Dark Rearing as a Means of Mimicking 'Physiological Hypoxia': a Rationale for Non-invasive Treatment of Retinopathy of Prematurity

Koina, M.[1], Chan-Ling, T.[2], Barnett, N.[3], Maccarone, R.[4], Provis, J.[5], Hu, P.[2], Natoli, R.[5], Bisti, S.[4], Linsenmeier, R.[6] and Adamson, S.[2]

[1] ACT Pathology, The Canberra Hospital, Australia, [2] Retinal & Developmental Neurobiology Laboratory, University of Sydney, Australia, [3] Queensland Eye Institute, Brisbane, QLD, Australia, Australia, [4] University of L'Aquila, Italy, [5] John Curtin School of Medical Research, Australian National University, Australia, [6] Department of Biomedical Engineering, Northwestern University, United States

An adverse side-effect of supplemental O_2 therapy for very premature infants is elevation of retinal oxygen levels, causing *delayed retinal vascularization* - the initiating event in retinopathy of prematurity (ROP). Because photoreceptors are depolarized and consume more O_2 in the dark, we hypothesized that keeping neonates in total darkness during O_2 therapy would normalize the pathogenic levels of retinal O_2. We propose that photoreceptor depolarization acts as a 'metabolic sump', depleting O_2 and preventing the down-regulation of vascular endothelial growth factor (VEGF). As proof of principle, we showed that dark rearing (DR) from birth to P11 resulted in higher superficial and deep retinal vascular density. Using a rat 50/10 model of oxygen-induced retinopathy (OIR), we showed that DR resulted in significantly smaller areas of non-vascularized peripheral retina, compared with OIR animals raised in normal cyclic light during Phase 1 OIR. This preservation of retinal vasculature in OIR+DR animals protects retinal neurons, vessel integrity, and supporting cells including astrocytes and pericytes during the hypoxic Phase 2 OIR. Moreover, DR mitigates the pathology typically seen after oxygen administration including formation of pre-retinal vessels, and reduces tissue hypoxia and expression levels of *HIF1Φ#177;*, *VEGF$_{164}$*, and *AP1/Jun* mRNA. Electroretinography and Transmission Electron Microscopy indicate that DR has no harmful effects on retinal function and structure respectively. The findings suggest that DR is an effective intervention during oxygen administration and mitigates iatrogenic ROP disease, post oxygen administration. Taken together, we suggest that DR offers a viable, non-invasive treatment for prevention of the disease and that DR could serve to supplement other strategies to minimize the damaging effects of ROP.

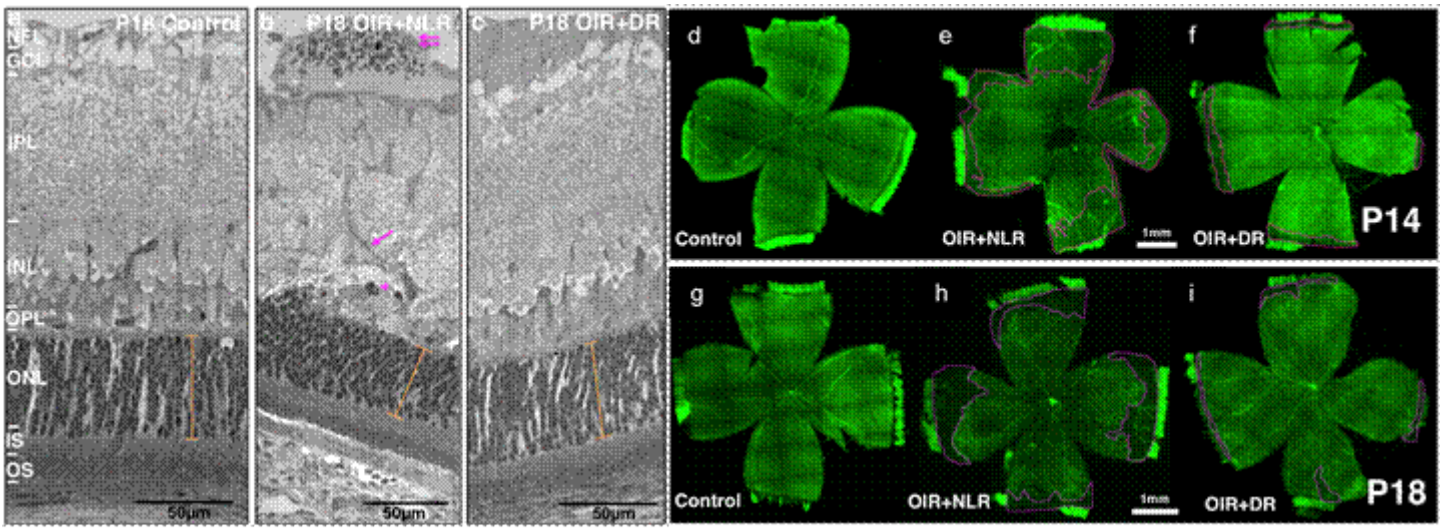

Figure Legend. *a - TEM of P18 control animal showing the normal ultrastructure of the retina, b - TEM of P18 animal OIR in normal light demonstrating abnormal vessel formation (double arrow) confirming our chosen model system, c - TEM P18 OIR in Dark Rearing conditions demonstrating essentially normal ultrastructure. d - f P14 animals ROP phase1, g-i P18 ROP phase animals whole retina stained with GS Isolectin B4 it can be seen that the outer portion of the retina is avascular (outlined in pink) in the OIR - normal light animals and this is largely eliminated by dark rearing.*

This research was supported by the National Health & Medical Research Council of Australia Project Grant #1005730 and Fellowship #571100, Baxter Charitable Foundation, The Bonnie Babes Foundation (Donors Lucy and Malcolm Turnbull), Rebecca Cooper Medical Foundation, the Sydney Medical School Foundation, the Brian M. Kirby Foundation Gift of Sight Initiative, the Alma Hazel Eddy Trust and the Ronald Geoffrey Arnott Foundation to TCL. SA was the recipient of the University of Sydney Post-Graduate Award, the Brian M. Kirby Foundation Scholarship and project support from the NWG Macintosh Memorial Fund