Background Data for P23H Line 1 Heterozygous Rats Raised Under Dim Light Conditions for the First 60 Days Post Partum

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1 Introduction

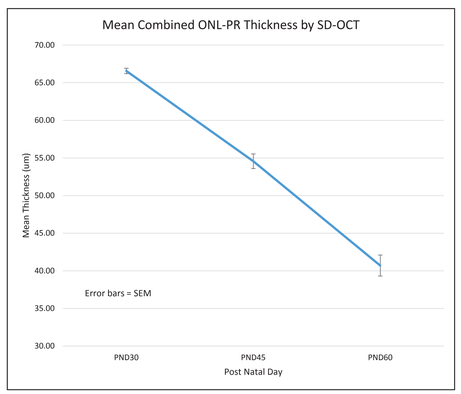
The P23H-1 transgenic rat exhibits retinal degeneration of the photoreceptors/outer nuclear layer due to insertion of a mutated mouse rhodopsin gene and has been used as a model for mechanistic and efficacy studies for autosomal dominant retinitis pigmentosa. The reported rate of outer nuclear layer (ONL) thickness loss is approximately 60% by post natal day 30 (PND30) and 80% by PND60. The objective was to determine if low light housing conditions during the 1st month post partum, when ocular structures are still under development, would delay the initial rate of degeneration, preserving the ONL cell layer enough to support a dose response neuroprotection efficacy regimen commencing in the PND21-PND30 range.

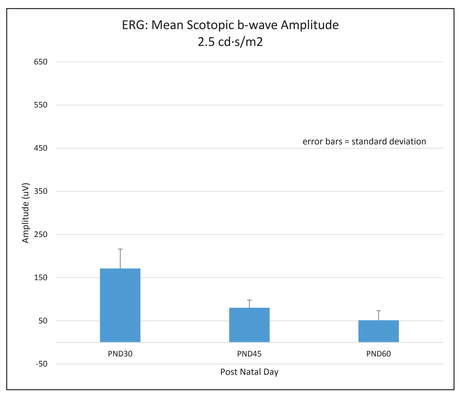
2 Methods

P23H-1 homozygous males (sourced from the RRRC) were mated to wild type Sprague Dawley females (Charles River, St. Constant, QC). The hemizygous P23H-1 pups were born and raised under a 12hr light/dark cycle with a maximum of 20 lux of room light during the light cycle until PND30, after which standard room light intensity (ca. 200 lux) was used. Photoreceptor (PR)/Outer Nuclear Layer (ONL) thickness was measured by spectral domain optical coherence tomography (SD-OCT) at approximately PND30, 45 and 60 (2 week intervals). The combined thickness measurement of the ONL-PR layers was done for consistency due to loss of definition of the PR layer over time. Electroretinograms (ERG) were recorded during the same period with a stimulus intensity (SI) of 2.5 cd·s/m^2. Microscopic evaluations of H&E-stained eye sections were evaluated at PND60.

3 Results

The rates of reduction in ONL-PR thickness were 18% and 25% between PND30 and PND45, and PND45 and PND60, respectively. Total reduction was 38% over 4 weeks. Previously reported ONL thinning ranged from approximately 50% (LaVail *et al* 2018, histologically) to approximately 21% (Ohran *et al* 2015, SD-OCT) for the same 4 week time period. Mean ONL-PR thickness was approximately 40 µm at PND60 with the dim light protocol while ONL thickness alone ranged from 10 to 23 µm in standard room light for LaVail and Ohran, respectively. Note that the photoreceptor contribution to the overall thickness in a healthy eye is about 20-25%, which should be considered when comparing these values to the literature. Reduction in scotopic ERG b-wave amplitude was 18% over 4 weeks from PND30 - PND60, *vs* approximately 48% reported by LaVail et al (2.4 cd·s/m^2 SI) and approximately 25% reported by Ohran (12 cd·s/m^2 SI, approximately 5x).





4 Discussion and Conclusion

Overall the rate of physical degeneration appeared to be slowed by housing the rat pups in low light for the first 30 days post partum given the absolute ONL thickness at that point and the number of ONL cell layers remaining by PND60 *vs* previously reported values. The rate of ONL-PR thinning in the 1st 60 days post partum appeared to be sufficient to detect a dose response, but the ERG changes compared to the literature did not support an obvious advantage in the short term at least, as the values by PND30 were already quite low. This was possibly influenced by the different breeding stock, anesthetic regimens, light stimulus intensities (much higher in Ohran paper), and/or the differences in light exposure from birth to 60 days post partum between these 3 references.

In summary, the P23H Line 1 rat was considered to be suitable for use as a physical retinal degenerative model for evaluation of neuroprotection using in-vivo and post mortem parameters. More work is required to optimize the functional ERG parameter to determine if it could be a reliable endpoint.

Microscopic evaluation of the H&E sections showed the number of ONL cells on PND60 reduced to 5-7 layers (10-12 is the normal amount for wild type Sprague Dawley), which was more than the 2-4 layers reported by LaVail (Ohran did not report this value).

References:
LaVail M, *et al.*, Phenotypic characterization of P23H and S334ter rhodopsin transgenic rat models of inherited retinal degeneration. Exp Eye Res, 167 (2018) 56-90.
Orhan E, *et al.* (2015) Genotypic and Phenotypic Characterization of P23H Line 1 Rat Model. PLoS ONE 10(5): e0127319. doi:10.1371/journal.pone.0127319