How Overexpression of miR-9 in Juvenile Songbirds Affects Neural Pathway Development Within Area X

Max Horowitz, Hannah Jarrell, XiaoChing Li, Ph.D.

Neuroscience Center of Excellence LSU Health Sciences Center

LSU Health NEW ORLEANS School of Medicine

Introduction

Language development in both songbirds and humans is heavily impacted by perceptual experience throughout the critical period of juvenile maturation (1). Similar to the way human infants learn from parents, perceptual experience in songbirds is guided through a conspecific "tutor", typically the father. Juvenile songbirds utilize trial and error techniques by producing simple vocalizations, called "subsongs," and comparing with the tutored song. This process is remarkably similar to the "babbling" phase observed in human language development (2).

Similarities in the language developmental stages between humans and songbirds are likely due to the convergent evolution of analogous brain regions. Basal Ganglia, subcortical nuclei that play a significant role in the neural development of human language, are also present in songbird brains. Area X is a specific basal ganglia nucleus of interest due to prior findings that conclude: lesions within Area X in the songbird brain limit juvenile songbirds in the latter stages of language development, causing stuttering abnormalities in adulthood (3).

FOXP2 is a genetic sequence, in both humans and songbirds, that facilitates the creation of neural pathways responsible for language development (4). Specifically, in humans, mutations within the FOXP2 sequence cause speech and language disorders, such as autism. Regulation of FOXP2 expression is carried out via specific microRNAs post-transcriptionally. miR-9 is an evolutionarily successful microRNA abundant within Area X, where it directly regulates FOXP2 expression and subsequently, the development of language in juvenile songbirds. Inducing overexpression of miR-9 has been proven to result in both, downregulation of FOXP2 expression, and more variable (less effective) song production in adults (5).

This project's objective is to observe the effect overexpression of mirR-9 has on the structural development of neural circuitry within Area X.

Methods

Subject Rearing:
Juvenile Zebra Finches were injected twice at 26-28 days of age. One injection contained a miR-9 virus and was introduced to a single hemisphere of Area X. The second virus, sans miR-9, was introduced to the opposite hemisphere. Following injection, Juveniles were isolated with a "tutor" throughout the critical period of language development

Imaging:
Half of the birds were sacrificed at 60 days of age, while the other half were sacrificed at 100 days. Area X was then sectioned by diving the hemispheres hemispheres. Neurons were then stained with darp32, a primary antibody, for neuron identification. darp32 was then accompanied with a fluorescent secondary antibody in order to provide a fluorescent stain under the fv1200 confocal microscope.

Tracing:
The confocal microscope captured images of Medium Spiny Neurons (MSNs) from Area X. These images were imported into Imaris 9.2.1 for 3D construction of the neuronal structure. From Imaris, quantitative data analyzing spines and dendrites were exported. Data exported from Imaris indicating spine structure included spine density, spine head volume, and spine length. Data exported from Imaris indicating dendrite structure included dendrite length sum, number of branch points and Scholl interactions.

Induced overexpression of miR-9 in juvenile Zebra Finch

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Juvenile is tutored during critical period

↓

Images of Medium Spiny Neurons within Area X are captured for tracing

↓

Images were traced in 3D for quantitative analysis on structural development

Informational Models

Figure 1: FOXP2
The FOXP2 genetic sequence resides on the 7th chromosome on the longer arm (q) at position 31.1. (6)
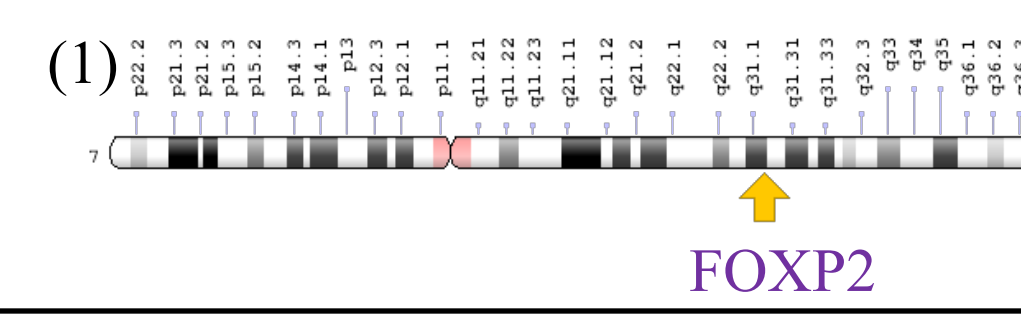

FOXP2

Figure 2: Area X
Area X is located in the basal brain region and is here being modeled as the injection site (5)
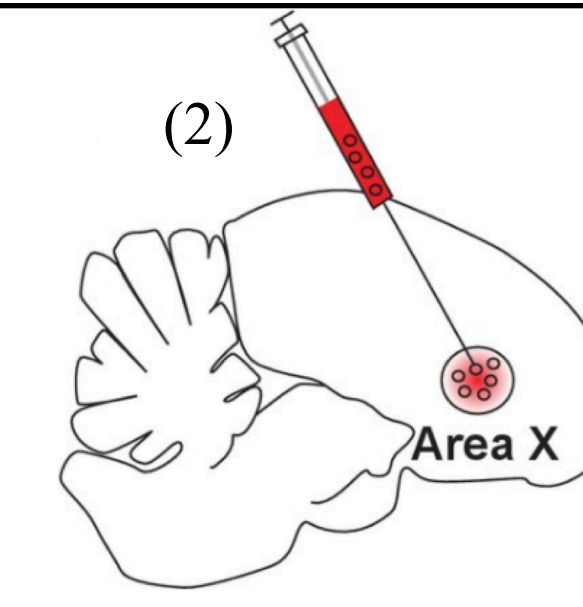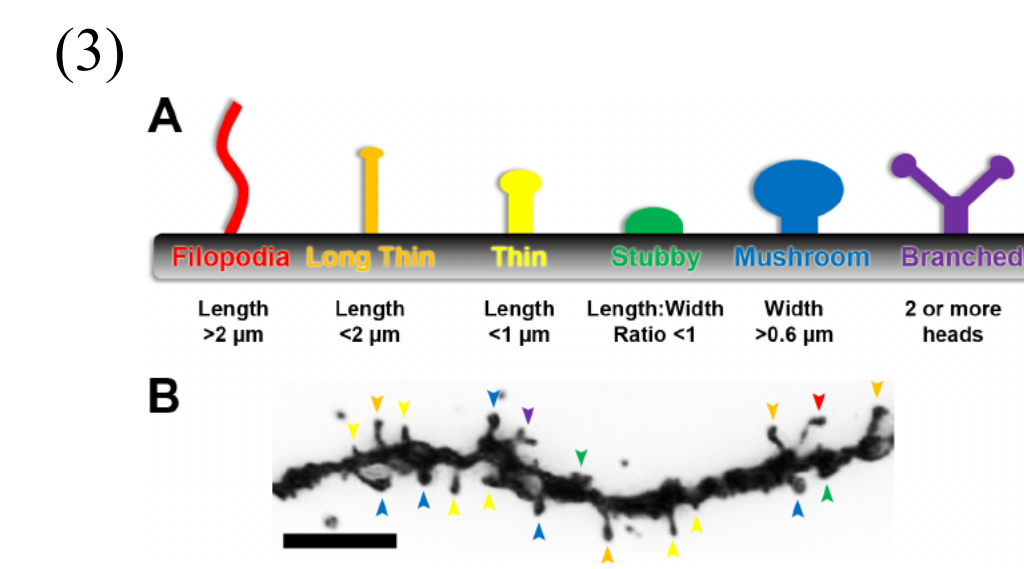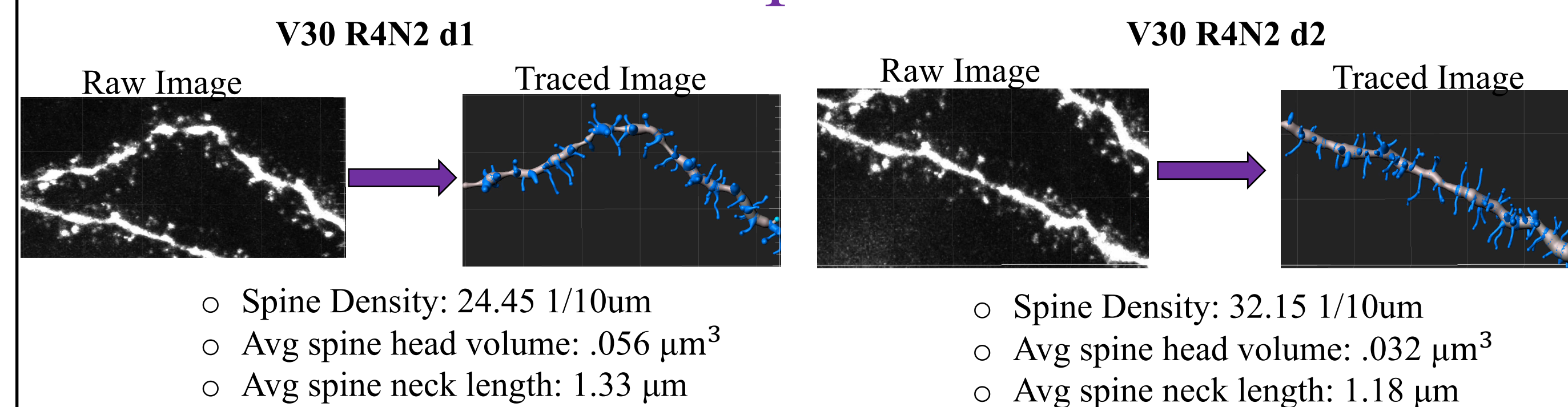

Area X

Figure 3: Dendritic Spines
Dendritic spines are small protrusions off dendrites that have direct contact with a synapse. Spine anatomy consists of a head and neck of which varies in size from spine to spine. As a synaptic connection strengthens, head size grows and neck length shortens as the shape develops (7). Model A shows shape progression while B labels each spine shape on a theoretical image.
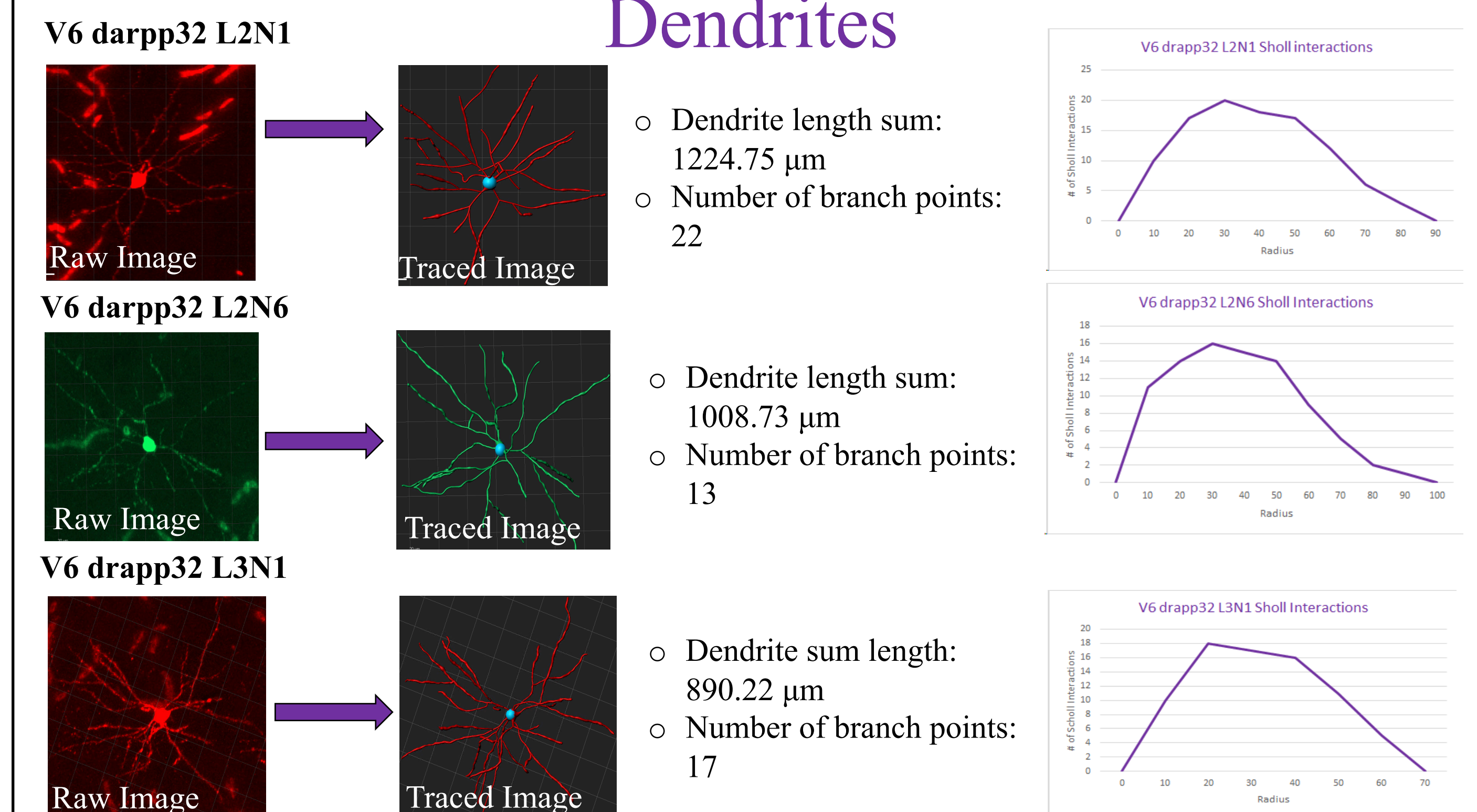

Data

Spines

V30 R4N2 d1

Raw Image → Traced Image

○ Spine Density: 24.45 1/10um
○ Avg spine head volume: .056 μm^3
○ Avg spine neck length: 1.33 μm

V30 R4N2 d2

Raw Image → Traced Image

○ Spine Density: 32.15 1/10um
○ Avg spine head volume: .032 μm^3
○ Avg spine neck length: 1.18 μm

Dendrites

V6 darpp32 L2N1

Raw Image → Traced Image

○ Dendrite length sum: 1224.75 μm
○ Number of branch points: 22

V6 drapp32 L2N1 Sholl interactions

V6 darpp32 L2N6

Raw Image → Traced Image

○ Dendrite length sum: 1008.73 μm
○ Number of branch points: 13

V6 drapp32 L2N6 Sholl Interactions

V6 drapp32 L3N1

Raw Image → Traced Image

○ Dendrite sum length: 890.22 μm
○ Number of branch points: 17

V6 drapp32 L3N1 Sholl Interactions

Data Interpretation

Dendrite analysis is split into two objectives
1. To analyze size of the dendrite
   - The **sum of the dendrite length** includes the length of all branched dendrites (primary, secondary, and tertiary). The dendrite length sum is measured in μm.
2. To analyze complexity of the dendrite
   - The **number of branch points** indicates how many locations a new branch was formed.
   - **Sholl interactions** indicate individual dendrite branches that cross a certain radius. A higher sholl interaction indicates a higher dendritic complexity at a given radius.

Spine analysis is split into two objectives.
1. To summarize spinal activity on the entire dendrite
   - **Spine density** is collected to represent the strength of connection between the dendrite and the synapse. Spine density is measured in spines per 10 μm of dendrite length.
2. To analyze the spines independently
   - **Spine head volume** is collected to analyze the synaptic strength associated with each spine. Spine head volume is measured in μm^3
   - **Spine neck length** is collected to distinguish the spine shape. Spine neck length is measured in μm.

Discussion

The results of this project are still unfolding as data analysis is still in progress. It is essential to uphold the blind perspective upon analysis in order to ensure a non-biased evaluation of the eventual results. In doing so, no conclusive statement can be made regarding the presented data as the origin of each image remains confidential. When data analysis is complete, and revelation of bird ID can be made, precise and consistent results will hold significant potential application. The similarities in language development between humans and songbirds allow findings in songbird language development to have human relevance. Understanding the structural effect that FOXP2 gene expression has on the neural pathway development could improve understanding in mild language defects, such as stuttering, and more notable language defects seen in autism spectrum disorders.

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