Spinule-Bearing vs. non Spinule-Bearing Presynaptic Boutons in Developing Visual Cortex

Maryam Al Darraji and Marc Nahmani

Division of Sciences & Mathematics, School of Interdisciplinary Arts & Sciences, University of Washington I Tacoma, Tacoma, WA

INTRODUCTION

Spinule-Bearing vs. non Spinule-Bearing Presynaptic Boutons in Developing Visual Cortex

Maryam Al Darraji and Marc Nahmani

Synapses are neuronal junctions that allow electrical signals to transmit from one neuron to another. Synapses are essential because they are responsible for all our motor and sensory functions. Previous research has shown that 25 – 75% of the synapses in the brain contain mysterious finger-like projections called 'spinules' that originate from one neuron and extend into the presynaptic bouton (neurotransmitter-releasing side) of another. However, the role of these spinules remains unclear. We hypothesized that if spinules acted as 'anchors' to make boutons more stable, the inclusion of a spinule would make these boutons' volumes larger, with consequently larger postsynaptic densities (PSDs; synaptic junctions), versus boutons without spinules. As a first step toward understanding spinules' function, we analyzed Spinule Bearing Boutons (SBB) versus non-Spinule Bearing Boutons (non-SBBs), to determine how boutons with spinules differ than those who do not, and how spinules might be impacting the overall function of the postsynaptic neuron. We analyzed SBBs and non-SBBs within the primary visual cortex of a postnatal day 60 (p60) ferret brain, an age when the levels of synaptic plasticity are declining. We compared the volumes and areas of non-SBB verses SBB along with their PSDs. We found that boutons from SBBs at p60 were three times larger with two times larger PSDs versus non-SBBs. These data suggest that the presence of a spinule is a marker for larger boutons. In addition, since a larger bouton is a stronger bouton (i.e., releasing more neurotransmitters), and larger boutons have larger PSDs with more receptors present to bind these neurotransmitters, the presence of a spinule may also be a marker for a stronger bouton. In conclusion, we find that spinules may indicate the presence of the strongest, most important boutons in a neuronal circuit.

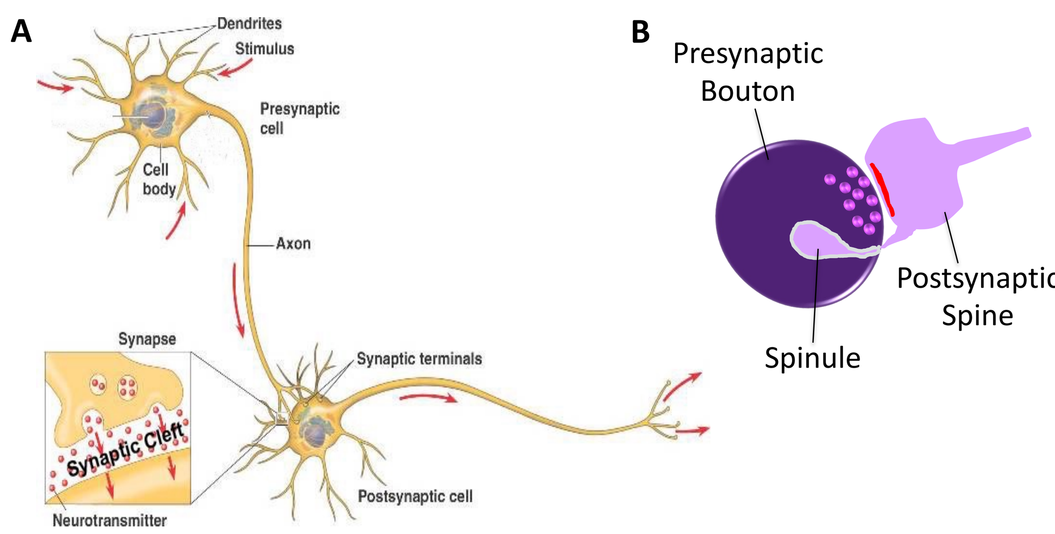

Figure 1. **A**, Carton image of neuronal synapse (non-SBB). **B**, carton of bouton encapsuling spinule (SBB). (Nashman B. 2019)

METHODS

Electron Microscopy

- Image volume of an adult ferret V1 primary visual cortex.

Fiji (ImageJ) Analysis

- We located excitatory synapses within the image volume
- If the presynaptic bouton lacked a spinule was included in our analysis

Excitatory synapses are found via the following criteria

1. 3+ Vesicles: Axon terminal or bouton contained at least 3 vesicles near the bouton's membrane opposite to PSD (near means within 2.5 vesicle distance from the membrane).
2. Parallel membranes: Presynaptic object (axon terminals or boutons) and presynaptic object (spines and dendrites) had parallel membranes at the synapse.
3. Thick asymmetric PSD: Postsynaptic density (PSD) – location of postsynaptic receptors (darkly shaded or black)
4. Regions of interest (ROI's) were drawn around the Non-SBB excitatory synapses and recorded for later analysis

Reconstruct

- Image volume was uploaded into Reconstruct for 3D analysis
- Stack's resolution of 5nm x 5nm x 15nm (Skipped every third image)
- Made traced of 10 non-SBB (Bouton and PSD)
- Both volume and surface area were recorded for statistical analysis

METHODS

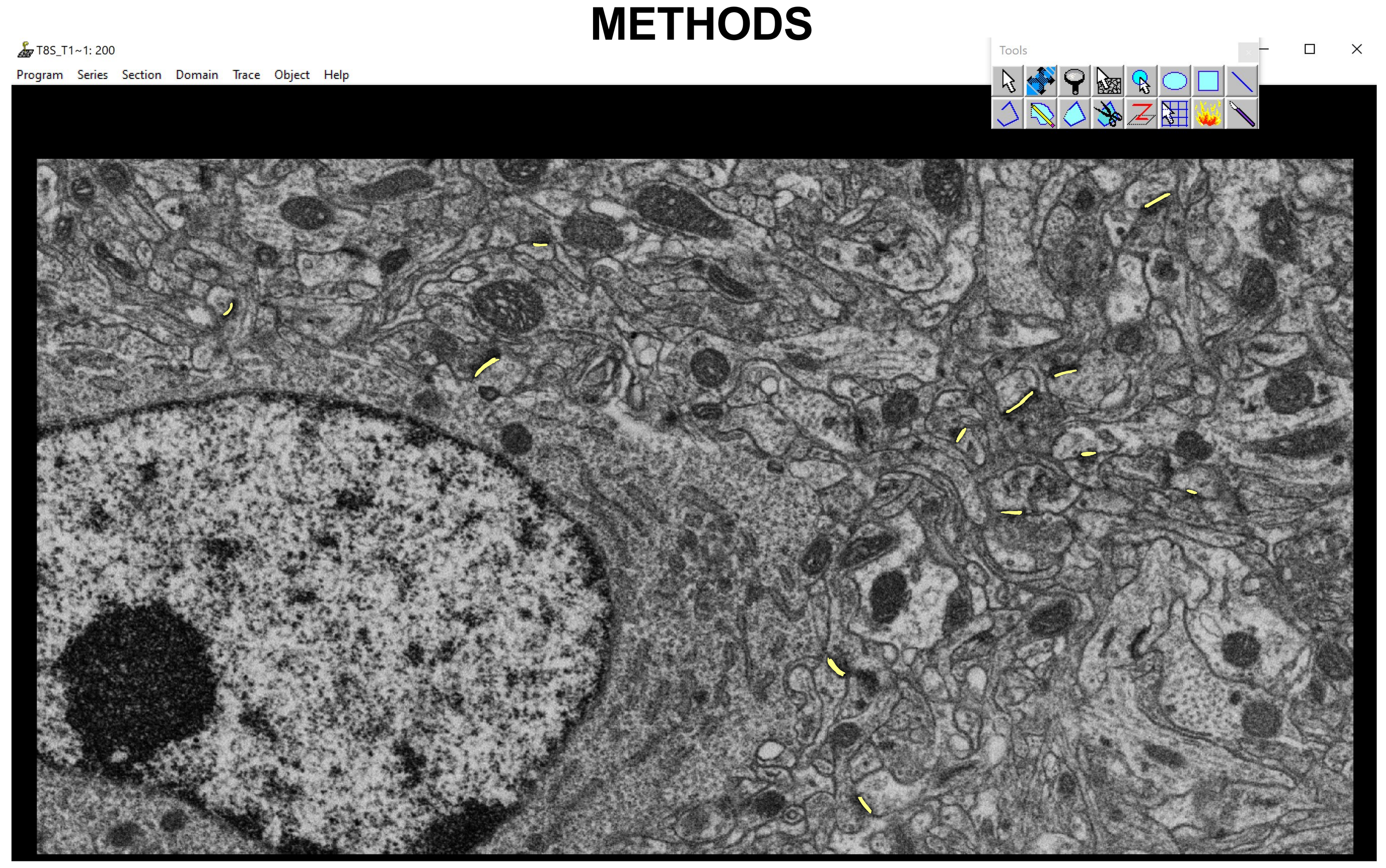

Figure 2. A visual representation of synapses in one electron microscope image showing non-SBBs.

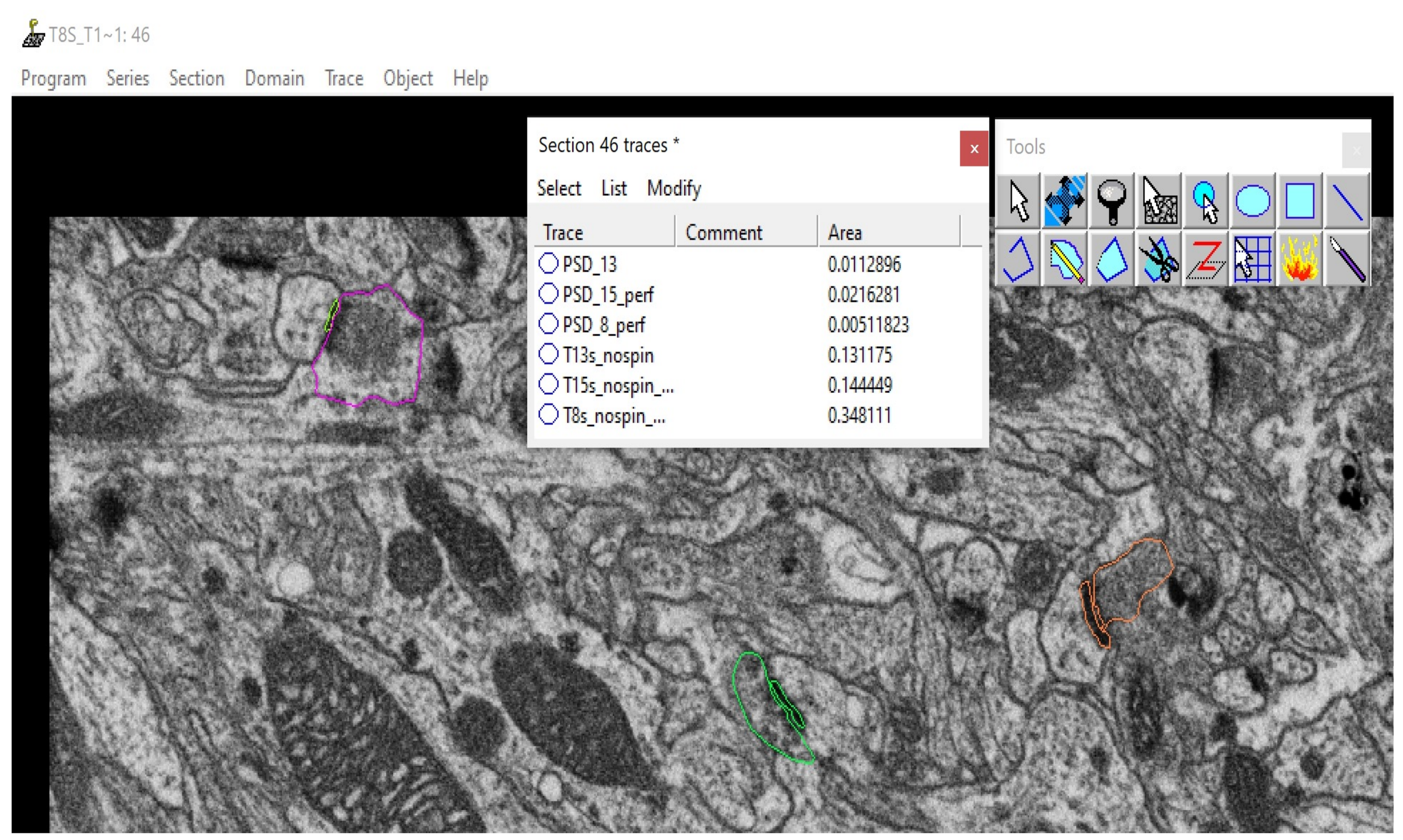

Figure 3. Analysis of non-SBBs through success tracing of the outer membrane of boutons and PSDs.

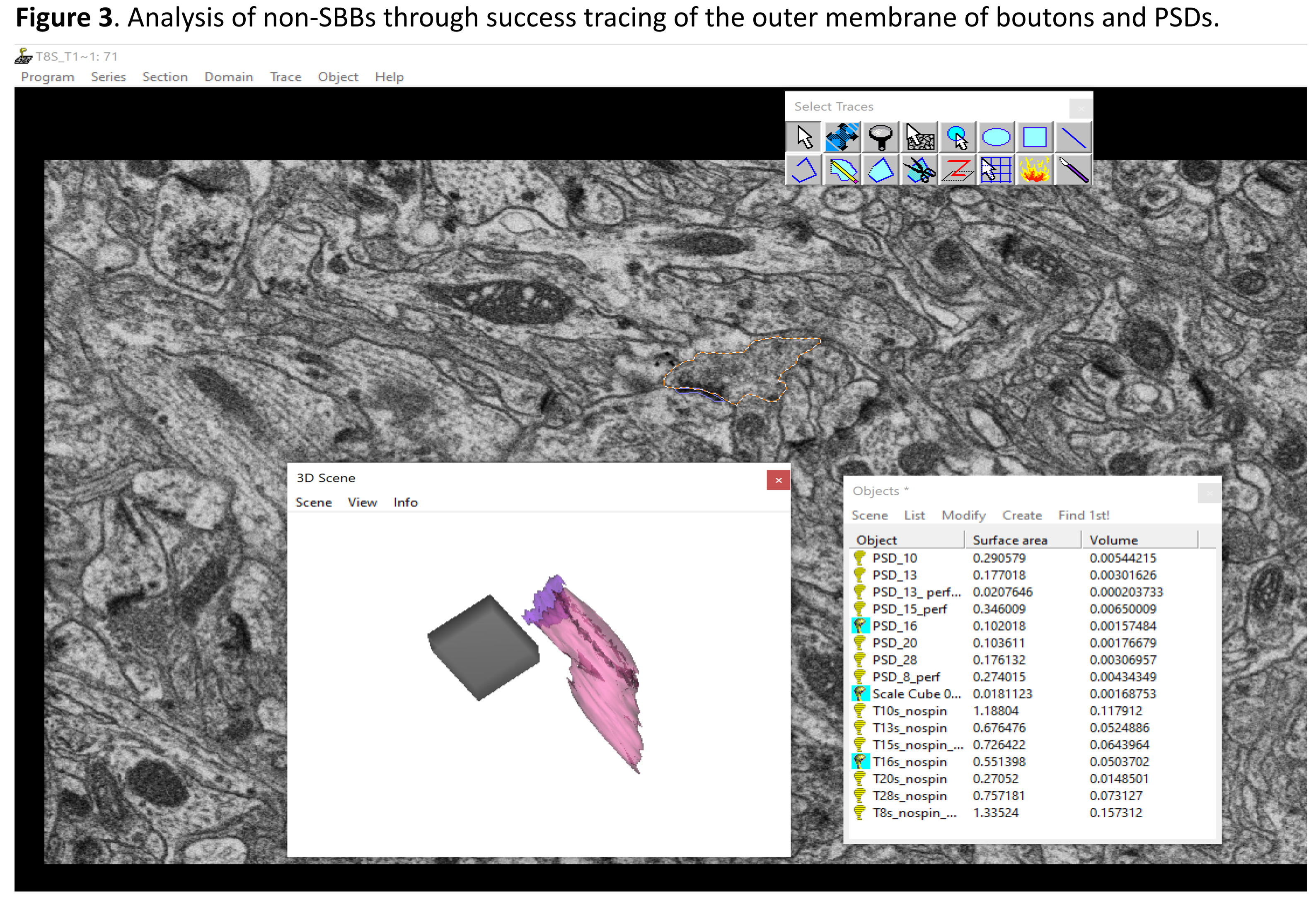

Figure 4. Example 3D reconstructions of a non-SBB from dozens of individual traces.

RESULTS

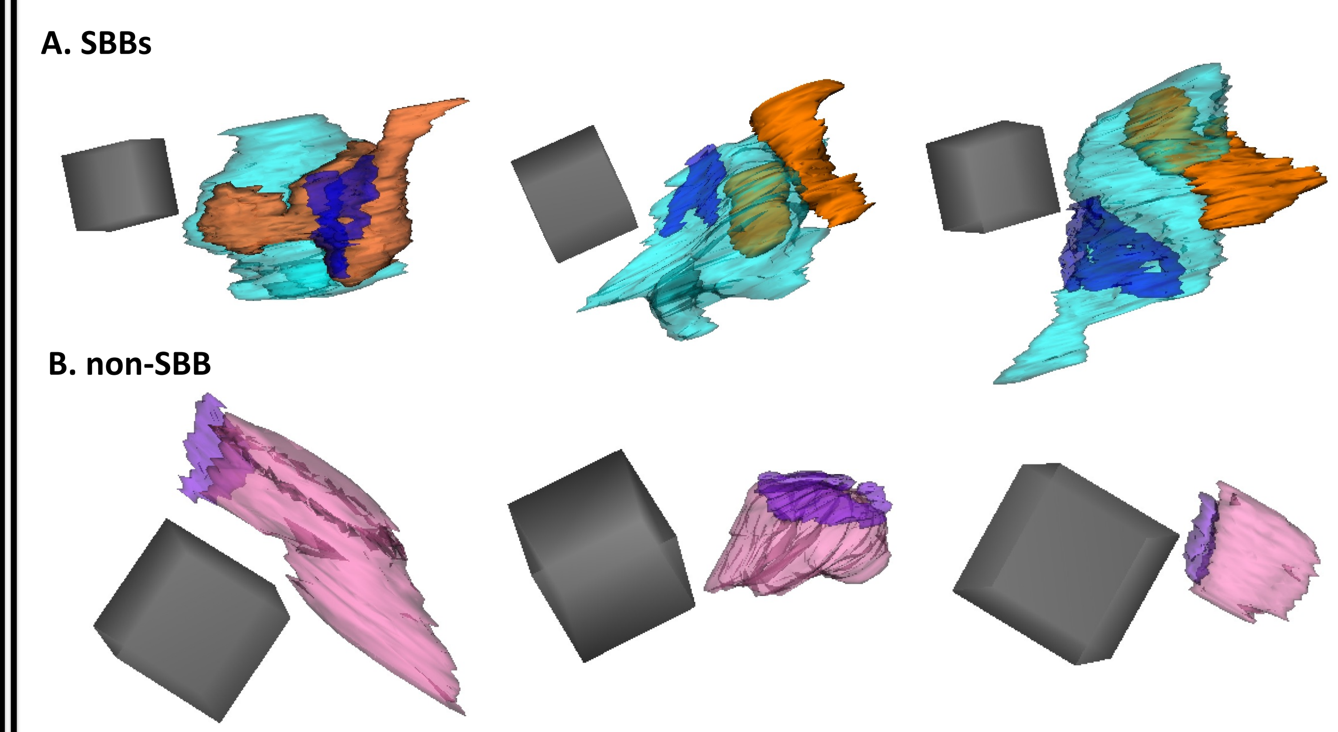

Figure 5. 3-Dimensional representation of the comparison between SBB vs. non-SBB. Presentation of the spinules in orange color.

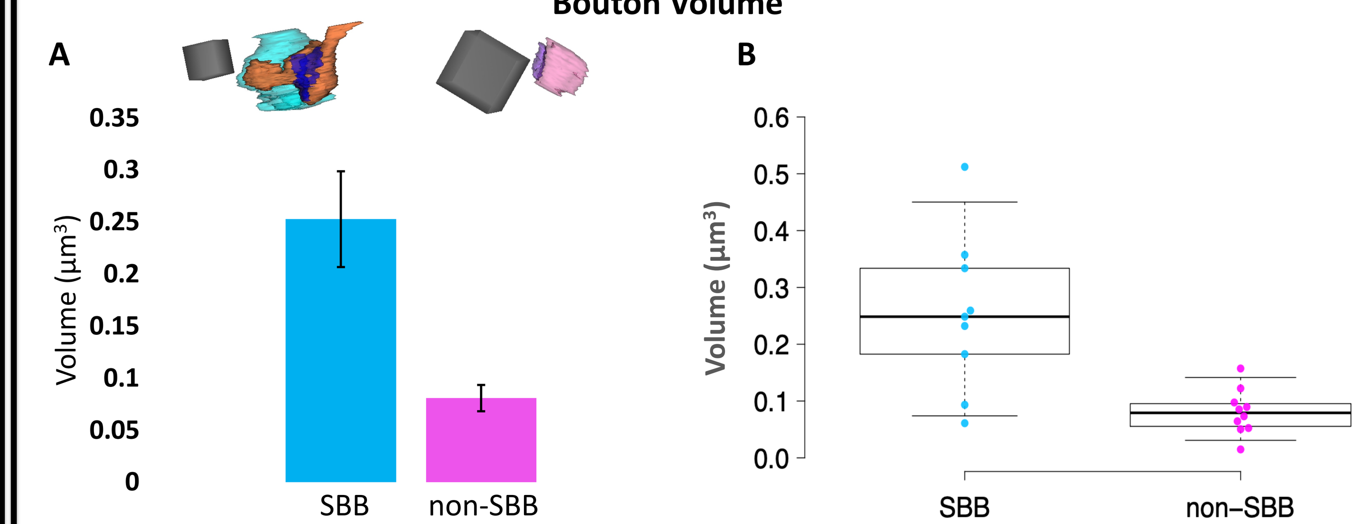

Figure 6. Bouton Volume (µm³) in SBB are three-times larger than PSD of non-SBB synapses. **A**, Bar plot showing the mean +/- 0.25 of SBB, mean +/- 0.08 of non-SBB, and the standard error with reconstructed PSDs of SBB vs. non-SBB . **B**, Box plot showing identical data as showing in A. n=8 SBB and 10 non-SBB of PSD sample point.

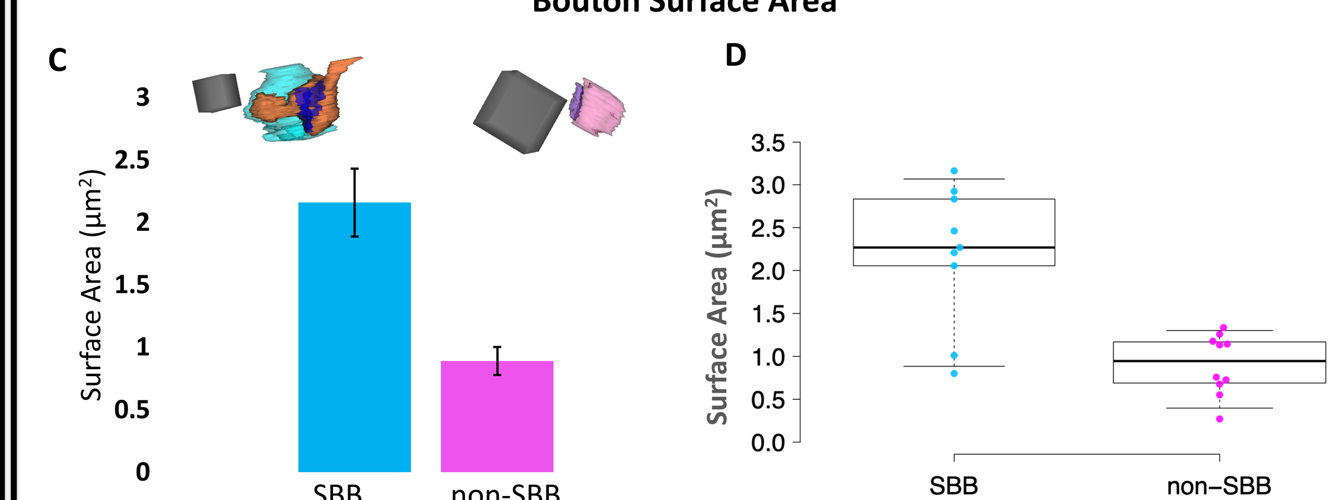

Figure 7. Bouton Surface Area (µm²) in SBB are three-times larger than PSD of non-SBB synapses. **C**, Bar plot showing the mean +/- 2.16 of SBB, mean +/- 0.89 of non-SBB, and the standard error with reconstructed PSDs of SBB vs. non-SBB . **D**, Box plot showing identical data as showing in C. n=8 SBB and 10 non-SBB of PSD sample point.

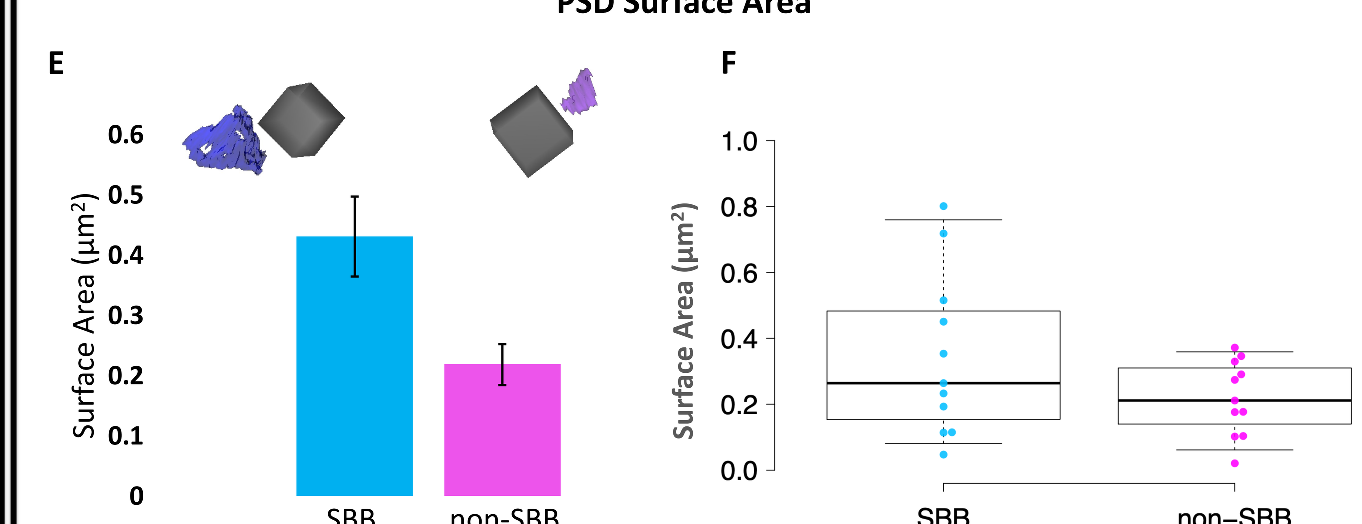

Figure 8. PSD Surface Area (µm²) in SBB are two-times larger than PSD of non-SBB synapses. **E**, Bar plot showing the mean +/- 0.43 of SBB, mean +/- 0.22 of non-SBB, and the standard error with reconstructed PSDs of SBB vs. non-SBB . **F**, Box plot showing identical data as showing in E. n=8 SBB and 10 non-SBB of PSD sample point.

RESULTS

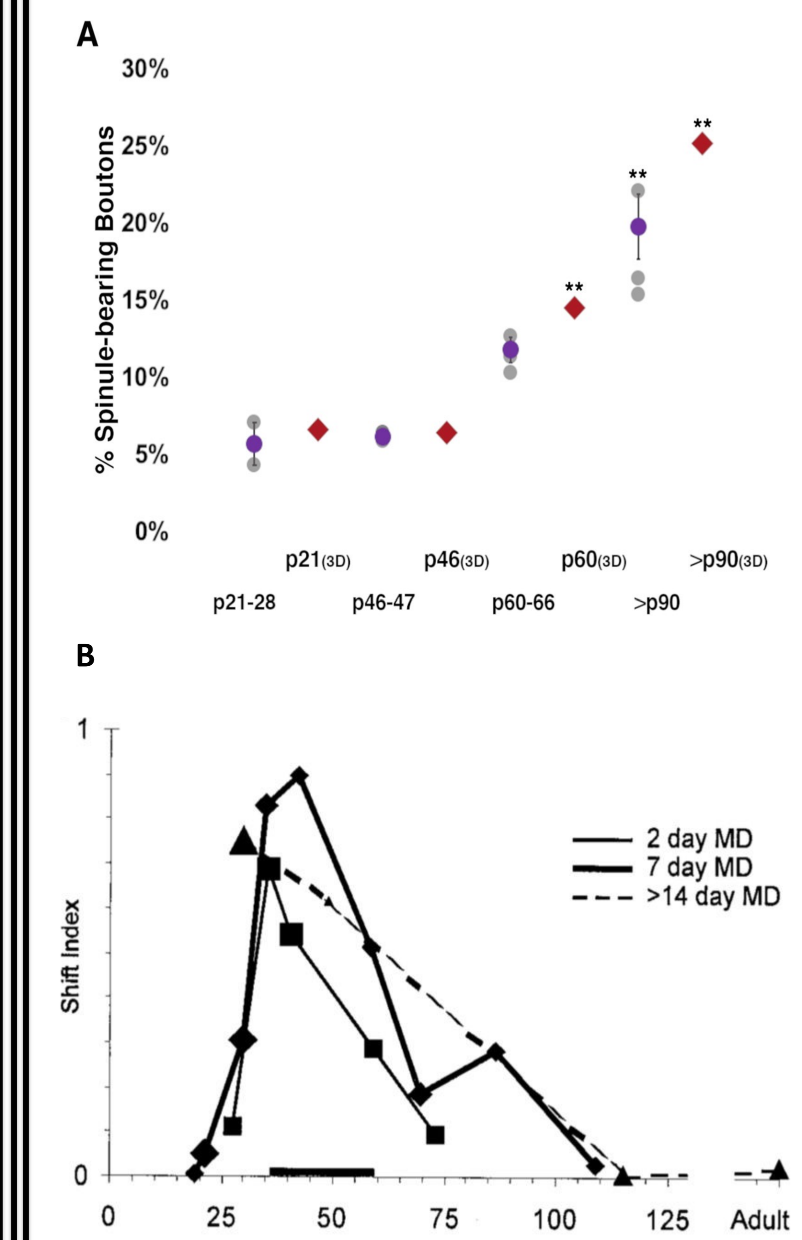

Figure 9. An inverse relationship between the spinule prevalence and plasticity. **A**, Percentage of SBBs as boutons age; spinule prevalence within boutons progressively increases. **B**, Shift index plot; the larger the shift index, the greatest the effect. As bouton become older, levels of plasticity decrease. (Issa et al., J Neuroscience, 1999).

CONCLUSIONS

- Spinules are very likely acting as an anchor
- Bouton's volume and surface area are larger in SBB (three-times larger than non-SBB)
  - Stronger bouton due to ability of releasing more neurotransmitter
- PSD's surface area are larger in SBB (two-times larger than non-SBB)
  - Allowing more receptors to bind those neurotransmitters
- An indication of an inverse relationship between plasticity and bouton's age

FUTURE RESEARCH

For future proposal, will need to examine the boutons and PSD across all ages (i.e., p21, p46, and >p90) to analyze non-SBBs. This will indicate whether spinules are making boutons larger or the larger boutons are targeted by spinules.

ACKNOWLEDGEMETNT

Special Thank you to Dr. Marc Nahmani for his extraordinary mentorship, guidance and support throughout this research.

REFERENCES

1. Nashman B. 2019. Modeling Your Brain On A Computer With Computational Neuroscience. Medium. [accessed 2022 Jun 8]. https://medium.com/@bennashman/modeling-your-brain-on-a-computer-with-computational-neuroscience-57596c919c70.
2. Naoum P. Issa, Joshua T. Trachtenberg, Barbara Chapman, Kathleen R. Zahs, and Michael P. Stryker. 1999. The Critical Period for Ocular Dominance Plasticity in the Ferret's Visual Cortex. J Neurosci. 19(16): 6965-6978.