Differentiation of Retinal Ganglion Cell Subtypes from Human Induced Pluripotent Stem Cells

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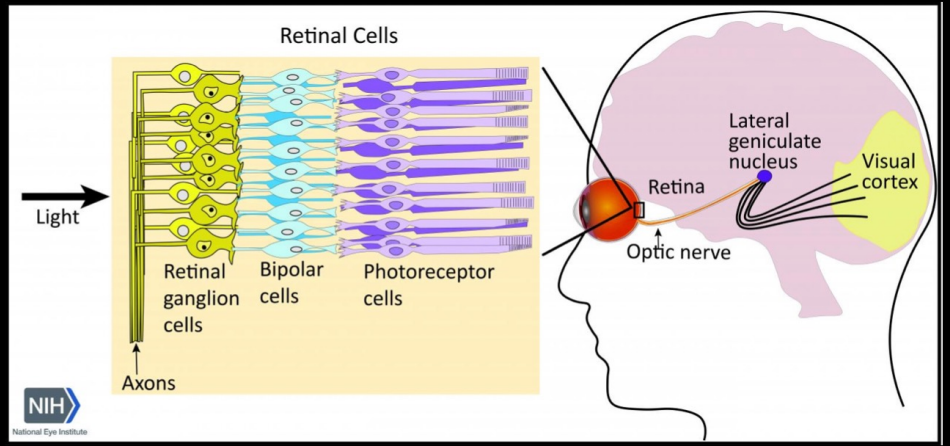
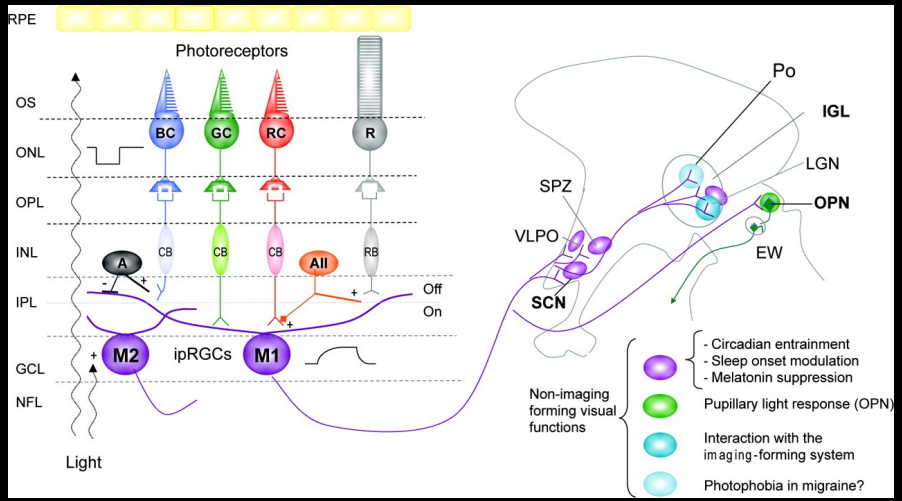
Background

Retina



Guo X, Zhou J, Starr C, Mohns EJ, Li Y, Chen E, Yoon Y, Kellner CP, Tanaka K, Wang H, Liu W, LR, Demb JB, Crair MC, and Chen B. "Preservation of vision after CaMKII-mediated protection of retinal ganglion cells." Published online July 22, 2021 in Cell. DOI: 10.1016/j.cell.2021.06.031

Intrinsically Photosensitive Retinal Ganglion Cells

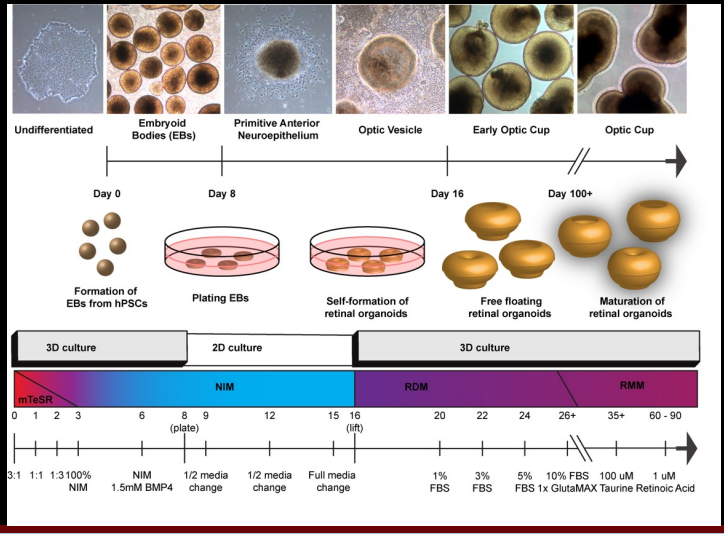


The Melanopsin System: Phototransduction, projections, functions, and clinical implications, Eduardo E. Benarroch Neurology Apr 2011, 76 (16) 1422-1427; DOI:10.1212/WNL.0b013e31821671a5

Background

Cell Line: WTC-11 Brn3b:Thy1 Opn4:tdTomato

- **WTC-11-** Human induced pluripotent stem cell line
- **Brn3b-** Mature RGC marker
- **Thy1-** Cell surface antigen
- **Opn4-** Melanopsin
- **tdTomato-** Red fluorescent protein



Undifferentiated Stem Cells

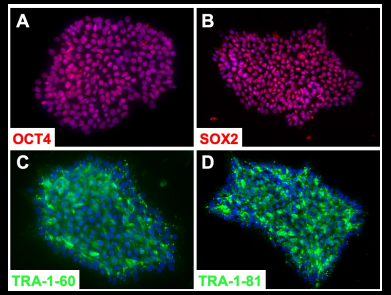


Figure 1.
(A-D) Immunostaining confirmed undifferentiated hiPSC identity through expression of specific markers such as (A) OCT4, (B) SOX2, (C) TRA-1-60, and (D) TRA-1-81.

OCT4 (Red): Pluripotency-associated transcription factor
SOX2 (Red): Neural progenitor and stem cell-associated transcription factor
TRA-1-60 (Green): Pluripotency-associated surface marker
TRA-1-81: (Green): Pluripotency-associated surface marker

*All cells stained with DAPI to visualize cell nuclei

Early Neural Induction

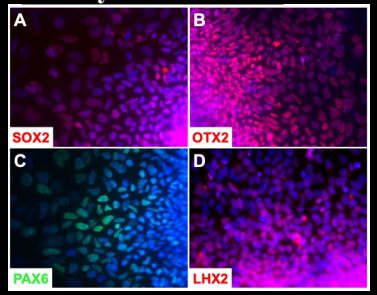


Figure 2.
(A-D) Immunostaining confirmed early neural progenitor cell identity after 10 days of differentiation through expression of specific markers such as (A) SOX2, (B) OTX2, (C) PAX6, and (D) LHX2.

SOX2 (Red): Neural progenitor and stem cell-associated transcription factor
OTX2 (Red): Rostral neural progenitor transcription factor
PAX6 (Green): Rostral neural progenitor transcription factor
LHX2: (Red): Rostral neural progenitor transcription factor

*All cells stained with DAPI to visualize cell nuclei

Retinal Organoids

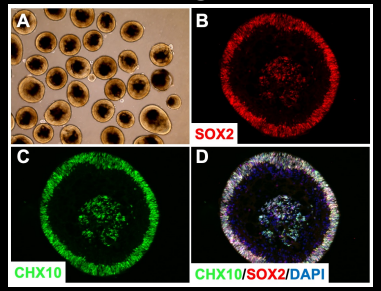


Figure 3.
(A) Brightfield imaging of retinal organoids demonstrated morphological characteristics at Day 30 of differentiation.
(B-C) Immunostaining confirmed early retinal progenitor cell identity of organoids through expression of (B) CHX10 and (C) SOX2.
(D) Immunostaining demonstrated co-expression of (B) CHX10, (C) SOX2, and DAPI on an isolated retinal organoid.

SOX2 (Red): Neural progenitor and stem cell-associated transcription factor
CHX10 (Green): Retinal progenitor transcription factor

Differentiation of RGC's

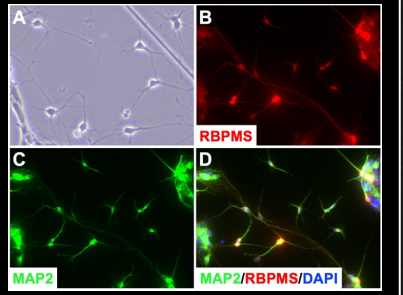


Figure 4.
(A) Brightfield imaging demonstrated morphological characteristics of hiPSC-derived RGCs with three-dimensional cell bodies, projecting lengthy neurites.
(B-C) Immunostaining confirmed RGC identity of cells through expression of cell-type specific markers, such as (B) RBPMS and (C) MAP2, as well as visualization of numerous neurites.
(D) Immunostaining demonstrated co-expression of (B) RBPMS, (C) MAP2, and DAPI in hiPSC-derived RGCs.

MAP2 (Green): Neuronal cytoskeleton marker
RBPMS (Red): RGC specific cell marker, typically expressed later than MAP2

RGC Subtypes

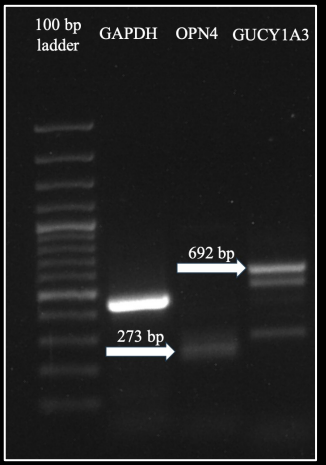


Figure 5.
RT-PCR analysis of differentiated RGCs demonstrated expression of markers associated with various RGC subtypes. Intrinsically-photosensitive RGCs are identified by the expression of *OPN4*, while midget RGCs are identified by the expression of *GUCY1A3*, highlighting RGC diversity in the population

Thank You Meyer Lab

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