3D Morphological Analysis of Biocytin Injected Hypoglossal Neurons

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Pediatric dysphagia is a condition characterized by difficulties in feeding and swallowing in infants and children. The tongue is a major structure involved in feeding and swallowing, and abnormalities in tongue control occur in patients suffering from dysphagia. Hypoglossal nerve is a cranial nerve that originates from the hypoglossal nucleus in the brain stem and innervates the tongue; it is responsible for all the motor supply of the tongue. To study the effects of dysphagia on neurons from the hypoglossal nucleus, we are using a mouse model of DiGeorge Syndrome (22q11 Deletion Syndrome) that display the feeding and swallowing difficulties seen in 22q11DS patients, mimicking a pediatric dysphagia. The goal of this research is to analyze the dendritic morphology of motoneurons localized in the hypoglossal nucleus, which have undergone electrophysiological measurements and filed with biocytin, between a control group/wild type (WT) and mice with the genes deletion (LgDel). The specific dendritic parameters that will be observed are length, branching points, straightness, and volume.

The samples are prepared after they undergo patch-clamp measurements – the cells are filled with biocytin and Alexa Fluor 647. After the samples are prepared, the images are acquired utilizing confocal and fluorescence microscopy techniques with the Leica TCS SP8 Multiphoton Confocal using single-molecule detectors. Using this microscope, we acquired overviews with the 10x objective and high-resolution 3D data sets using the 20x objective. The 3D data sets were analyzed on Imaris, a 3D visualization and analysis software; we used Imaris to qualitatively view the neurons and create 3D reconstructions of neuronal morphology. These 3D reconstructions allowed us to obtain quantitative data of dendritic parameters that were further analyzed. Initial findings suggest there is a trend that shows less complexity of dendritic branching of LgDel neurons that is an indication of changes in the afferent activity of this neurons. Our next steps for this project will be to label the motoneurons responsible for protrusion and retraction of the tongue. This will allow the researcher to correlate the morphology of a neuron to the specific function of that neuron.