Morphometric Analysis of Spinule Bearing & Non-Spinule-Bearing Perisomatic Inhibitory Boutons in CA1 Hippocampus

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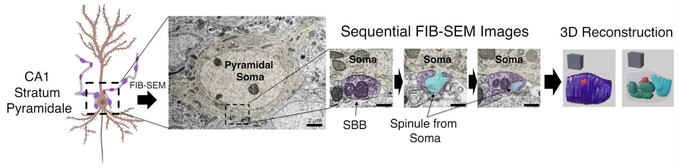
ABSTRACT

Synapses, the pivotal junctions for brain communication, relay electrical impulses between neurons. Proper synapse function is vital for cognition, memory, and essential brain activities. Inhibitory synapses are important for regulating the timing of neuronal communication and preventing seizure-like behaviors. Despite the importance of inhibitory synapses, key structures in their anatomy known as synaptic spinules remain unexplored. Synaptic spinules are finger-like projections from one neuron that are embedded into the neurotransmitter-releasing end (presynaptic bouton) of another neuron. Spinules could enhance cortical synapse stability and potentially revolutionize our understanding of neuronal communication. As a first step in exploring these possibilities, we sought to quantify the presence and effect of spinules on inhibitory synapses within the CA1 hippocampus, the well-characterized memory formation center of the brain. To this end, we completed a morphometric analysis of 5 spinule-bearing inhibitory boutons (SBBs) and 5 non-spinule-bearing boutons (non-SBBs) surrounding a soma within a large electron microscopy image volume in CA1 hippocampus of an adult male mouse. We analyzed these boutons to quantify their surface area, volumes, and spinules. We discovered that 65% of perisomatic inhibitory boutons in our volume were SBBs, with 57% of SBBs containing a spinule from its postsynaptic soma partner, with smaller percentages coming from adjacent dendrites and other somas. Our results did not show a significant difference between the size of SBBs compared to non-SBBs. Yet, these analyses demonstrate that inhibitory SBBs represent a subset of inhibitory synapses in CA1 that may play a role in neuronal circuit stability.

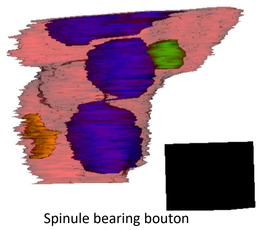
METHODS

For this study, we chose one pyramidal soma and analyzed 20 inhibitory perisomatic presynaptic boutons (n=20) for their postsynaptic partners, presence of a spinule, and spinule origin. This analysis was performed by making regions of interest (ROIs) in Fiji (ImageJ). Next, we randomly selected 10 boutons (n = 5 SBBs & 5 non SBBs) and reconstructed their volumes, PSDs, mitochondria, and spinules (if present) using Reconstruct software (Fiala et al., 2005).

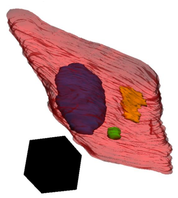


SPINULES AT INHIBITORY CORTICAL SYNAPSES

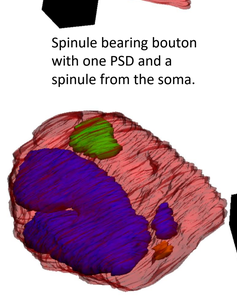
Spinule Bearing Boutons (SBBs)



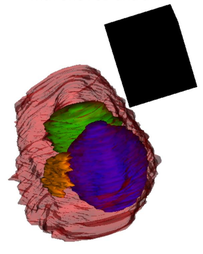
Spinule bearing bouton with one PSD and a spinule from the soma.



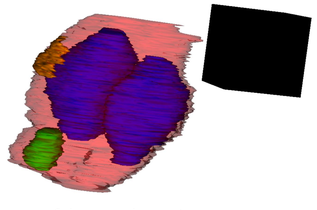
Spinule bearing bouton with one PSD and a spinule from the soma.



Spinule bearing bouton with one PSD and a ...

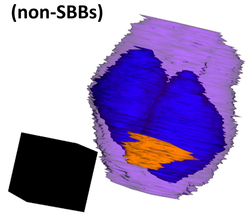


Spinule bearing bouton with one PSD and a spinule from the soma.

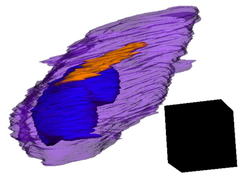


Spinule bearing bouton with one PSD and a spinule from the soma.

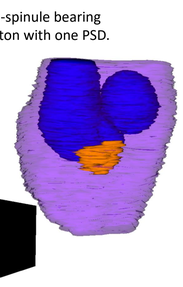
Non Spinule Bearing Boutons (non-SBBs)



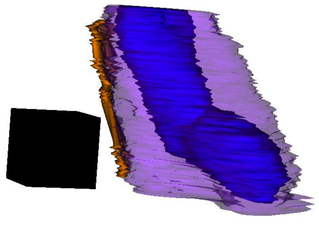
Non-spinule bearing bouton with one PSD.



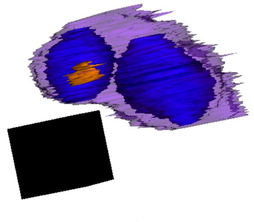
Non-spinule bearing bouton with one PSD.



Non-spinule bearing bouton with one PSD.

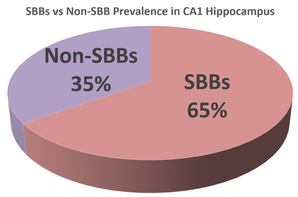


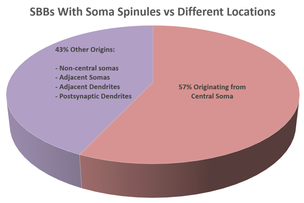
Non-spinule bearing bouton with a perforation of the PSD (three PSDs present).



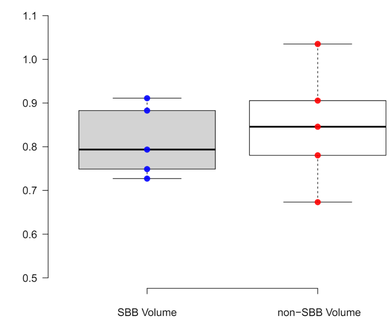
Non-spinule bearing bouton with one PSD.

QUANTIFICATION OF SYNAPTIC ULTRASTRUCTURE





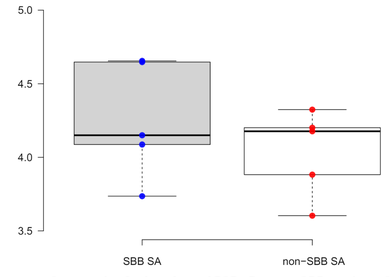
Comparative Volumes Between SBBs and non−SBBs



Volume differences between SBBs and non-SBBs mapped along a Box-whisper plot (frequency histogram) that does not demonstrate a significant volume difference ($p < 0.05$).

Data collected from 5 SBBs and 5 non-SBBs.

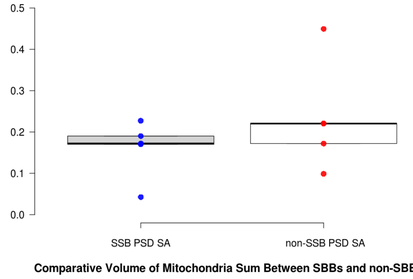
Comparative Surface Area Between SBBs and non−SBBs



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 ($p < 0.05$).

Data collected from 5 SBBs and 5 non-SBBs.

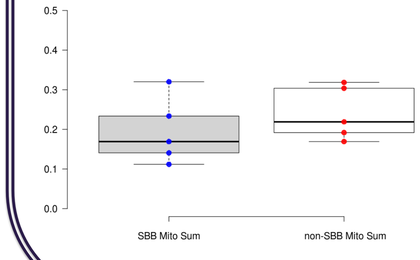
Comparative Surface Area of PSDs Between SBBs and non-SBBs



Surface area differences between SBBs PSDs and non-SBBs PSDs mapped along a Box-whisker plot (frequency histogram) that demonstrates there is no significant surface area difference ($p < 0.05$).

Data collected from 5 SBBs and 5 non-SBBs.

Comparative Volume of Mitochondria Sum Between SBBs and non-SBBs



Volume differences between SBBs mito sums and non-SBBs mito sums mapped along a Box-whisker plot (frequency histogram) that demonstrate there is no significant mitochondrial sum difference ($P < 0.05$).

Data collected from 5 SBBs and 5 non-SBBs.

CONCLUSIONS

- 65% of inhibitory boutons in CA1 are spinule bearing, demonstrating they are important structures in the hippocampus.
- Post synaptic density (PSD) surface area did not show significant difference between SBBs and non-SBBs, potentially due to mix of bouton types.
- 57% of SBBs contain soma spinules, suggesting an unexplored form of communication between boutons and somas.
- Parent study with n=135 reconstructions found significant size differences for SBB vs Non-SBBs.

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 Dr. Nahmani for the opportunity and guidance.