Loss of Xbp1 in retinal cells accelerates age-related deterioration in the mouse visual system

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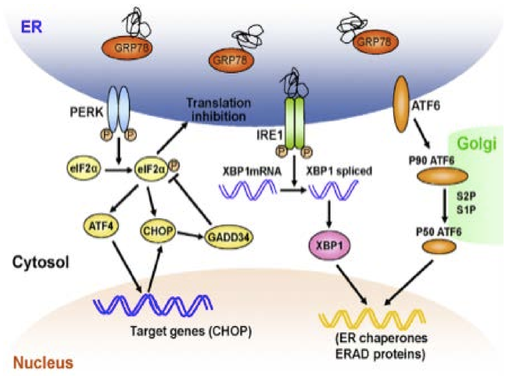
Introduction

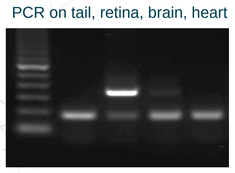
X-box binding protein 1 (Xbp1) is a critical component of the Unfolded Protein Response to reduce endoplasmic reticulum (ER) stress. We hindered cellular stress responses by deleting Xbp1 from a subset of retinal cells to determine if naturally occurring stress over a long period (i.e. more than one year) contributes to age-related abnormalities in retinal structure and functional decline in mice.



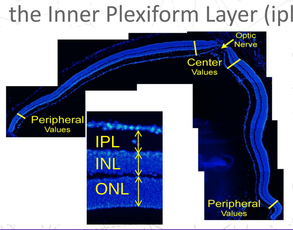
Zhang et al., 2014

Methods

- Conditional knockout (cKO) of Xbp1 was achieved by crossing mice with floxed alleles of Xbp1 with a retina-specific Cre line (Chx10-cre).
- Cre deletes Xbp1 from a subset of retinal cells, including most or all bipolar cells, prior to maturation. Retinal morphology was examined with immunohistochemical markers in wild type (WT). and Xbp1 cKO in retinal sections in adolescent and 12-15 month old mice.
- PCR demonstrates cre-mediated recombination is specific to retina.



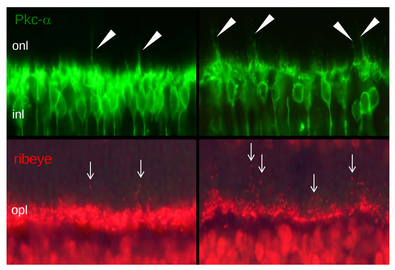
- Visual function was assessed by both dark and light adapted electroretinogram (ERG) in both cKO and WT.
- Sections stained with DAPI were used to measure thickness of the Outer Nuclear Layer (onl), Inner Nuclear Layer (inl), and the Inner Plexiform Layer (ipl)



Layer measurements were 300 microns from the optic nerve and 300 microns from the peripheral edges

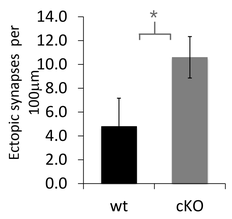
Results

Bipolar cells in Xbp1 fl/fl; Chx10-cre retina extend dendrites into onl and have more ectopic synapses than in WT



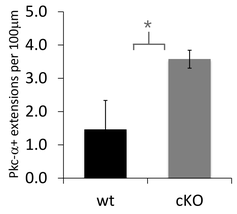
Cryosections of 12-14 month retina labeled with antibodies against Pkc-α (green), ribeye (red), and counterstained with DAPI

Extensions from bipolar cells into the outer nuclear layer (onl; arrowheads) as well as the ectopic synapses outside the outer plexiform layer (opl) and within the onl (arrows)

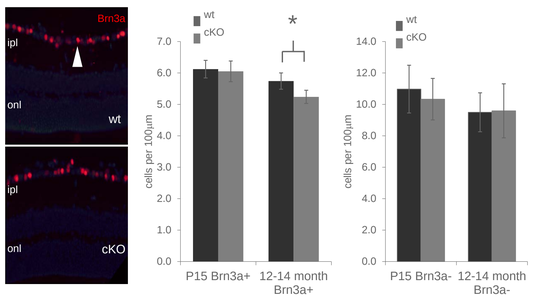


There are significantly more ectopic synapses in the Xbp1 cKO than in WT.

The number of extensions from Pkc-α positive bipolar cells into the onl also reveals significantly more extensions in Xbp1 cKO than in WT retina, with more than twice as many extensions in the Xbp1 cKO than in WT.



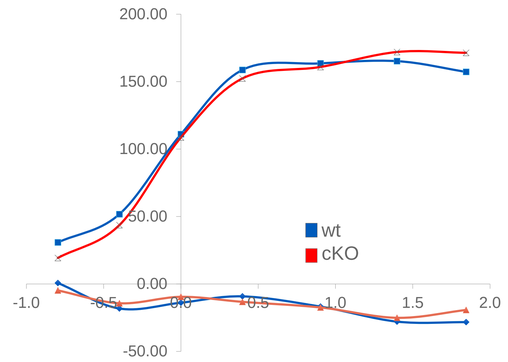
Conditional knockout of Xbp1 results in fewer ganglion cells in 12 month old mice



We find significantly fewer Brn3a-positive cells within the ganglion cell layer at 12-14 months. There is no difference in Brn3a-negative cells at P15 or at 12-14 months.

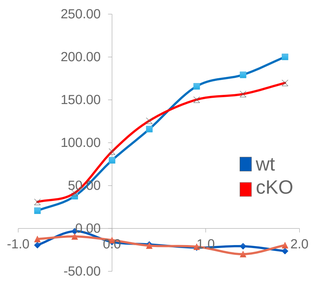
Results

In light adapted ERG 6-8 month old WT and Xbp1 cKO are indistinguishable



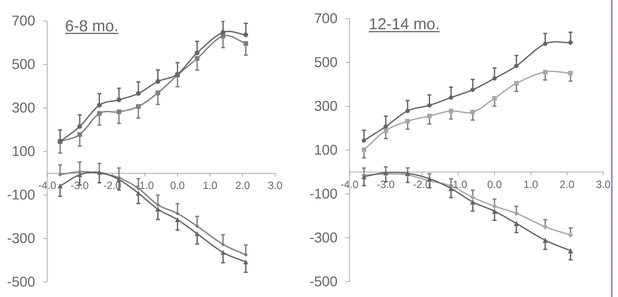
Light adapted ERG responses for 6-8 months WT (blue) and 6-8 months cKO mice (red) over 7 steps of increasing stimulus intensity. There is no difference in a-wave (bottom lines) or b-wave (upper lines) amplitude at any intensity

Possibly reduced response in light adapted ERG 12+ month old Xbp1 cKO



There is an apparent small reduction in light adapted b-wave (upper lines) at higher intensity in 12+ month old cKO mice (red)

Dark adapted ERG response indicates that the retinal function declines in Xbp1 cKO by 12 months of age

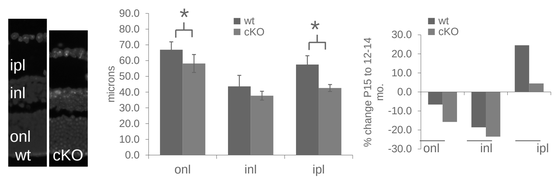


Graphs of the 10-step dark adapted ERG responses for 6-8 month old mice (left) and 12-14 month old mice (right)

In 12-14 months aged mice, significantly smaller responses in Xbp1 cKO (grey) compared to WT (black) are observed for both the a-wave and b-wave responses at higher flash intensities.

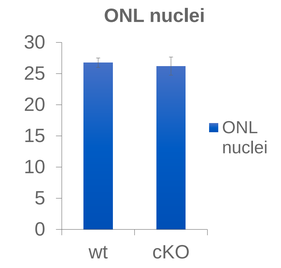
Results

Conditional knockout of Xbp1 results in retinal deterioration in 12 month old mice

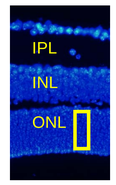


DAPI stained cryosections of WT and Xbp1 cKO retina at 12-14 months that closely represent the average retinal layers for each genotype. The outer nuclear layer (onl; $p < 0.02$) and inner plexiform layer (ipl; $p < 0.001$) are significantly thinner in Xbp1 cKO. Graph (right) of the percent change in layer thickness from P15 to 12-14 months for WT and Xbp1 cKO retina.

Outer Nuclear Layer (onl) cell number in Xbp1 cKO is not significantly different than WT at 12+ months



Comparison of ONL cell counting between wild type and Xbp1 cKO. Each sample counted a three nuclei wide column of the onl 300 microns from the optic nerve.



Dapi stained retinal sections showing the location of the counted area.

Conclusion

- The loss of Xbp1 compromises the ability of a cell to respond to the chronic stresses related to aging. Eventually, retina lacking Xbp1 in a subset of cells are less functional than WT littermates.
- We find that bipolar cells in the Xbp1 cKO are less functional than WT cells at an advanced age, and display several morphological attributes that mimic much older animals.
- We suggest that routine long term stress is detrimental to the visual system and age-related decline is accelerated in the absence of a robust ER stress response system.