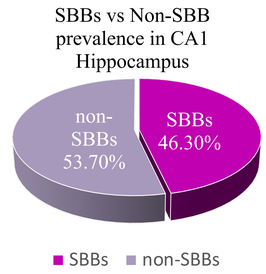


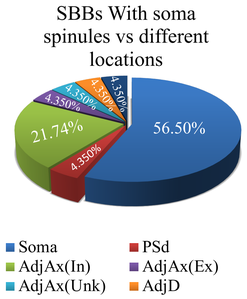
Non-spinule bearing bouton with one PSD demonstrating the smaller volume of Non-SBBS



Spinule bearing bouton with one PSD and a spinule from an Adjacent Axon demonstrating larger volume of SBBs

Data





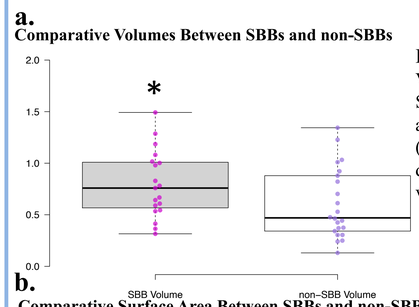


Figure 3a: Volume differences between SBBs and non-SBBs mapped along a Box-whisker plot (frequency histogram) that demonstrates a significant volume difference (p=0.05)

Data collected from **19** SBBs, and **22** non-SBBs

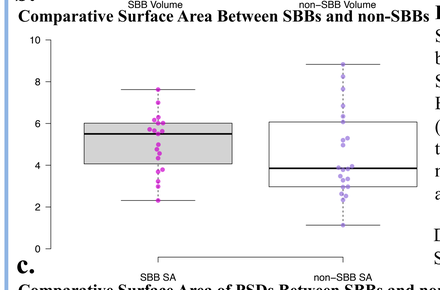


Figure 4b: Surface Area differences between SBBs and non-SBBs mapped along a Box-whisker plot (frequency histogram) that demonstrates there is no significant surface area difference

Data collected from **19** SBBs, and **22** non-SBBs

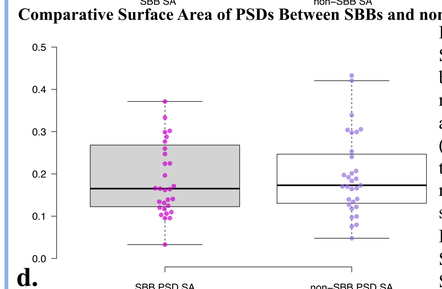


Figure 5c: Surface Area difference between SBBs PSDs and non-SBBs PSDs mapped along a Box-whisker plot (frequency histogram) that demonstrates there is no significant PSD surface area difference

Data collected from **31** SBB PSDs and **31** non-SBBs PSDs

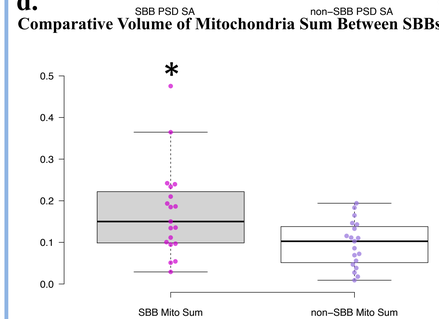


Figure 6d: Volume differences between SBBs Mito Sums and non-SBBs Mito Sums mapped along a Box-whisker plot (frequency histogram) that demonstrate there is a significant mitochondria sum difference (p=0.02)

Data collected from **19** SBB Mito Sums and **19** non-SBB Mito Sums

Conclusions

- **46%** of inhibitory boutons in CA1 are SBBs, demonstrating they are important structures n the hippocampus.
- SBBs are **33%** larger in volume than non-SBBs, showing there is correlation between spinule presence and bouton volume
- Post synaptic density surface area did not show significant difference between SBBs and non-SBBs, potentially due to mix of bouton types.
- **56%** of SBBs contain Soma spinules, this suggests these spinules could potentially represent an unexplored form of communication between boutons and somas.

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 causes spinules to form?
- Do specific type of spinules found in boutons change their physiological behaviors?
- Do spinules participate in a form of molecular communication?
- Do spinules participate in bouton growth? If not, are large, mature boutons involved in spinule 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).
- Thank you to Dr. Nahmani for the opportunity and the guidance that was provided