Diminished Pupillary Light Reflex at High Irradiances in Melanopsin-Knockout Mice

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In the mammalian retina, a small subset of retinal ganglion cells (RGCs) are intrinsically photosensitive, express the opsin-like protein melanopsin, and project to brain nuclei involved in non–image-forming visual functions such as pupillary light reflex and circadian photoentrainment. We report that in mice with the melanopsin gene ablated, RGCs retrograde-labeled from the suprachiasmatic nuclei were no longer intrinsically photosensitive, although their number, morphology, and projections were unchanged. These animals showed a pupillary light reflex indistinguishable from that of the wild type at low irradiances, but at high irradiances the reflex was incomplete, a pattern that suggests that the melanopsin-associated system and the classical rod/cone system are complementary in function.

It had long been assumed that rods and cones were the only intrinsically photosensitive cells in the mammalian retina. However, this assumption was questioned when mice lacking functional rods and cones were shown to retain circadian photoentrainment and the pupillary light reflex (1, 2). Most recently, a small subset of rat RGCs have been shown to be directly photosensitive (3, 4). These RGCs express melanopsin (4–7), an opsin-like protein (8), and project to retino-recipient areas of the brain responsible for non–image-forming visual functions (4–6), raising the possibility that these RGCs serve as photoreceptors for these functions. The action spectra of these RGCs are consistent with an opsin: vitamin A–based photopigment underlying the process (2, 3). To determine whether melanopsin and the RGCs expressing it indeed have physiological roles, we have examined the pupillary reflex of mice lacking this gene.

Previously, we have generated mice in which the melanopsin gene (mop) is replaced by a tau-LacZ coding sequence (4). We bred these animals to homozygosity. We found that the RGCs that would normally express melanopsin are still present in mop+/− (tau-LacZ+/−) mice, as revealed by blue X-Gal labeling (9) (Fig. 1A). Their morphology and number (~600 per mouse retina) are similar to those in mop+− (tau-LacZ+−) mice and, by implication (4), wild-type animals. Their axons converge on the optic disk and still innervate the olivary pretectal nuclei (OPN) (Fig. 1B), the retino-recipient area responsible for the pupillary light reflex (10, 11), and the suprachiasmatic nuclei (SCN) (Fig. 1C), the circadian pacemaker in the brain. In both structures, the spatial pattern of the innervation is similar to that previously observed in mop+− mice (4). Thus, the absence of melanopsin does not affect the genesis, survival, or connectivity of the melanopsin-associated RGCs.

The genetic deletion does, however, eliminate the intrinsic photosensitivity of these RGCs. For wild-type and mop+− mice, RGCs retrograde-labeled from the SCN and tested under synaptic blockade (9) were tonically depolarized by light (13 of 13 cells tested for wild type, and 3 of 3 cells tested for mop+−) (Fig. 1D), just as in rat (3, 4). In contrast, none of 10 cells tested for mop+− mice were photosensitive under the same conditions (Fig. 1E). The morphologies of recorded cells were indistinguishable across genotypes and closely resembled those of the photosensitive RGCs innervating the SCN in rat (3, 4).

We compared the consensual pupillary light reflex of mop+−, mop−−, and mop+− RGCs...
animals (9). All three genotypes showed a light-dependent pupillary constriction, but the minimal pupil area attained by dark-adapted mop/−/− animals in 1 min of steady bright light was three times that of mop+/− and wild-type animals (Fig. 2, A and B). This difference was not due to an intrinsic defect in the iris sphincter of mop/−/− animals, because parasympathetic activation by topical application of carbachol (9) elicited equally strong constrictions in mop/−/− and wild-type animals (Fig. 2A). Nor was it due to an alteration in circadian entrainment (pupillary recordings in the day and night revealed a similar phenotype) or to the mixed C57Bl6/129 genetic background of the three genotypes (littermates were used and both parental strains assessed) (9). Thus, the impaired response of mop/−/− mice appeared to have resulted directly from a loss of intrinsic photosensitivity of the melanopsin-expressing RGCs.

With a stimulus of bright, steady monochromatic light, the pupil constriction in wild-type mice was greater than that in mop/−/− animals during both the transient phase and steady state of the response (Fig. 2C, compare open and filled circles). With dimmer light (green squares), the reflex was slower, but also became indistinguishable in speed and amplitude between the two genotypes, indicating that melanopsin exerted an influence only at high irradiances. With an intense, 100-mW white flash (Fig. 2D, circles), which should give little opportunity for the rods and cones to light-adapt (12, 13), the maximal constriction of the mop/−/− pupil remained weaker than that of the wild type. This result suggests that there is probably a genuine limit to how far rods and cones can drive the mouse pupil reflex—a ceiling independent of adaptation.

The complete step irradiance–response relations (9, 14) for mop+/+ and mop/−/− animals are similar (Fig. 3A and supporting online text). The relation for mop/−/− mice is also similar to that of the wild type at irradiances less than 10^{11.5} photons cm^{-2} s^{-1}, but diverges at higher irradiances (supporting online text), approaching a minimum attainable pupil size that is larger than that of wild-type mice. Mice lacking detectable rods and cones (rd/rd cl) retain a pupillary light reflex, with an action spectrum (2) resembling that for the intrinsic photosensitivity of melanopsin-expressing RGCs in rat (3). The rd/rd cl pupil reflex
shows greatly reduced sensitivity, but nonetheless reaches the same maximum response as that of the wild type at high irradiances (2). Thus, the rd/rd cl phenotype is the complement of the mop−/− phenotype. To test this apparent complementarity quantitatively, we measured the irradiance–response relation for the pupillary reflex of rd/rd cl mice (9) under conditions identical to those for mop−/− and wild-type animals (Fig. 3B) and then summed it with the relation for mop−/− mice (15). The resulting irradiance–response relation provides a good prediction of that measured from wild-type mice (Fig. 3C). This agreement suggests that the rod/cone and melanopsin systems together provide the full dynamic range of the normal pupillary reflex. We cannot rule out the existence of a flex. We cannot rule out the existence of a third photodetection pathway, but if the melanopsin system. Data were interpolated where the two mouse lines did not receive identical irradiances.

16. With the \( \lambda_{\text{max}} \) for the action spectrum of the photosensitive RGCs at 484 nm (3) and that for the pupil response of rd/rd cl mice at 479 nm (2), the sensitivities of the two systems in the wavelength range of 480 to 500 nm can be compared directly without corrections because sensitivity changes little around \( \lambda_{\text{max}} \).

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Materials and Methods
Supplementary Text
Fig. S1
References
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