Quantitative Three-Dimensional Characterization of Synaptic Spinules Within Inhibitory Synapses in Hippocampus

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Abstract

Inhibitory synapses are critically important connections between neurons that release gamma-aminobutyric acid (GABA), which regulates neuronal activity, behavior, cognition, and prevents seizure-like activity. Presynaptic boutons (neurotransmitter releasing side of a synapse) often receive finger-like projections from surrounding neurons, called spinules, which may represent an unexplored from of neuronal communication and/or regulate synaptic strength and stability. Yet while spinules within select excitatory boutons have been quantified, there is no published data on spinules within inhibitory synapses. Hence, we performed a study with the goal of quantifying the proportions of inhibitory presynaptic boutons within the memory center of the brain (CA1 hippocampus), to determine differences between spinule-bearing boutons (**SBBs**) and non spinule-bearing boutons (**non-SBBs**). Toward this end, we analyzed perisomatic inhibitory synapses within a large TEM image volume of CA1 from an adult mouse brain. We categorized inhibitory bouton synapses based on their spinules and postsynaptic partners. In addition, we three-dimensionally reconstructed 50 boutons, 71 synapses, and 28 spinules, and quantified their surface areas and volumes. We discovered that 58% of perisomatic inhibitory boutons in our volume were SBBs, and that SBBs were 2X larger than non-SBBs. In addition, we found that 60% of spinules within perisomatic inhibitory SBBs originated from somas, whereas ≤13% projecting from other sources. Together, 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 unique excitatory to inhibitory communication in CA1.

Methods

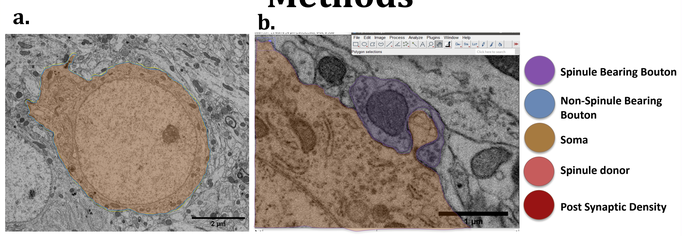


Figure 1 a, b: Using ImageJ (FIJI), we analyzed **3** individual somas in the CA1 hippocampal area (**a.**) for inhibitory synapses. Each synapse releasing inhibitory bouton was described (n=**122**) and recorded based on PSD identity, and characteristics of any occurring spinules (**b.**)

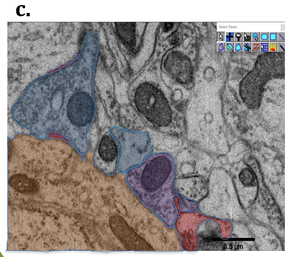
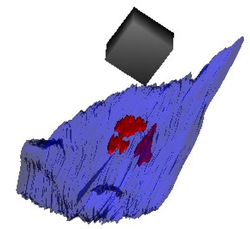


Figure 2 c: After randomly selecting **25** SBBs and **25** non-SBBS we used a software called Reconstruct to trace the outline of each bouton, PSD (n=**71**), and spinule (n=**28**), forming 3D reconstructions (**c.**). These models were measured for volume and surface area for comparing size-based metrics.

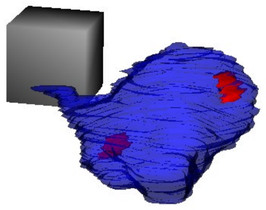
Reconstructions

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

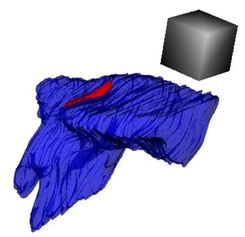
Non-SBBs



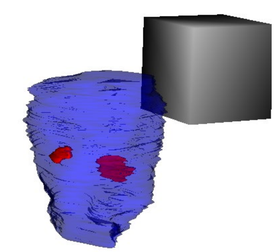
Non-Spinule Bearing Bouton, with two PSDs.



Non-Spinule Bearing Bouton, with two PSDs.

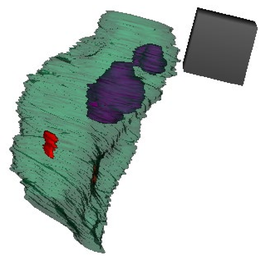


Non-Spinule Bearing Bouton, with one PSD.

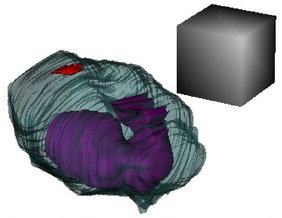


Non-Spinule Bearing Bouton, with two PSDs, demonstrating the smaller relative volume of non-SBBs.

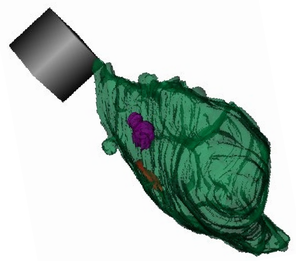
SBBs



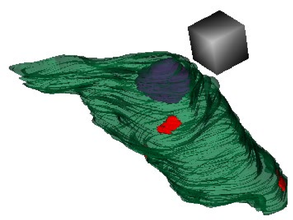
Spinule Bearing Bouton with two PSDs and a spinule from a soma.



Spinule Bearing Bouton with a spinule from a soma, and two PSDs.



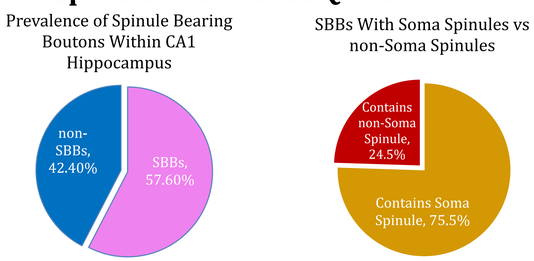
Spinule Bearing Bouton with a spinule from a soma and an "anterograde" spinule.



Spinule Bearing Bouton, with three PSDs, demonstrating the larger relative volume of SBBs.

Data

Spinule Prevalence Quantification



Inhibitory boutons analyzed: 122
Total spinules analyzed: 79
Total PSDs analyzed: 170

3-Dimensional Morphology Quantification

Comparative Analysis of SBB and non-SBB Volume

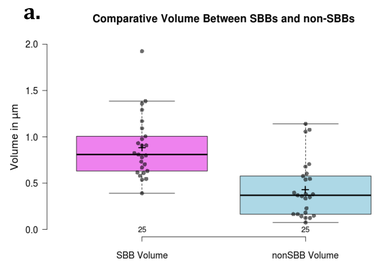


Figure 3 a, b: Volume differences between SBBs and non-SBBs can be mapped along a Box-whisker plot, or a frequency histogram demonstrating the statistically significant volume difference.

Data collected from **25** non-SBBs, and **25** SBBs

3-Dimensional Analysis of PSD Surface Area

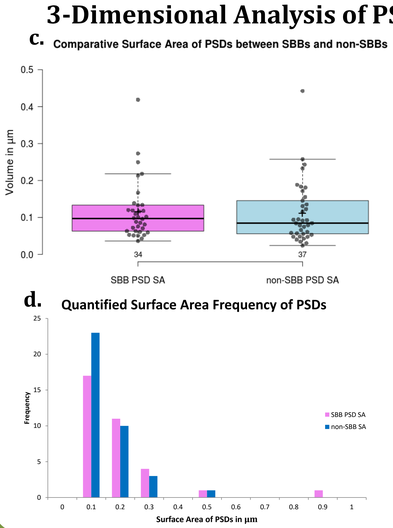


Figure 4 c, d: Surface area differences between SBB PSDs and non-SBB PSDs when mapped along a Box-whisker plot, or frequency histogram demonstrate that there is no significant statistical difference between SBBs or non-SBBs.

Data collected from **33** non-SBB PSDs, and **36** SBB PSDs

Conclusions

- **58%** of inhibitory boutons in this brain region contain spinules, which demonstrates that they are ubiquitous structures within the hippocampus.
- SBB are **207%** larger in volume than non-SBBS, implying correlation between spinule presence and bouton volume.
- Post synaptic density surface area was not significantly different between SBBs and non-SBBs, potentially due to PSD size differences of distinct bouton subsets.
- **75%** of perisomatic SBBs contain Soma spinules, suggesting these spinules may represent an unexplored form of communication and/or impart stability to mature, functionally important synapses.

Future Directions

- Measure mitochondria to determine if specific functional subsets of inhibitory boutons differ in their spinule number or characteristics.
- Do spinules induce bouton growth? Or do large, functionally mature boutons necessitate spinule formation?
- Do spinules participate in a form of molecular communication?
- What causes spinules to form?
- Several novel types of spinules were observed during analysis such as spinules between somas, raising new directions for future studies.

Acknowledgements

- Focused ion beam scanning electron microscopy volume from CA1 hippocampus of an adult mouse kindly provided by Dr. Shu-Hsien Sheu (Sheu et al., 2022, Cell 185, 3390–3407).
- Special thanks to Dr. Nahmani for his mentorship and guidance through this research.