SNARE proteins in retinal synaptic vesicle release

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Introduction

Exocytosis is the process of vesicle fusion and is the final step in the secretory pathway. Exocytosis is driven by the continuous assembly and disassembly of the SNARE complex. Traditionally, the SNARE complex is comprised of **syntaxin-1, SNAP-25** and **synaptobrevin 2**. Nonconventional photoreceptor synapses have SNARE complexes containing **syntaxin-3** rather than syntaxin-1, while much debate surrounds the SNAP isoform. Alongside the core proteins, regulators such as Munc18-1 play critical roles in traditional exocytosis. We hypothesize that non-traditional SNARE proteins such as syntaxin-3, SNAP-23 and the SNARE regulator Munc18-1 play key roles in photoreceptor neurotransmitter release. Elucidating the role of these SNARE proteins and regulators will be important in understanding the ribbon synapses of the retina and how photoreceptor neurotransmitter release is maintained. This knowledge will be critical to understanding synaptic transmission impairments that often precedes retinal degeneration which is the leading cause of vision loss worldwide.

Methods

To study the function and morphology of these proteins in retinal ribbon synapses, we developed an in vivo conditional knockout (cKO) model and will selectively remove these proteins from photoreceptor cells in mice.

Morphological (optical coherence tomography, electron micrographs) and **functional** (visual acuity by optokinetic tracking, light response in electroretinograms) investigations were done to address changes following specific protein removal.

Results

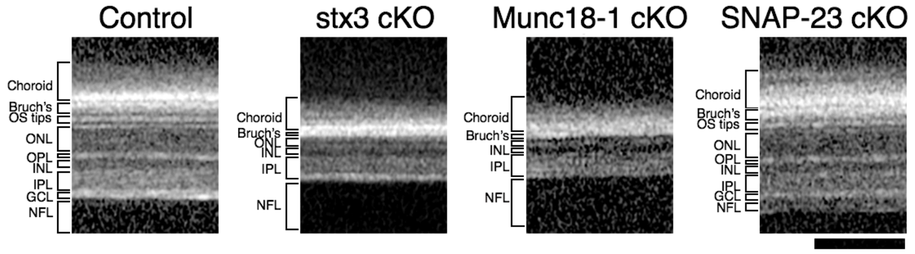

Figure 1a: Decrease in retinal thickness of stx3 and Munc18-1 cKO observed by optical coherence tomography. Scale bar = 200µm.

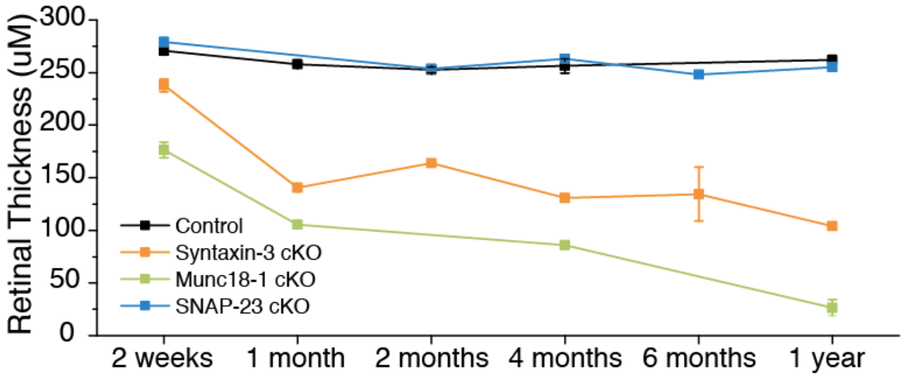

Figure 1b: Tracking of average retinal thickness 1.5mm away from optic nerve over time. Stx3 cKO shows moderate loss to retinal thickness while Munc18-1 cKO shows severe loss of retinal thickness.

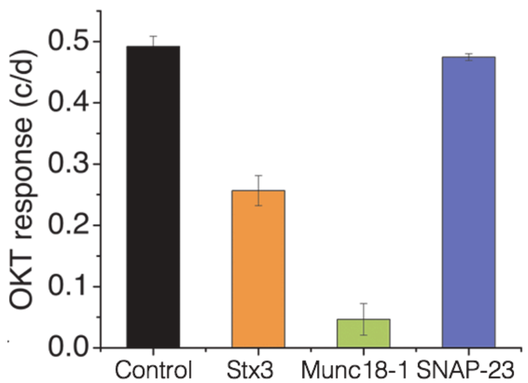

Figure 2: Visual acuity measured by optokinetic tracking response. A moderate decline in visual acuity is seen in syntaxin-3 cKO mice, while Munc18-1 cKO mice are blind. No changes were observed in visual acuity of SNAP-23 cKO mice

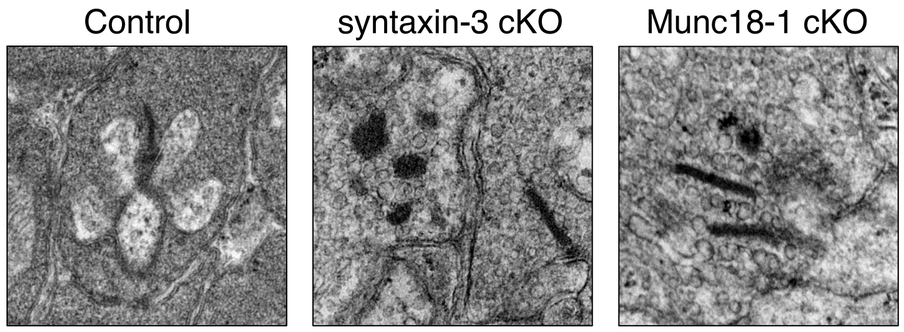

Figure 3: Electron micrographs of control, syntaxin-3 cKO, and Munc18-1 cKO photoreceptor terminals. Photoreceptor terminal with invaginated post-synapses and presence of single ribbon clearly observed in control retina. Stx3 cKO and Munc18-1 cKO lack defined post-synaptic connections. Duplicated and rounded ribbons also observed in both stx3 and Munc18-1 cKO retina.

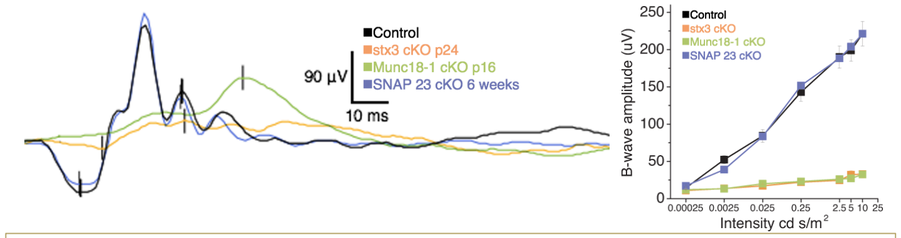

Figure 4: Electroretinogram recordings shows decline in functional photoreceptor response to light in Munc18-1 cKO and stxbp1 cKO retinas. Sample electroretinogram trace (left) and average b-wave amplitude across light intensities (right). B-wave indicative of photoreceptor synaptic transmission is severely attenuated in both stx3 and stxbp1 cKO retina.

Summary of Findings

Syntaxin-3 and **Munc18-1** are critically important to retinal synaptic transmission. Loss of these proteins leads to

- Progressively decreases in retinal thickness
- Impairments to synaptic structure and shorter synaptic ribbons
- Decreases to response to light and visual acuity

Acknowledgements

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