

Title:

Dendrite Morphometry Analysis for Spine Clusterization in Hippocampal Neurons

Authors:

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Introduction: (211/250 words)

Dendritic spines play an important role in synaptic transmission, brain plasticity, and communication. Branching patterns and dendritic complexity are directly associated with both the volume and distribution of neurons receiving and processing information, meaning that defects in neuron structure, plasticity and connectivity can lead to various intellectual disabilities [1]. Currently, dendritic spines are commonly classified into four categories based on their head and neck properties: mushroom, thin, stubby, and filopodia [1]. However, recent studies on spine shape reveal the limitations of this classification method, especially given that spines more accurately exist on a continuum of shapes and sizes rather than in discrete categories [2]. New super-resolution imaging techniques show that certain spine shapes are often overrepresented due to previous limitations in resolution. In addition, long-term observation of dendritic spines reveal their plasticity [2]. Given this information, current dendritic spine analysis may often be biased and therefore not reproducible.

The objective of this project is to apply dendritic spine clusterization to a dataset of hippocampal cultured neurons from postnatal mice to observe dendritic spine clusterization. We focus on comparing the results of dendritic spine clustering using a morphological clusterization approach versus more traditional classifications, aiming to expand understanding on the morphological variations in dendritic spines. Our results will be important in areas including computational biological analysis, neuroscience, and intellectual disabilities.

Materials and methods: (259/250 words)

The methodology of this study closely follows recent studies, which show the potential of utilizing the morphological properties of dendritic spines to perform clusterization of spines into shape-based groups as an alternative to classification. Specifically, a detailed algorithm for clustering both active (potentiated synapses with cLTP stimulation) and non-active neurons based on spine shape taxonomy was implemented [2].

First, morphological feature data is utilized from a previous dataset, which consists of proposed dendritic spines from hippocampal cultured neurons from postnatal mice, imaged using various methods, including in-vitro two-photon structural imaging, in-vitro confocal structural imaging, and in-vivo functional imaging [3]. Semi-automatic morphological feature extraction is performed using SpineJ, an imageJ plugin designed to collect morphometric data from high-resolution dendritic spine images [4]. For each spine, 12 features are collected.

Next, a probabilistic PCA (PPCA) algorithm is applied to reduce the dimensionality of the data and determine the most significant features to be used for clustering. Applying a PPCA model for data reduction may be beneficial for quantifying uncertainty in the measurements, as well as better capturing variability in the data that may be simplified using a deterministic model.

After determining important features, both “crisp” (deterministic) and “hierarchical” (probabilistic) clustering algorithms will be applied to the data. The result of grouping the dendritic spines using the proposed clusterization techniques will be compared to the groupings created using the traditional four-class method.

Results (254/350 words)

Figure 1 shows the morphological feature collection pipeline. The first step is filtering and spine segmentation, during which parameters including segmentation coefficient and minimum segmentation size (in pixels) are selected. Additionally, spine reconnection is done to ensure that neck features are preserved. The next step is spine identification, which includes manual identifications of spines and neck definition. The features collected include: Neck length (nm), Spine Length (nm), Ratio (%), Minor axis (nm), Major axis (nm), Aspect Ratio, Perimeter head (nm), Area head (nm), Smallest neck width (nm), Median neck width (nm), Average neck width (nm), and FWHM (nm)). These measurements are used to calculate additional parameters specified in existing literature, including width to length ratio (WLR), length to width ratio (LWR), and length to area ratio (LAR) [2]. In total, 15 morphological features were collected from 10 spines for the initial experimentation. These steps will be followed for all images in the dataset to collect adequate feature information for PPCA dimensionality reduction and clustering.

Conclusions and Discussions

We will continue to collect data, and perform clusterization of important morphological features to expand this methodology for the application of identifying morphological patterns and clusters that can be attributed to diseased and healthy individuals. This project could expand understanding on the morphological variations in dendritic spines that are associated with intellectual disability, potentially contributing to the development of targeted interventions and treatment approaches, and improving our understanding of the neurological basis of intellectual disabilities.

Figure

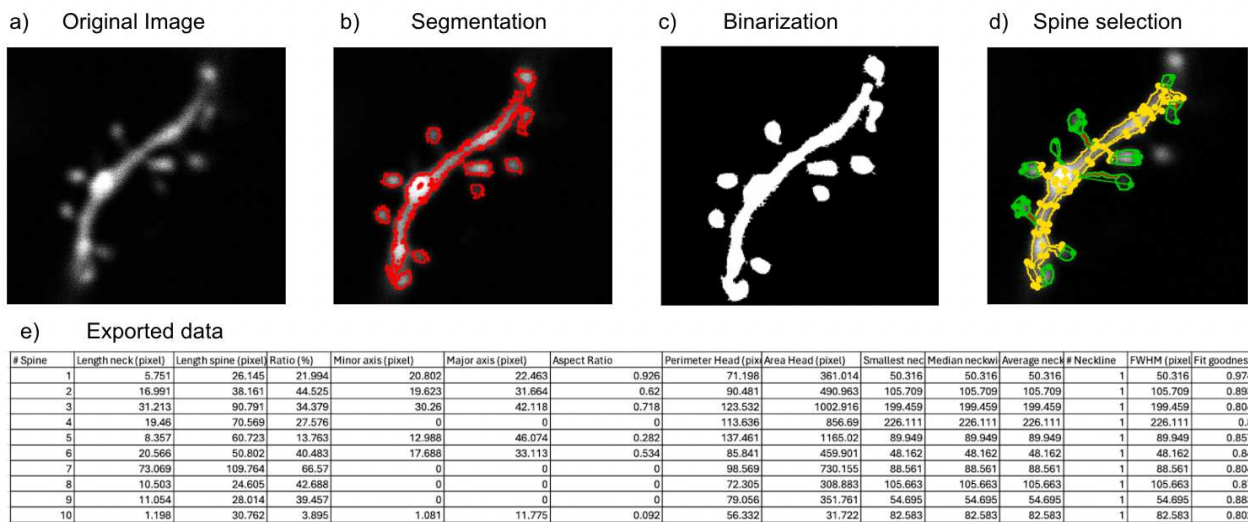


Figure 1: Dendrite morphometry data collection pipeline for in-vitro two-photon structural image of hippocampal neurons from postnatal mice. 10 dendrites were identified in the .tif image, and the 15 morphological features are specified in e). This analysis was performed using the SpineJ plugin in ImageJ.

