Comprehensive Labelling of Melanopsin Expressing Retinal Ganglion Cells And Mapping their Central Projection in Mouse.

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Abstract

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.

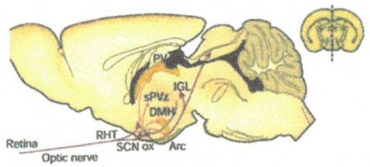
We generated a mouse with targeted insertion of Cre-recombinase *Opn4$^{Cre/+}$* into the native *melanopsin* locus and bred it with a *Z/EG (lacZ EGFP)* or *Z/AP (lacZ human alkaline phosphatase)* mouse. This strategy allows Cre-dependent expression of green fluorescent protein (GFP) or human placental alkaline phosphatase (AP) from a strong *β-actin* promoter. 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. An average of 131 GFP expressing cells/mm2 (±25.4, SD, n = 3) were found in these retina, 42.6% of which also expressed immunologically detectable levels of melanopsin. Within *Opn4$^{Cre/+}$;Z/AP* mice, the strong innervation of mRGCs in the SCN is much more apparent. Additionally, mRGCs axon termini also sparsely innervate various other hypothalamic regions. Surprisingly, the AP staining also revealed extensive projections 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.

Introduction

The mammalian circadian clock is an endogenous oscillator that shows approximately 24 hour rhythms. Its phase can be affected by external cues, most strongly by light. The central circadian clock is located in the Suprachiasmatic Nucleus (SCN) within the hypothalamus.

The SCN clock receives external light cues that trigger entrainment to photic cycles through a set of photoreceptors in the retina referred to as melanopsin expressing Retinal Ganglion Cells (mRGCs). It was previously known that these cells project through the retinohypothalamic tract (RHT) leading to the SCN and to the Olivary Pretectal Nucleus (OPN) which regulates pupil constriction. However, complete mRGC projection to the brain was unknown. The diagram below is a sagital section that illustrates what was previously assumed to be the targets of the mRGCs.



Adapted from Panda et al. Nature 2002

Opn4$^{Cre/+}$;Z/EG and *Opn4$^{Cre/+}$;Z/AP* Breeding Strategy

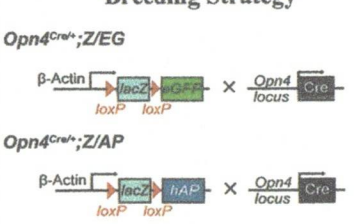


Figure 1. Strategy for Labelling Melanopsin Retinal Ganglion Cells (mRGCs).
A mouse with a Cre recombinase "knocked in" the *melanopsin* locus was bred with a *Z/EG* or *Z/AP* mouse allowing for the Cre dependent expression of EGFP or Alkaline Phosphatase (AP) respectively.

Genetic and Immunohistochemical Co-labelling of mRGCs

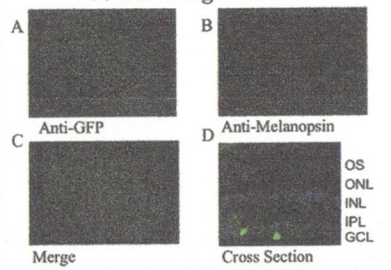


Figure 2. Immunohistochemical co-labelling of Melanopsin Retinal Ganglion Cells. A-C. Retina Flatmount A. Anti-GFP expression (green) B. Anti-melanopsin expression (red) C. Merged D. Cross section of the retina. Anti-GFP (green), DAPI (blue). Scale bar = 50μm.

Genetic Labelling of mRGCs

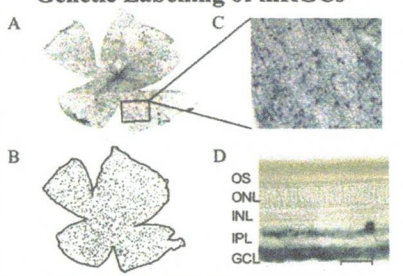


Figure 3. *Opn4$^{Cre/+}$;Z/AP* retina stained with AP substrate. A and B. Uniform distribution of melanopsin expressing retinal ganglion cell somas throughout the mouse retina. Each retina contained 1556 (+72) (average + S.D., n = 4) AP stained cells. C. Magnified view of the AP stained cells show labelling of the soma, dendrites and axons. D. AP stained cells are restricted to the ganglion cell layer (GCL) and the innermost region of the inner nuclear layer (INL). Scale bar = 50μm.

Hypothalamic Targets

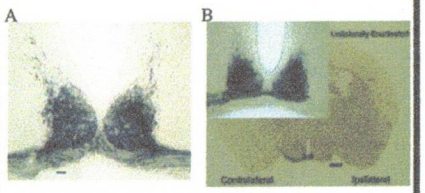


Figure 4. Genetic Labelling of mRGCs in the Hypothalamus.
Opn4$^{Cre/+}$;Z/AP mice stained with AP substrate. A. Coronal brain section showing Suprachiasmatic Nucleus (SCN) staining. Scale bar 5μm. B. Coronal brain section of a unilaterally enucleated mouse showing equivalent AP staining of both sides of the SCN with contralateral staining only along the optic track. Scale bar 50μm.

Lateral Geniculate Nucleus (LGN) and Pretectal Targets

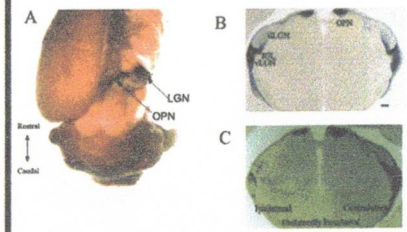


Figure 5. Genetic Labelling of mRGCs in the LGN and Pretectum. *Opn4$^{Cre/+}$;Z/AP* brain is stained with AP substrate. A. Wholemount B. Coronal brain section C. Coronal brain section of a unilaterally enucleated mouse heavily stained along the contralateral side and minimal staining along the ipsilateral side. Scale bar = 20μm.

Superior Colliculus (SC) Targets

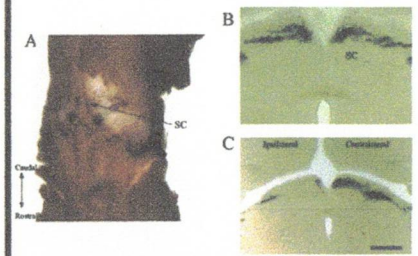


Figure 6. Genetic Labelling of mRGCs in the SC. *Opn4$^{Cre/+}$;Z/AP* mice stained with AP substrate. A. Wholemount dorsal side up with several tracks of mRGC projections leading into the Superior Colliculus (SC). B. Coronal brain section showing the SC area. C. Coronal brain section through a unilaterally enucleated mouse showing strong contralateral staining and minimal ipsilateral staining. Scale bar = 50μm.

Conclusion

We created transgenic reporter mouse lines that allowed us to specifically label mRGCs and therefore comprehensively study their projections. In our *Opn4$^{Cre/+}$;Z/AP* mouse, mRGCs were evenly distributed across the retina with approximately 1500 cells per retina which is about two times more than were previously labeled in *Opn4^{tmLacZ}* mouse (Hattar *et al.* 2006). In our mouse line, we found mRGC projections in the SCN (Figure 4A) and OPN (Figure 5 A and B) as reported before, and some regions that were not previously reported as well. These regions located in the hypothalamus and thalamus consisted of the Lateral Geniculate Nucleus (LGN; Figure 5 A and B) which receives and processes visual information for image formation, the Intergeniculate Leaflet (IGL; Figure 5 A and B) that contributes to photoentrainment, and the Superior Colliculus (SC; Figure 6 A and B) in the Tectum which contributes to rapid eye movements.

Through unilateral enucleation it is apparent that the SCN is innervated equally by both contralaterally and ipsilaterally projected mRGCs (Figure 4B). Unilateral staining also reveals the *LGN to be contralaterally innervated* (Figure 5C). The IGL and OPN receive both contralaterally and ipsilaterally mRGC projections (Figure 5C). Further down the path the SC is also receiving projections from mRGCs contralaterally as shown through unilateral enucleation (Figure 6C).