Comprehensive map of melanopsin-expressing retinal ganglion cells in mouse

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Summary. Melanopsin is an opsin class of photopigment exclusively expressed in a small subset of retinal ganglion cells (mRGCs) that are intrinsically photosensitive. These mRGCs project their axons to the suprachiasmatic nucleus (SCN) and a few other brain regions that directly or indirectly regulate all non-image forming visual processes including circadian photoentrainment, pupil constriction, pineal melatonin regulation and light regulation of activity/rest. Identifying the full complement of mRGCs and their central projection is critical to understanding the cellular basis of melanopsin function. However, the existing methods to mark melanopsin cells and map their projections are insufficient. Therefore, we have generated transgenic reporter lines to specifically label the mRGCs and comprehensively study the projections of mRGCs in adult mice.

We generated a mouse with targeted insertion of *Cre*-recombinase into the native *melanopsin* locus and bred it with a *Z/EG* or *Z/AP* mice. This strategy allows *Cre*-dependent expression of green fluorescent protein (GFP) or human placental alkaline phosphatase (AP) from a strong *β-actin* promoter. In the resultant mice melanopsin cells are marked with a GFP or an AP marker which can be visualized by fluorescence or histochemical staining respectively.

In the retina of *Opn4$^{Cre/+}$;Z/EG* mice, GFP expressing cells were mostly found in the retinal ganglion cell (RGC) sub-layer, and these cells had extensive dendritic arborization characteristic of the mRGCs. In *Opn4$^{Cre/+}$;Z/AP* mice, the mRGCs strongly innervate the SCN. Additionally, mRGCs axon termini also sparsely innervate various other hypothalamic regions. Surprisingly, the AP staining also revealed extensive projection of the mRGCs in the lateral geniculate complex which is involved in image-forming vision. This implies mRGCs may play some role in patterned vision. In summary, we found that the projection patterns of mRGCs were much more extensive than previously reported.

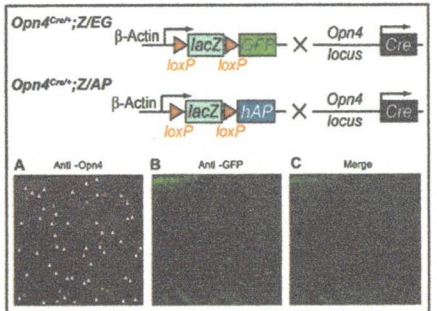


Figure 1. Strategy for Labeling Melanopsin Retinal Ganglion Cells (mRGC). Breeding scheme for the Cre dependent expression of EGFP or Alkaline Phosphatase (AP) in the mRGCs. Representative flat mount retinal sections from *Opn4$^{Cre/+}$;Z/EG* mice co-labelled with a purified rabbit polyclonal antibody raised against an N-terminus epitope of mouse melanopsin. (A) Anti-Opn4-immunofluoresence (red), (B) GFP expression (green), (C) merge. We found 110–130 GFP positive cells/mm², which amounts to ~1,500 cells in the adult mouse retina (based on an area of 14 mm²). Of these cells 86.4% were double labelled (white arrows in A) and 10.2% were GFP positive (green arrows) but lacked detectable melanopsin immunostaining, presumably due to a very low level of melanopsin expression undetectable by the antibody.

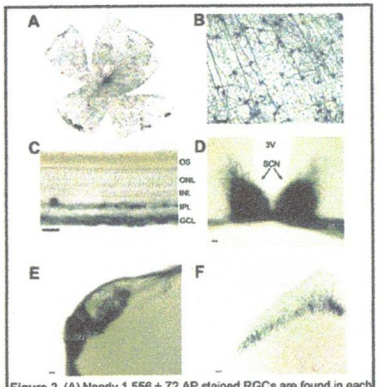


Figure 2. (A) Nearly 1,556 ± 72 AP stained RGCs are found in each retina. (B) AP labels the soma, dendrites, and axons of mRGCs. (C) Labeled cell bodies are restricted to the ganglion cell layer (GCL) and inner nuclear layer (INL), with dendrites in both sublaminae of the inner plexiform layer (IPL). (D–F) AP-stained coronal brain sections demonstrate dense mRGC innervation of the suprachiasmatic nuclei (SCN, D), intergeniculate leaflet (IGL, E), ventral and dorsal lateral geniculate nuclei (vldLGN, E), and sparse innervation of the superior colliculus (F). 3V, third ventricle; ot, optic tract. Scale bars represent 50 μm (B, D–F) and 25 μm (C).

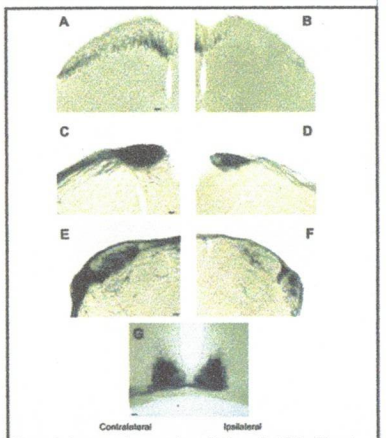
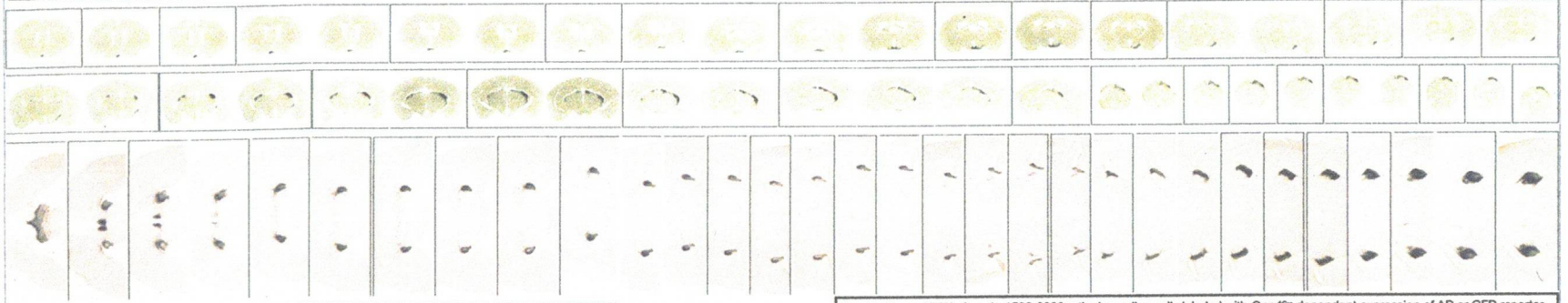


Figure 3. Anatomy of monocular projections of mRGCs. Comal sections from unilaterally enucleated mice stained for AP. mRGC innervations to the (A,B) superior colliculus (SC), (C,D) olivery pretectal nuclei (OPN), and (E,F) lateral geniculate nucleus (LGN) are predominantly contralateral. As shown previously the (G) SCN receives bilateral innervation of mRGC from each retina.

Figure 4. Serial coronal brain sections of *Opn4$^{Cre/+}$;Z/AP* mice showing extensive monocular central projections of the mRGCs. Average section thickness 150 μm.



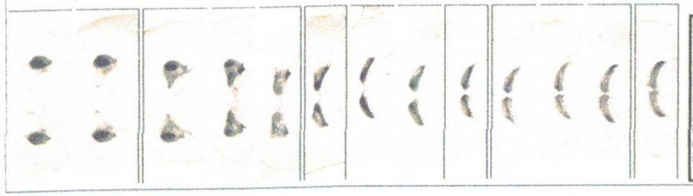


Figure 5. Serial horizontal brain sections of adult 5 months old *Opn4$^{Cre/+}$;Z/AP* mice stained with chromogenic AP substrate showing extensive projections of the mRGCs in the hypothalamus, thalamus and in the pretectal regions. Average section thickness 100 μm. Only the brain regions with detectable AP staining are shown.

Conclusions: **(1)** We found ~1500-2000 retinal ganglion cells labeled with *Opn4^{Cre}* dependent expression of AP or GFP reporter. However, significant number of cells also stained positive for GFP or melanopsin alone. Insufficient melanopsin expression might account for GFP-only cells. There are reports of silencing of *Z/AP* and *Z/EG* transgenes in adult neurons, which might account for GFP-only RGCs. Therefore, we suspect the actual number of RGCs with active melanopsin promoter might be slightly higher than detected here. **(2).** mRGCs from each retina project almost bilaterally to the SCN. Beyond the SCN the mRGC projections are largely contralateral. **(3)** mRGCs sparsely innervate large areas of the hypothalamus. In the thalamus extensive mRGCs projections are found in the ventral LGN, IGL and in the dorsomedial portion of the dLGN. **(4)** Beyond the thalamus the mRGCs *extensively innervate the OPN, habenula and the SC region. In summary,* we found that the projection patterns of mRGCs were much more extensive than previously reported.

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