Unraveling Cognitive Mysteries: Dendritic Spine Morphology in Pitt-Hopkins Syndrome (PTHS)

Dan Tsuma, Ben Philpot
Philpot Lab, Neuroscience Center, Department of Cell Biology and Physiology

Background

Pitt-Hopkins Syndrome (PTHS), a rare neurodevelopmental disorder resulting from TCF4 gene haploinsufficiency, leads to profound cognitive challenges, including intellectual disability and learning and memory deficits. Understanding the neural basis of these cognitive impairments is essential for advancing therapies. Dendritic spines, minute protrusions on neurons, are key players in synaptic connections and memory formation, making their morphology a vital focus. Changes in spine characteristics are linked to various neurological disorders, including autism spectrum disorder. This research explores how TCF4 haploinsufficiency in PTHS may affect dendritic spine morphology, specifically in the hippocampus, but also in the cortex, and its role in learning and memory deficits. By comparing dendritic spine characteristics between a novel PTHS mouse model, which exhibits TCF4 haploinsufficiency, and wildtype mice, we aim to test the hypothesis that TCF4 haploinsufficiency leads to a reduction in spine number and concurrent alterations in their size and shape. Through Golgi staining, widefield microscopy, and future image analysis, this investigation lays the groundwork to elucidate the intricate relationship between dendritic spine structure and cognitive function in PTHS, potentially paving the way for therapeutic advancements and improved quality of life for affected individuals.

Methods

Sample Collection

- Brain tissues were collected from both PTHS (Pitt-Hopkins Syndrome) and wildtype mice, maintaining the blind nature of the experimentation, where sample identities remained undisclosed to researchers.

Golgi-Cox Staining

- Employed the FD Rapid GolgiStain™ Kit, a modified version of the traditional Golgi-Cox staining method developed in 1987, renowned for its efficiency.

Tissue Preparation

- Fresh brain tissue was chosen for the study, as this method is not suitable for previously formalin-fixed or fresh-frozen brains. Brains were rapidly extracted from the skull without prior fixation to ensure optimal results and minimize post-mortem alterations.

Impregnation Solution

- One half of each brain, regardless of its origin (PTHS or wildtype), was submerged in the impregnation solution, created by mixing equal volumes of Solutions A and B, which had been combined and left unstirred for 24 hours. The impregnation phase, conducted in a blinded manner, spanned two weeks, with samples gently swirled, but not shaken, several times each week.

Tissue Transition

- Following impregnation, the brain halves, again blind to the experimenters, were transferred to Solution C for an additional 72 hours, up to a maximum of one week.

Sectioning

- The brain halves were subsequently frozen in isopentane cooled with dry ice and were sectioned into slices with thicknesses ranging from 100 to 200 µm. Care was taken to avoid the use of anti-roll.

Slide Mounting

- The obtained sections, regardless of their origin, were mounted on gelatin-coated slides using Solution C. Excess solution was meticulously removed, and sections were left to air-dry overnight at room temperature, maintaining the blinding of sample identities.

Staining

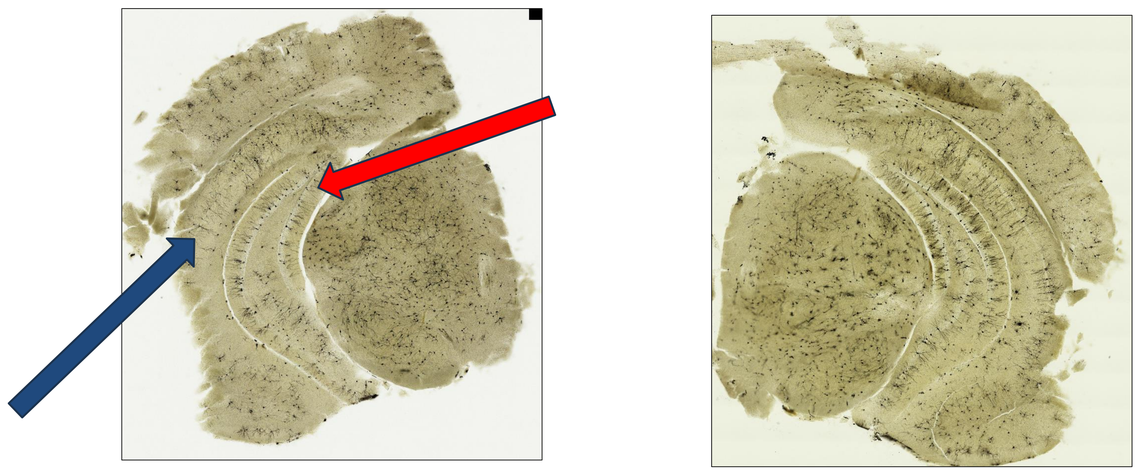
- On staining days, an identical staining protocol was applied to both PTHS and wildtype sections. Precise steps were taken, encompassing water baths and ethanol immersion, ending with xylene treatment and coverslipping with Eukitt.

Imaging

- All sections were imaged, without knowledge of their origin, using the Nikon Eclipse Ti2, a widefield microscope available in the UNC Neuroscience Microscopy Core. Imaging included both hippocampal and cortical regions to visualize dendritic spine morphology in a non-biased manner.

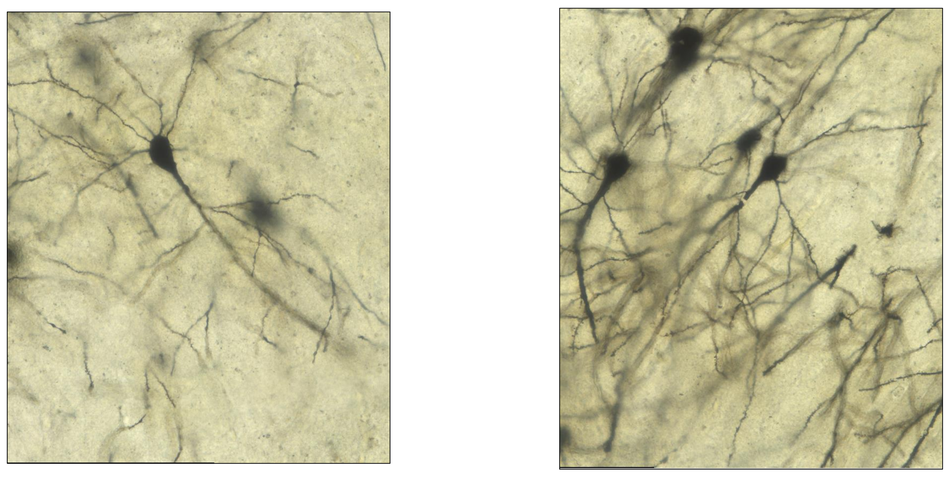
Results

Figure 1: Golgi-Cox Stained Mouse Brain Sections



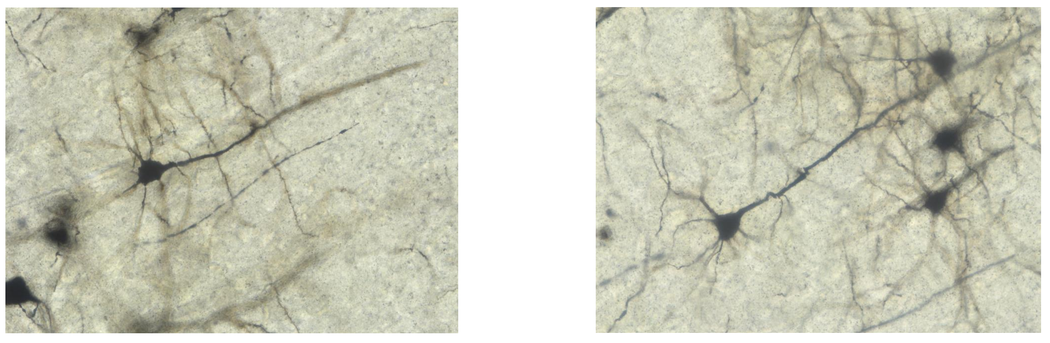
Caption: Both the left image and the right image are stained mouse brain sections, with a red labeled arrow indicating the hippocampus and a blue labeled arrow indicating the cerebral cortex

Figure 2: Hippocampal Neurons in Golgi-Stained Sections



Caption: Both the left image and the right image are stained hippocampal neurons, corresponding to the two brain sections above (**Fig 1**)

Figure 3: Cortex Neurons in Golgi-Stained Sections



Caption: Both the left image and the right image are stained cortex neurons, corresponding to the two brain sections above (**Fig 1**)

Conclusions/Next Steps

Our results, captured by the Nikon Eclipse Ti2, affirm the successful Golgi-Cox staining of entire brain sections, particularly in the hippocampal and cortex regions relevant to learning and memory deficits in Pitt-Hopkins Syndrome (PTHS). This validation underscores the reliability of the staining method, enabling us to move forward confidently with our blind experiment. With this foundation in place, our next step involves developing a pipeline for quantifying dendritic spine density in PTHS and wildtype mice. By testing our hypothesis that TCF4 haploinsufficiency leads to reduced spine numbers and alterations in size and shape, we aim to elucidate the role of dendritic spine morphology in PTHS.

Acknowledgements

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Microscopy was performed at the UNC Neuroscience Microscopy Core.