Melanopsin in cells of origin of the retinohypothalamic tract

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All known eukaryotic organisms exhibit physiological and behavioral rhythms termed circadian rhythms that cycle with a near-24-hour period; in mammals, light is the most potent stimulus for entraining endogenous rhythms to the daily light cycle. Photic information is transmitted via the retinohypothalamic tract (RHT) to the suprachiasmatic nucleus (SCN) in the hypothalamus, where circadian rhythms are generated, but the retinal photopigment that mediates circadian entrainment has remained elusive. Here we show that most retinal ganglion cells (RGCs) that project to the SCN express the photopigment melanopsin.

The phase of circadian rhythms in rodents is shifted most effectively by light ranging from 480–511 nm, consistent with an opsin-based photopigment[1–3]. However, mice lacking rods and cones have normal circadian entrainment, suggesting that a novel photopigment mediates phase-shifting in response to light[4]. Recently, melanopsin, an opsin-based photopigment, was localized to the RGC layer of rodents and primates[5]. We therefore tested whether RGCs that express melanopsin project to the SCN.

We injected the right SCN of 10 rats with FluoroGold (FG) to retrogradely label the retinohypothalamic RGCs. Four of the injections were confined to the SCN and did not include the optic chiasm or optic tract (**Fig. 1a**). In these animals, FG labeled a distinct subset of widely distributed RGCs, corresponding to type III or W cells, as previously reported[6].

For *in situ* hybridization, we used a 957-base-pair mouse melanopsin riboprobe[5]. Melanopsin transcript occurred in a pattern similar to that previously described[5], with a scattered population of cells showing intense hybridization, predominantly in the RGC layer (**Fig. 1b**).

In doubly labeled sections, 74.2 ± 0.3% (mean ± s.e.m.) of retrogradely labeled RGCs also expressed melanopsin mRNA (**Fig. 1c**), with a similar percentage of double labeling in eyes ipsilateral and contralateral to the FG injection. Although the extent of retrograde labeling differed between cases, approximately 70% of RGCs that were intensely labeled for melanopsin mRNA were also retrogradely labeled. Both calculations are likely to underestimate the actual percentage of colocalization, because technical factors limit the efficiency of the combined labels. Therefore, most RGCs that project to the SCN express melanopsin, and a majority of melanopsin-containing RGCs project to the SCN.

These observations suggest that RGCs that contain melanopsin are particularly well poised to provide photic information to the SCN. Melanopsin in these retinohypothalamic RGCs may therefore mediate the photic entrainment of circadian rhythms in mice lacking rods and cones. Although a high percentage of RHT RGCs express melanopsin, RHT cells may also receive other photic signals through rods and cones in intact animals. In addition, the photopigments cryptochrome 1 and 2 have been localized to RGCs of the mouse retina[7]. Further experiments will be necessary to determine whether cryptochromes are involved in circadian photic entrainment. However, melanopsin may now be considered a primary candidate photopigment for mediating circadian entrainment.

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Competing interests statement

The authors declare that they have no competing financial interests.

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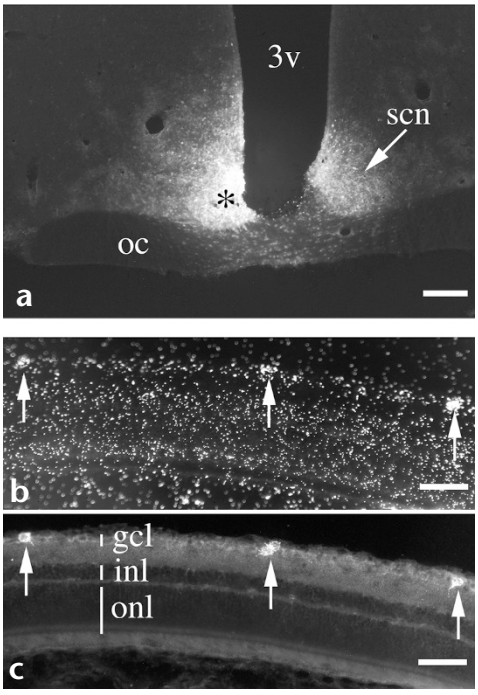

Fig. 1. Colocalization of retrogradely labeled FluoroGold (FG) and melanopsin transcript in retinal ganglion cells of rat. (**a**) The suprachiasmatic nucleus (asterisk) was injected by glass micropipette with 3 nl of 5% FG, resulting in retrograde labeling of the contralateral suprachiasmatic nucleus (arrow) due to reciprocal innervation. The injection avoided the optic chiasm. (**b**) *In situ* hybridization for melanopsin localized with NTB-2 emulsion autoradiography, demonstrating a group of three intensely labeled cells (arrows) in the ganglion cell layer. Light diffuse labeling over all three cellular layers[5] was similar to labeling seen with sense probe. (**c**) All three intensely labeled RGCs were retrogradely labeled with FG (arrows). 3v, third ventricle; oc, optic chiasm; scn, suprachiasmatic nucleus; gcl, ganglion cell layer; inl, inner nuclear layer; onl, outer nuclear layer. Scale bar, 200 μm (**a**), 50 μm (**b**, **c**).