Morphometric analysis of synaptic plasticity in higher cortical centers

Ignacio Novoa Cornejo [1], Vidhi Patel [2], Kathiravan Kaliyappan[1], Vijaya Prakash Krishnan Muthaiah[1]

[1] Department of Rehabilitation Sciences, School of Public Health and Health Professions, State University of New York at Buffalo, Buffalo, NY, United States.

[2] Department of Biomedical Research, State University of New York at Buffalo, Buffalo, NY, United States.

Introduction

Synaptic plasticity (SP) refers to neurons' ability to alter synaptic connectivity over time [1]. SP changes underlying cognitive impairment following blast traumatic brain injury (TBI) is still unclear [2]. As we focus on blast overpressure-induced alteration of SP and dendritic architecture in higher cortical centers, in this study, we optimized the analysis of spine density in rat brain sections following Golgi-Cox staining. The neuronal microstructure was assessed with Golgi staining, which selectively stains a small percentage (1–3%) of neurons and their entire dendritic arbor [3].

This study analyzed the variables such as dendritic length, dendritic density, and spine identification using morphometric measures. For this study, we followed the Risher et al., 2014 method of Sholl analysis (SA), dendritic length, spine count, and spine density to describe the dendritic arborization and quantify the neuronal plasticity from various brain sections.

Objective

To develop an image analysis pipeline to quantify the characteristics of dendritic architecture.

Methods

The analysis was performed on one image acquired at 100x from the Golgi-stained brain sections from normal rat. Data collection started by following the Golgi-Cox staining protocol [4]. The image analysis started with importing the image into ImageJ Fiji[5]. Using the software with SNT plugin to do the Sholl analysis[4] to obtain dendritic length, spine count, and spine density for each one, we used "Dendritic spine counter" plugin in ImageJ. We worked in optimization process for three neuron from auditory cortex. These metrics will be used for our pre-clinical study on investigating the alteration of SP post-blast-induced TBI.

Data Analysis

Data were analyzed using GraphPad Prism-9. We used descriptive statistics and then created graphs to observe the behavior in each variable.

A. Neuron 2

B. Segmentation image of neuron 2.

C. Neuron 2, with marked dendritic in blue, and green points represent spines. (Using Dendritic spine counter.)

A

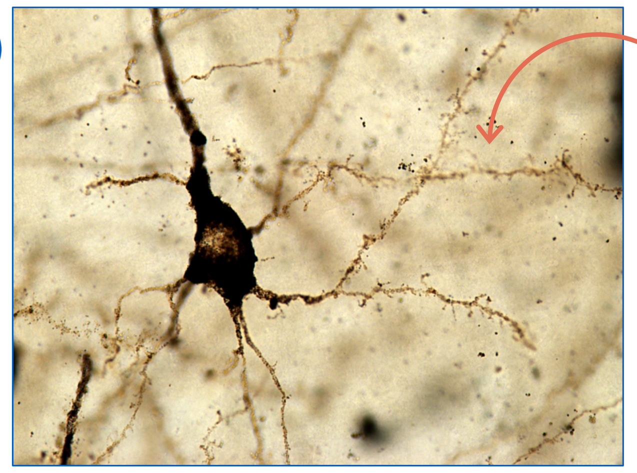
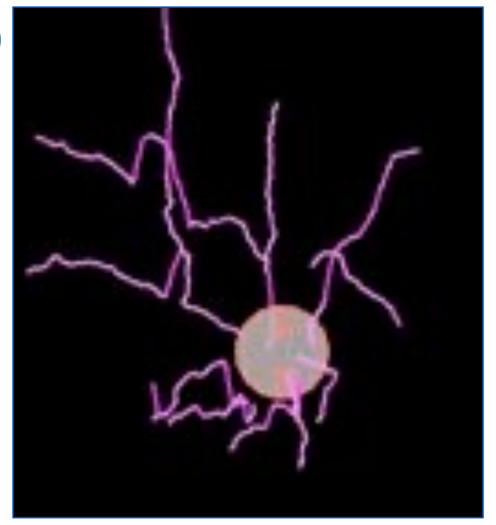


Figure A: Represents neuron 2. In a 16-Bit composite image, before going to grayscale and 8-Bit for analysis.

B



C

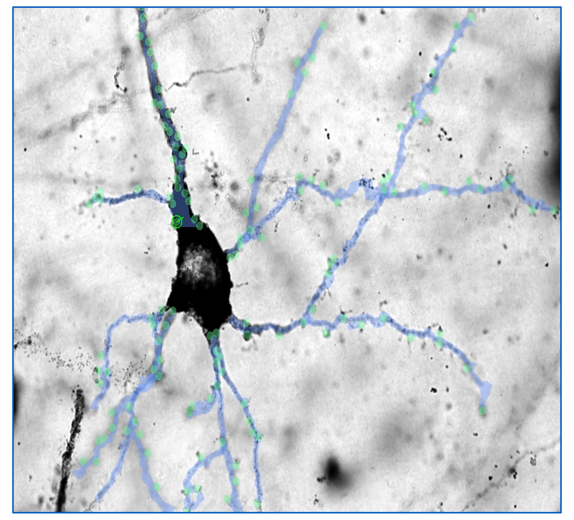
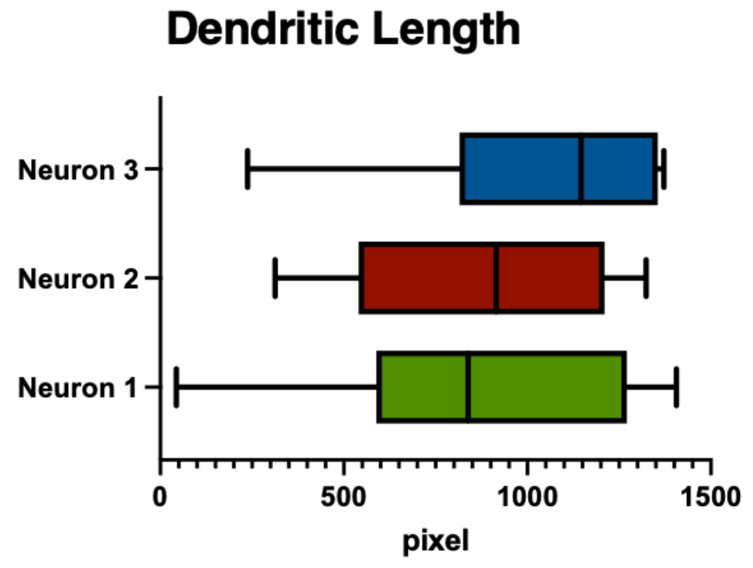


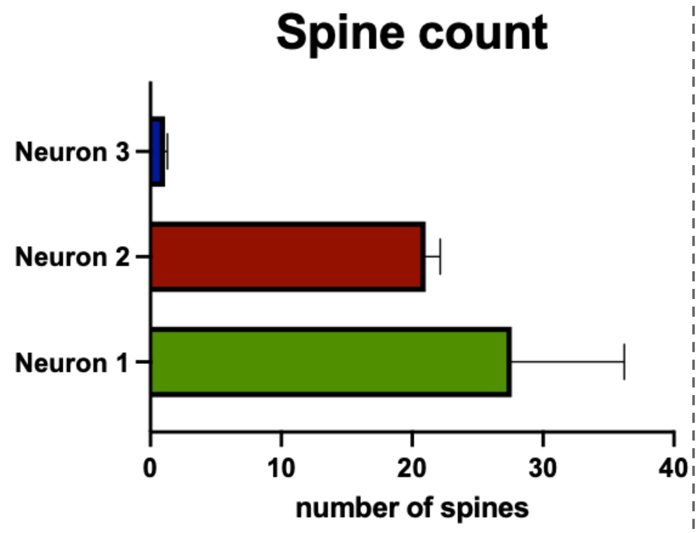
Table 1 – Summary of Means

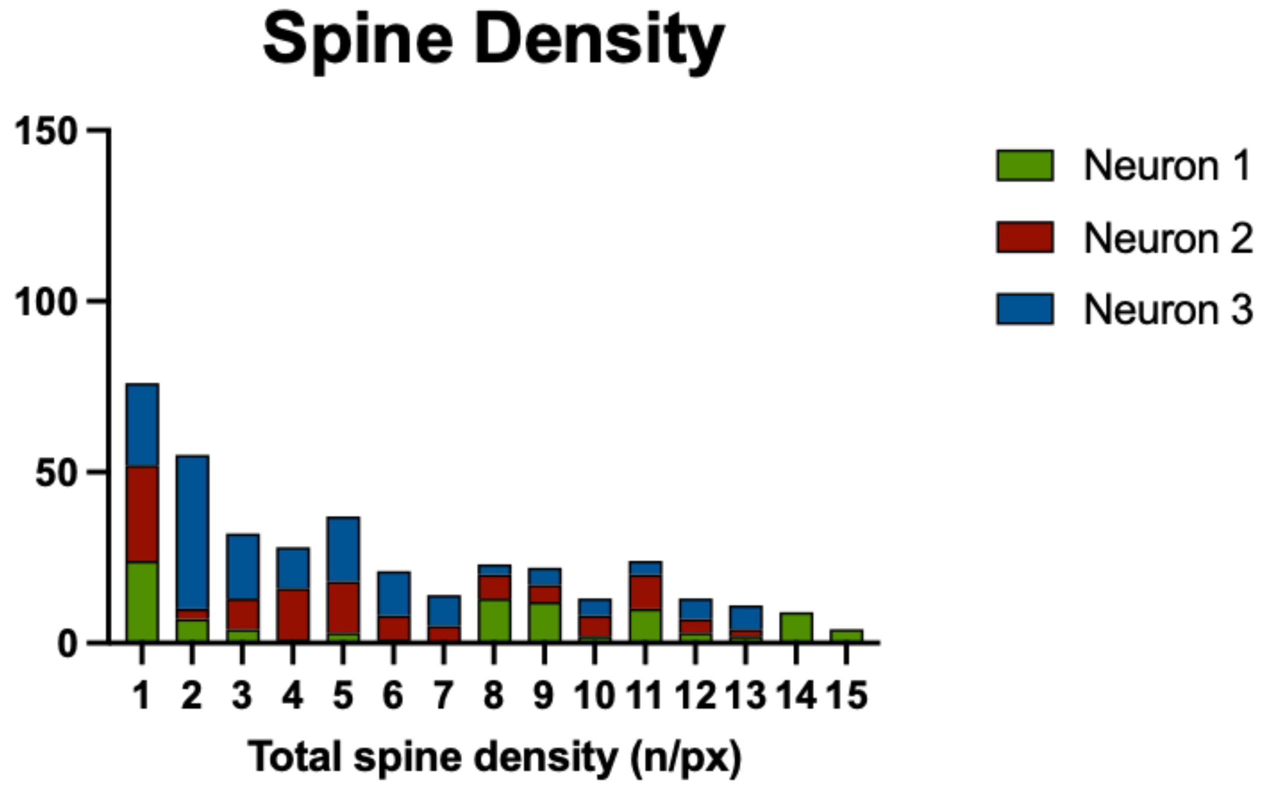
	Neuron 1	Neuron 2	Neuron 3
Dendritic Length	885.3	878.8	1008
Spine Count	27.61	21.04	1.116
Spine Density	6.333	8.923	13.15
Sholl Analysis	7.500	3.000	5.750

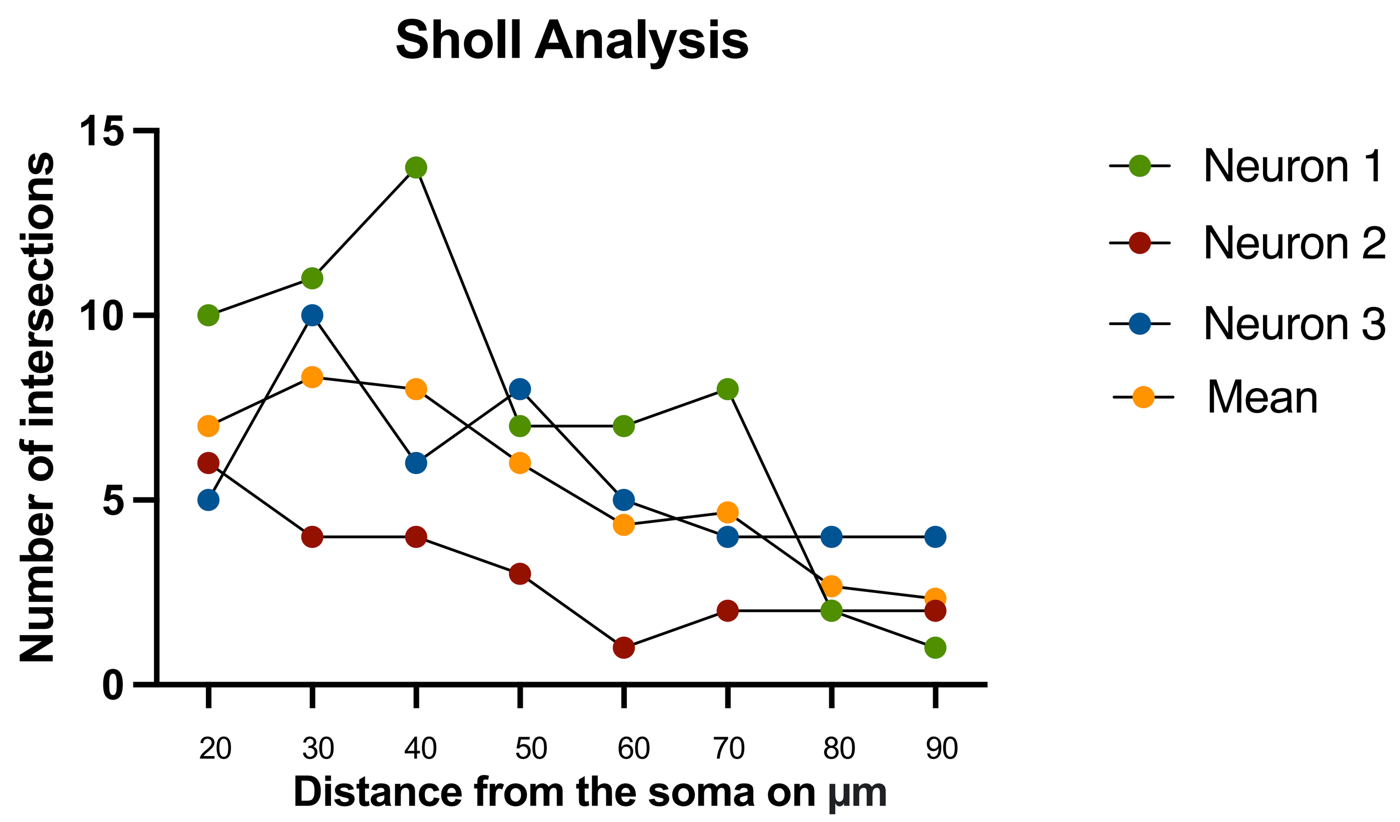
Summary table of means for neuron 1, 2 and 3.

Results







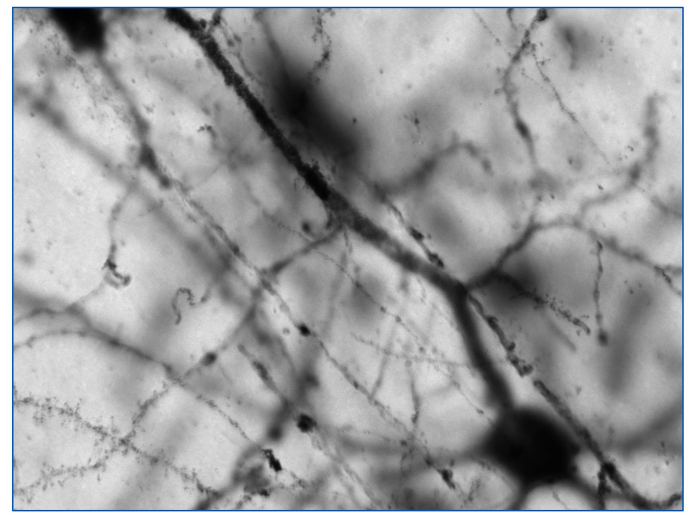


The results for dendritic length are in pixels (1 px is 264.5833 Micrometers). The Spine count is in nominal values. Moreover, the Spine density is the relation between numbers and pixels. Finally, the Sholl analysis represents the number of intersections versus the distance from the soma on μm. The number of intersections decreases to more distance from the soma (yellow dot=mean).

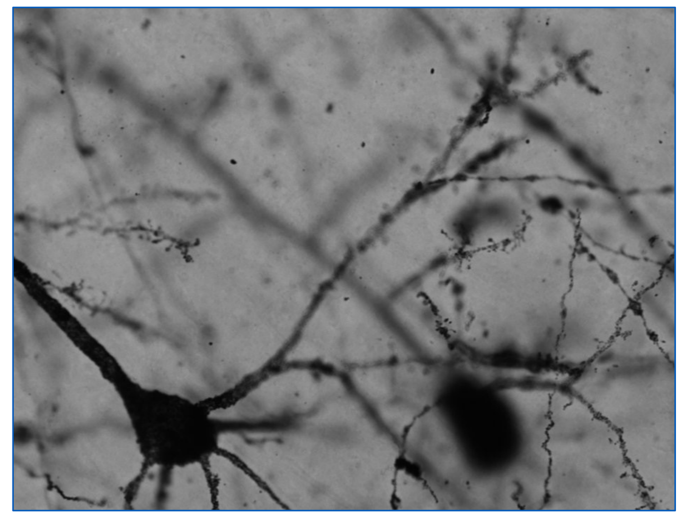
Conclusion

The neuron analysis with SNT that generate the Sholl analysis and Dendritic spine counter to obtain the quantitative variables from neurons is an excellent tool to describe synaptic plasticity in higher cortical centers due to the number of variables that it gives us and the optimization time of the image that will allow us to increase the number of neurons analyzed.

Graphic Elements



Neuron 1



Neuron 2

References

1.Shefa U, Kim D, Kim MS, Jeong NY, Jung J. Roles of Gasotransmitters in Synaptic Plasticity and Neuropsychiatric Conditions. Neural Plast. 2018 May 6;2018:1824713

2.Humeau, Y., & Choquet, D. (2019). The next generation of approaches to investigate the link between synaptic plasticity and learning. *Nature neuroscience*, *22*(10), 1536-1543.

3.Dudink, I., White, T. A., Ardalan, M., Mallard, C., Ballerin, G., Creed, S. J., Pham, Y., Sutherland, A. E., Castillo-Melendez, M., Allison, B. J., & Miller, S. L. (2022). An Optimized and Detailed Step-by-Step Protocol for the Analysis of Neuronal Morphology in Golgi-Stained Fetal Sheep Brain. *Developmental neuroscience*, *44*(4-5), 344–362

4.Risher, W. C., Ustunkaya, T., Singh Alvarado, J., & Eroglu, C. (2014). Rapid Golgi analysis method for efficient and unbiased classification of dendritic spines. *PloS one*, *9*(9), e107591.

5. Arshadi, C., Günther, U., Eddison, M., Harrington, K. I. S., & Ferreira, T. A. (2021). SNT: a unifying toolbox for quantification of neuronal anatomy. *Nature Methods*, *18*(4), 374–377.