Non-Image-Forming Light Driven Functions Are Preserved in a Mouse Model of Autosomal Dominant Optic Atrophy

Georgia Perganta, Alun R. Barnard, Christiana Katti, Athanasios Vachtsevanos, Ron H. Douglas, Robert E. MacLaren, Marcela Votruba, Sumathi Sekaran

Abstract

Autosomal dominant optic atrophy (ADOA) is a slowly progressive optic neuropathy that has been associated with mutations of the OPA1 gene. In patients, the disease primarily affects the retinal ganglion cells (RGCs) and causes optic nerve atrophy and visual loss. A subset of RGCs are intrinsically photosensitive, express the photopigment melanopsin and drive non-image-forming (NIF) visual functions including light driven circadian and sleep behaviours and the pupil light reflex. Given the RGC pathology in ADOA, disruption of NIF functions might be predicted. Interestingly in ADOA patients the pupil light reflex was preserved, although NIF behavioural outputs were not examined. The B6; C3-OPA1Q285STOP mouse model of ADOA displays optic nerve abnormalities, RGC dendropathy and functional visual disruption. We performed a comprehensive assessment of light driven NIF functions in this mouse model using wheel running activity monitoring, videotracking and pupillometry. Opa1 mutant mice entrained their activity rhythm to the external light/dark cycle, suppressed their activity in response to acute light exposure at night, generated circadian phase shift responses to 480 nm and 525 nm pulses, demonstrated immobility-defined sleep induction following exposure to a brief light pulse at night and exhibited an intensity dependent pupil light reflex. There were no significant differences in any parameter tested relative to wildtype littermate controls. Furthermore, there was no significant difference in the number of melanopsin-expressing RGCs, cell morphology or melanopsin transcript levels between genotypes. Taken together, these findings suggest the preservation of NIF functions in Opa1 mutants. The results provide support to growing evidence that the melanopsin-expressing RGCs are protected in mitochondrial optic neuropathies.

Introduction

Autosomal dominant optic atrophy (ADOA) is the most common form of inherited optic neuropathy[1,2,3]. It is clinically characterized by a moderate to severe decrease in visual acuity, central visual field deficits, colour vision defects and temporal or diffuse optic nerve pallor[1,3,4]. ADOA is associated with a specific degeneration of retinal ganglion cells (RGCs)[4,5]. Mutations in the optic atrophy 1 (OPA1) gene have been identified in most of patients with ADOA[4-7]. The OPA1 protein is a dynamin-related guanosine triphosphatase (GTPase) anchored to the inner mitochondrial membrane.

Mouse models of OPA1 ADOA have been generated to characterise further the pathophysiology of the disease[8,9]. In the B6; C3-OPA1Q285STOP model, abnormalities in the optic nerve were detected by 9 months[8] without significant RGC loss[10]. Evidence of dendritic pruning localized to sublamina b of the retinal inner plexiform layer was observed from 10 months[10]. By 24 months, severe optic nerve degeneration was found[11]. Visual dysfunction has been described in this mouse model by both electrophysiological and behavioural testing. A specific deficit in the photopic negative response of the electroretinogram which reflects ganglion cell function, was detected in Opa1 mutants from 12 months[12]. Importantly, deficits in the visual evoked potential (VEP) suggested light information was not being correctly relayed to the brain[12]. Concordantly, a reduction in visual acuity detectable by optokinetic drum screening was described[8].

A subset of RGCs is intrinsically photosensitive and expresses the photopigment melanopsin[13,14,15]. These cells are primarily responsible for providing the photic input to non-image-forming (NIF) behaviours. Intrinsically photosensitive RGCs are directly light sensitive but also act as conduits for rod and cone derived responses via dendritic inputs[16,17,18]. Ablation of the melanopsin expressing RGCs resulted in: (i) reduced entrainment to normal light/dark (LD) cycles[16,17,18], (ii) complete loss of circadian phase shift responses[17], (iii) absence of the suppression of locomotor activity to acute light exposure at night (negative
RGCs and NIF light-driven functions in ADOA. The results support a preservation of the melanopsin equivalent to intensity used throughout (unless otherwise stated) was 200 lux and exposed to a 12:12 light/dark (LD) cycle for 10 days. The light individually placed in cages equipped with a steel running wheel suffering.

Process (PPL 30/2812). All efforts were made to minimise efforts were made to minimise

Animals (Scientific Procedures) Act 1986, UK. The protocol was approved by the University of Oxford Local Ethical Review Animals (Scientific Procedures) Act 1986, UK. The protocol was approved by the University of Oxford Local Ethical Review

Embryonic lethal in homozygous animals. Food and water were available ad libitum.

Circadian behaviour

Mice (Opa1+/+: n = 6; Opa1+/−: n = 7; 11–13 months old) were individually placed in cages equipped with a steel running wheel and exposed to a 12:12 light/dark (LD) cycle for 10 days. The light intensity used throughout (unless otherwise stated) was 200 lux (equivalent to ~60 µW/cm²/s or 14.2 log quanta/cm²/s). Activity levels, the length of the active phase and period were calculated using ClockLab software (Actimetrics). A 3-h pulse of white fluorescent light (200 lux) was given at zeitgeber time (ZT) 14 (2 h after the lights were switched off on the night of day 10). The masking response was scored as the number of wheel revolutions during the light pulse expressed as a percentage of the number of revolutions made by the same animal during the same period on the previous night when there was no light pulse[22]. Total activity levels and activity levels in 1 h bins during the light pulse were also calculated. Animals were re-entrained to the normal LD cycle for 10 days.

Mice were subsequently released into constant darkness (DD) for 10 days. To induce phase shift responses animals were exposed to a 15-min monochromatic 480 nm or 525 nm (half peak bandwidth 10 nm) pulse of 1x10^14 photons/s/cm² irradiance at circadian time (CT) 16 (4 h after the onset of daily activity). Monochromatic light was applied in a specialised chamber with full internal reflectance using an LED light source (Honig Lichttechnik). In the control sham condition animals were handled in a similar way to the light pulse condition but maintained in darkness. There were at least 10 days between pulses. The magnitudes of the phase shifts were calculated using the difference between a regression line through steady state activity onsets prior to and after the pulse (ClockLab, Actimetrics). Transient responses in the first 2–3 days after the pulse were excluded.

Sleep screening

Mice (Opa1+/+: n = 5; Opa1+/−: n = 6; 11–13 months old) were placed in cages equipped with infrared cameras for monitoring activity (Senient Mini-night vision CCTV camera, Maplin, UK) and infrared LEDs. The output was recorded on a 16-channel digital hard-drive recorder (VXM4B-16, Videcon. PLC) at 25 frames per s with a resolution of 704x576 pixels. Animals were exposed to a 12:12 LD cycle for 15 days. To determine the modulation of sleep behaviour by light, animals were exposed to a 1-h white light pulse (200 lux) at ZT14[23]. The immobility-
defined sleep period for each mouse was assessed using video tracking software (ANY-maze, Stoeling). Sleep was defined as a period of immobility greater than 40 s. A previous study has confirmed that monitoring of immobility with videotracking has extremely high concordance (>95%) with EEG recordings for sleep determination[23]. Sleep latency (defined as the time between the onset of the light exposure and the first 2 min of continuous immobility) and total sleep, were also determined.

Pupillometry
The right eyes of unanaesthetised mice (Opal+/+; n = 5; Opal−/−: n = 5; 24 months) were filmed under infrared illumination by a camera (Cohu, San Diego, USA) fitted with a zoom lens (giving a field of view of 8.7 x 6.9 mm), positioned in a plane parallel to the pupil. White light was delivered to the eye being filmed through a fibre optic (Leica CLS 100x). Since the action spectrum of wildtype mouse pupil constriction can be largely described by the mouse’s scotopic sensitivity[24], measured spectral irradiances were weighted by the spectral absorbance of the murine rod visual pigment[23], as has been previously used[26]. Experiments were conducted during the light phase of the normal LD cycle to avoid any effects of circadian variation in the PLR[27]. After at least 1 h of dark adaptation, a light stimulus was delivered for 20 s. Animals were subjected to 14 different light intensities presented in ascending order of brightness with at least 2 min between stimulus presentations. The area of the pupil immediately before and 5 s after illumination was determined using Image J software. Pupil areas after illumination were expressed relative to the area of the pupil in darkness. Average irradiance/response curves were constructed for each genotype and fitted by a four term sigmoid relationship (SigmaPlot, Systat Software Inc., San Jose, USA).

Immunohistochemistry
Animals were sacrificed at ZT8–ZT10 (Opal+/+: n = 5; Opal−/−: n = 3; 11–13 months old). Retinae were dissected and fixed in 4% paraformaldehyde (PFA) overnight. Retinae were incubated with an N-terminal molecular melanopsin antibody (PAS8331; rabbit anti-mouse (1:500) in 1% Donkey serum, 0.1% Triton X-100 in PBS)[28]. Retinae were subsequently incubated with an Alexa Fluor 488 secondary antibody (donkey anti-rabbit IgG (1:400) in 0.1% Triton X-100 in PBS; Invitrogen). A fluorescent microscope (Zeiss Axioskop, Germany) was used to view the retinal flatmounts and images were captured with a Hamamatsu Orca camera. Cell counts were performed using Adobe Photoshop software. Confocal images were captured using a laser scanning confocal microscope (Zeiss LSM510, Germany). Soma diameter was measured using Image J software (NIH, USA).

Results
Opal mutation has no effect on entrainment to a normal LD cycle
Circadian phototainment is driven by both melanopsin and conventional RGC pathways [16,17,18]. The ability of Opal+/+ and Opal+/− mice to entrain to a 12:12 LD cycle was assessed (Figure 1A,B). Both genotypes largely confined their activity to the dark phase suggesting the animals were entrained to the normal LD cycle. The period length (τ) and length of the active phase...
were equivalent for wildtype and heterozygous mice (Figure 1C). Total activity (average number of wheel revolutions per day) was slightly higher in Opa1<sup>+/2</sup> animals relative to the wildtype controls but this difference was not significant (Figure 1C). When released into constant darkness (DD), the phase of activity onset for the first day in DD was coincident with lights OFF of the previous LD cycle for both genotypes (Figure 1A,B) again suggesting that the mice were entrained to the light signal.

Negative masking is preserved in Opa1<sup>+/2</sup> mice

Previous studies have shown that light of ~200 lux presented at night induced a complete negative masking response in wildtype mice[29] and in rodless/coneless mice[30] whereas negative masking was significantly impaired in melanopsin knockout mice[22]. We examined masking responses to 200 lux stimuli at ZT14 in Opa1<sup>+/+</sup> and Opa1<sup>+/2</sup> mice. Both genotypes significantly decreased their activity during the 3 h light exposure (data normalised to baseline from previous night; Figure 2A). Opa1<sup>+/+</sup> and Opa1<sup>+/2</sup> mice acutely stopped running during the first hour of the light pulse and activity levels subsequently increased, as has been previously shown in wildtype strains[22]. The baseline corrected activity levels during the light pulse was not significantly different between genotypes. An hourly breakdown of the number of wheel revolutions during the light pulse also showed no significant difference between genotypes (Figure 2B).

Figure 3. Phase shift behaviour in Opa1<sup>+/+</sup> and Opa1<sup>+/2</sup> mice.

Representative actograms from (A) Opa1<sup>+/+</sup> and (B) Opa1<sup>+/+</sup> mice in constant dark (DD) conditions. Animals were exposed to 15 min light pulses every ~15 days. Photon matched pulses at 480 nm (black arrow) or 525 nm (white arrow; 1 x 10<sup>11</sup> photons/s/cm<sup>2</sup>) were applied at CT16. Animals were also exposed to a dark sham pulse condition (grey arrow). (C) The size of the phase shift response are plotted for the 525 nm, 480 nm and sham conditions for Opa1<sup>+/+</sup> (n = 6) and Opa1<sup>+/−</sup> (n = 7) mice. A two-way ANOVA with genotype and wavelength as factors was performed. There was no significant effect of wavelength ($p=0.66$) or genotype ($p=0.17$) and the interaction of genotype and wavelength was not significant ($p=0.91$).

doi:10.1371/journal.pone.0056350.g003

Figure 4. Induction of sleep by acute light in Opa1<sup>+/+</sup> and Opa1<sup>+/2</sup> mice.

(A) The average immobility-defined sleep is plotted against zeitgeber time in a normal 12:12 h LD cycle (1 h resolution) for Opa1<sup>+/+</sup> (n = 5) and Opa1<sup>+/2</sup> (n = 6) mice. Animals were largely immobile in the day phase of the LD cycle. White background indicates the day portion and grey backround the night portion of the 24 h LD cycle. (B) The effect of the administration of the 1-h light pulse (white background) at ZT 14 during the night phase is shown. Both genotypes demonstrated an increase in immobility during the light pulse (10 min resolution). Quantification of (C) sleep latency and (D) total sleep during light exposure found no significant differences between genotypes (unpaired student’s t-test). All data are presented as mean ± SEM.

doi:10.1371/journal.pone.0056350.g004
Phase shift responses are maintained in Opa1+/− mice

Mice were released into constant darkness (DD) for a period of 10 days before application of phase shift light pulses. In DD, Opa1+/− and Opa1−/− mice displayed a wheel running activity rhythm with a period (τ) of ~23.7 h. The average τ was almost identical between wildtype and heterozygous mice in constant darkness (Figure 1C). The total activity and length of the active phase were also not significantly different between genotypes in DD (Figure 1C).

Light pulses of two different wavelengths were applied at CT16 to elicit phase shift behaviour. 480 nm light was applied to maximally stimulate the melanopsin-RGCs (I(max)~480 nm) and a 525 nm pulse was used to maximally stimulate the MW-sensitive cone (I(max)~508 nm) input to the melanopsin RGCs. The 480 nm pulse induced a delay in the phase of activity onset in both Opa1+/+ and Opa1−/− mice (Figure 3A,B). The average magnitude of the phase shifts was not significantly different between genotypes (Figure 3C). In both genotypes, the period of the activity rhythm was not altered following the light pulse (τ after 480 nm pulse: Opa1+/+: 23.6±0.09 h; Opa1−/−: 23.6±0.07 h). The pulse of 525 nm also successfully induced a phase delay in activity onset in Opa1+/+ and Opa1−/− mice (Figure 3A,B). There was no significant difference in the size of the phase shifts between genotypes (Figure 3C). Again the period length was not altered after the light pulse for each genotype (τ after 525 nm pulse: Opa1+/+: 23.6±0.09 h; Opa1−/−: 23.6±0.06 h). Importantly in a control sham pulse exposure, no obvious phase shift was observed in either wildtype or heterozygous animals (Figure 3C).

Acute light induction of immobility-defined sleep is present in Opa1+/− mice

Previous studies have shown that acute light exposure at night induces sleep in nocturnal species[19,31,32,33] and that this process is distinct from the process of negative masking[19,31] At 200 lux, light induces sleep in wildtype mice and in rodless/coneless mice but sleep induction is impaired in melanopsin knockout mice[31]. We examined the acute effects of 200 lux light on sleep in Opa1 mutant mice and wild-type controls by administering a 1 h light pulse 2 h after the onset of dark (ZT 14) when the homeostatic and circadian drives for wakefulness are high. We used video-tracking assessment of immobility as a measure of sleep which has been found to highly correlate with EEG/EMG recordings of sleep[23]. Opa1+/+ and Opa1−/− mice exhibited low immobility-defined sleep (i.e. high activity) during the dark period of a normal 12:12 LD cycle as would be expected for a nocturnal species (Figure 4A). In response to the light pulse, a sharp increase in immobility-defined sleep was observed in both genotypes within 30 min of light (Figure 4B). Immobility levels subsequently declined to ~50% of baseline by the end of the light pulse. The sleep induction response differed from negative masking where the suppression in activity levels was maintained during the entire first hour of the light pulse. This adds further support to acute light induced sleep and negative masking being distinct processes. The latency to the first 2 min of continuous immobility (sleep latency) was not significantly different between genotypes (Figure 4C). During acute light exposure, the total time spent asleep was also not significantly different between mice and mutant mice (Figure 4D) suggesting that both genotypes sustained the inhibitory response to light.

Pupil light reflex is preserved in Opa1+/− mice

Figure 5. Pupil light reflex in Opa1+/− and Opa1−/− mice. The average minimum pupil area expressed as a percentage of maximum dilation following illumination with various intensities of white light for Opa1+/− (n = 5) and Opa1−/− (n = 5) mice. All data are fitted with four term sigmoidal functions (solid lines) of the form y = y0+a/(1+exp−(x−x0)/b)) (goodness of fit of fitted curve to actual data (R²): Opa1+/+, 0.993 and Opa1−/− = 0.995). A 2-way ANOVA using intensity and genotype as factors showed a significant effect of light intensity (p < 0.0001) but no significant effect of genotype (p = 0.51) and no significant interaction between genotype and intensity (p = 0.99).

doi:10.1371/journal.pone.0056350.g005

Opa1 defect has no effect on melanopsin expression

Melanopsin cells were assessed immunohistologically in retinal wholemounts (Figure 6A,B). There was no significant difference in the total number of melanopsin cells between genotypes (average cell count per retina: Opa1+/−: 900±28.9 cells; Opa1−/−: 810±37.9 cells). Morphological characterisation of the melanopsin cells also revealed no significant difference in the mean soma diameter (Opa1+/+: 13.9±0.5 μm, n = 30; Opa1−/−: 14.0±0.5 μm, n = 30; data not shown). Furthermore, we observed no obvious differences in the stratification patterns of the melanopsin cells between genotypes (Figure 6C). Melanopsin positive dendrites were observed in both sublamba a (OFF) and sublamba b (ON) of the innerplexiform layer. Finally, there was no significant difference in melanopsin transcript levels between Opa1+/+ and Opa1−/− retinae (Figure 6D). As a control, we assessed Opa1 transcript levels and found an ~50% reduction in Opa1+/− retinae relative to wildtype controls (p < 0.005) as expected[8,9].
behaviours including circadian and sleep behaviours, have not been examined in patients. We performed a comprehensive assessment of NIF functions in a mouse model of ADOA. There was no significant difference in circadian photoentrainment, driven by melanopsin-expressing and conventional RGCs\cite{16,17,18}, between \textit{Opa1}\textsuperscript{+/+} and \textit{Opa1}\textsuperscript{+/-} mice. Negative masking, phase shift behaviour to 480 nm and 525 nm stimuli and acute light induction of sleep are likely to be entirely driven by intrinsically photosensitive melanopsin-expressing RGCs without significant input from conventional RGCs\cite{16,17,18}. These behaviours were also preserved in \textit{Opa1} mutant mice, suggesting preservation of melanopsin RGC derived responses. A previous study also assessed masking behaviour in the same \textit{Opa1} mutant line\cite{8} and suggested that masking in \textit{Opa1}\textsuperscript{+/-} mice was impaired relative to wildtype controls. It is likely that this discrepancy is due to the fact that the data were not corrected for individual differences in baseline activity. This may be important as we have noted that \textit{Opa1}\textsuperscript{+/-} animals display a slightly higher baseline activity level relative to \textit{Opa1}\textsuperscript{+/+} mice. In the present study this difference in baseline activity levels was not significant. The results overwhelmingly support a preservation of NIF light driven behaviour in \textit{Opa1} mutant mice.

It has been previously reported that RGC soma loss is minimal in heterozygous B6; C3-\textit{Opa1}\textsuperscript{Q285STOP} mice\cite{10}. In confirmation, we found no significant difference in melanopsin RGC numbers between wildtype and mutant mice. Hence, the lack of effect of the \textit{Opa1} mutation on circadian function maybe due to the lack of a significant effect on melanopsin RGC cell numbers. However,
previous studies have shown that visual function is disrupted in Opa1+/− mice [9,12] despite the maintained numbers of RGCs. It was suggested that the visual disruption may be due to the observed RGC axonal abnormalities and the significant progressive RGC dendropathy [10]. Interestingly, RGC dendropathy was restricted to sublamina b of the inner plexiform layer (IPL). Melanopsin-expressing photosensitive RGCs stratify in sublamina a (M1 cells), sublamina b (M2, M4 and M5 cells) or in both sublaminae (M3) of the IPL [13,36,37,38]. The different morphological melanopsin cell classes project to different brain centres and thus drive different NIF visual functions [13,36]. The suprachiasmatic nucleus, which controls circadian behaviour, receives projections mainly from M1 cells [39,40]. The olivary pretemporal nucleus which drives the PLR, receives projections from both M1 and M2 cells [39,40]. A specific deficit in the pupil light reflex may therefore be predicted in Opa1 mutant mice. The pupil light reflex is driven by melanopsin at high irradiance [34]. At lower irradiances both rod and cone input to the melanopsin cells and conventional RGCs contribute to the PLR [16,17,24,34]. Presently, a robust pupil light reflex was recorded in Opa1+/− mice, equivalent to wildtype controls, in response to a full irradiance series. This would suggest that all photoreceptor inputs to the PLR are preserved including dendritic inputs from the rod and cone pathways to the melanopsin RGCs. Immunohistological studies also revealed no apparent differences in melanopsin RGC dendritic projections to sublamina a and sublamina b of the IPL between Opa1+/+ and Opa1+/− mice. These data would suggest that melanopsin RGCs are perhaps not subject to the dendritic reorganisation observed in other RGCs that stratify in sublamina b [10].

Several studies have suggested that the PLR is also preferentially preserved in another mitochondrial optic atrophy – Leber’s hereditary optic neuropathy (LHON) [20,41,42,43]. La Morgia et al. (2010) also reported a relative sparing of melanopsin RGCs relative to the total RGC population in ADOA and LHON patients [20]. Preservation of melanopsin ganglion cells has been reported in other models of retinal disease/damage [44,45]. This may suggest that melanopsin RGCs are resistant in models of inner retinal damage, particularly in mitochondrial retinopathies.

It is unclear why melanopsin-expressing photosensitive RGCs may be less susceptible to mitochondrial impairment than other ganglion cells. It has been predicted that cells with a low energy demand may be affected least by loss of normal mitochondrial morphology or function. However, ultrastructural characterisation of the melanopsin cells has identified dendritic varicosities packed with mitochondria [46] arguing against this simple explanation. Melanopsin RGCs express the neuropeptide pituitary adenylate cyclase activating polypeptide (PACAP) [47]. Several studies have found that in vivo application of PACAP has retinoprotective effects [48,49,50]. However, it is unknown if melanopsin cells express PACAP receptors or if cellular expression of PACAP itself is neuroprotective in mitochondrial retinopathies.

We conclude that photic regulation of circadian behaviour, immobility-defined sleep induction and the pupil light reflex are preserved in a mouse model of ADOA despite previous reports of RGC pathophysiology [8,10] and visual dysfunction [12,35]. Melanopsin-expressing RGCs, which primarily drive these behaviours, may be resistant to damage in mitochondrial retinopathies. Identification of the possible neuroprotective mechanism involved could lead to exciting therapeutic strategies.

Acknowledgments

The videotracking system was kindly supplied by Russell Foster and Stuart Peirson with technical assistance provided by Simon Fisher and Sofia Godhino. Julian Partridge kindly performed irradiance calibration for the pupillometry studies and Chris Hull and David Crabbe helped with pupillometry data analysis and statistics.

Author Contributions

Conceived and designed the experiments: SS ARB RHD REM MV. Performed the experiments: SS GP ARB CK RHD MV. Analyzed the data: SS GP ARB CK RHD MV. Contributed reagents/materials/analysis tools: SS RHD REM MV. Wrote the paper: SS GP ARB RHD REM MV.

References

1. Kjer P (1959) Infantile optic atrophy with dominant mode of inheritance: a clinical and genetic study of 19 Danish families. Acta Ophthalmol Suppl 164: 1–147.
2. Klune LB, Glaser JS (1979) Dominant optic atrophy. The clinical profile. Arch Ophthalmol 97: 1680–1686.
3. Votruba M, Finke FW, Holder GE, Carter A, Bhattacharya SS, et al. (1998) Clinical features in affected individuals from 21 pedigrees with dominant optic atrophy. Arch Ophthalmol 116: 351–358.
4. Johnston PB, Gaster RN, Smith VC, Tripathi RC (1979) A clinicopathologic study of autosomal dominant optic atrophy. Am J Ophthalmol 88: 688–687.
5. Kjer P, Jensen OA, Klinken L (1983) Histopathology of eye, optic nerve and brain in a case of dominant optic atrophy. Acta Ophthalmol (Copenh) 61: 300–312.
6. Alexander C, Votruba M, Pesch UE, Thielson DL, Mayer S, et al. (2000) OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. Nat Genet 26: 211–215.
7. Delêtre C, Lenczars G, Griffoin JM, Giguere N, Lorenzo C, et al. (2000) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. Nat Genet 26: 207–210.
8. Davies VJ, Hollins AJ, Piechota MJ, Yip W, Davies JR, et al. (2009) Opa1+/− mice, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. Nat Genet 26: 211–215.
9. Votruba M, Finke FW, Holder GE, Carter A, Bhattacharya SS, et al. (1998) Clinical features in affected individuals from 21 pedigrees with dominant optic atrophy. Arch Ophthalmol 116: 351–358.
10. Johnston PB, Gaster RN, Smith VC, Tripathi RC (1979) A clinicopathologic study of autosomal dominant optic atrophy. Am J Ophthalmol 88: 688–687.
11. Kjer P, Jensen OA, Klinken L (1983) Histopathology of eye, optic nerve and brain in a case of dominant optic atrophy. Acta Ophthalmol (Copenh) 61: 300–312.
22. Mrosovsky N, Hattar S (2003) Impaired masking responses to light in melanopsin-knockout mice. Chronobiol Int 20: 989–999.

23. Fisher SP, Godinho SI, Polhemery CA, Hankins MW, Foster RG, et al. (2012) Rapid assessment of sleep-wake behavior in mice. J Biol Rhythms 27: 46–58.

24. Lucas RJ, Douglas RH, Foster RG (2001) Characterization of an ocular photopigment capable of driving pupillary constriction in mice. Nat Neurosci 4: 621–626.

25. Gavrovski VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K (2000) In search of the visual pigment template. Vis Neurosci 17: 509–528.

26. MacLaren RE, Pearson RA, MacNeil A, Douglas RH, Salt FE, et al. (2006) Retinal repair by transplantation of photoreceptor precursors. Nature 444: 203–207.

27. Zele AJ, Feigl B, Smith SS, Markwell EL (2011) The circadian response of intrinsically photosensitive retinal ganglion cells. PLoS One 6: e17860.

28. Pires SS, Hughes S, Turton M, Melyan Z, Peirson SN, et al. (2009) Differential expression of two distinct functional isoforms of melanopsin (Opn4) in the mammalian retina. J Neurosci 29: 12332–12342.

29. Mrosovsky N, Foster RG, Salmon PA (1999) Thresholds for masking responses to light in three strains of retinally degenerate mice. J Comp Physiol A 184: 423–428.

30. Mrosovsky N, Lucas RJ, Foster RG (2001) Persistence of masking responses to light in mice lacking rods and cones. J Biol Rhythms 16: 585–598.

31. Lupi D, Oster H, Thompson S, Foster RG (2008) The acute light-induction of sleep is mediated by OPM4-based photoreception. Nat Neurosci 11: 1068–1073.

32. Benca RM, Gilliland MA, Obermeyer WH (1998) Effects of lighting conditions on sleep and wakefulness in albino Lewis and pigmented Brown Norway rats. Sleep 21: 451–460.

33. Tsai JW, Hannibal J, Hagiwara G, Colas D, Ruppert E, et al. (2009) Melanopsin as a sleep modulator: circadian gating of the direct effects of light on sleep and altered sleep homeostasis in Opn4(-/-) mice. PLoS Biol 7: e1000125.

34. Lucas RJ, Douglas RH, Foster RG (2001) Characterization of an ocular photopigment capable of driving pupillary constriction in mice. Nat Neurosci 4: 621–626.

35. Heiduschka P, Schnichels S, Fuhrmann N, Hofmeister S, Schraermeyer U, et al. (2010) Electrophysiological and histologic assessment of retinal ganglion cell fate in a mouse model for OPA1-associated autosomal dominant optic atrophy. Invest Ophthalmol Vis Sci 51: 1424–1431.

36. Hattar S, Kumar M, Park A, Tong P, Tung J, et al. (2006) Central projections of melanopsin-expressing retinal ganglion cells in the mouse. J Comp Neurol 497: 326–349.

37. Ecker JL, Dumitrescu ON, Wong KY, Alten NM, Chen SK, et al. (2011) Melanopsin-expressing retinal ganglion-cell photoreceptors: cellular diversity and role in pattern vision. Neuron 67: 49–60.

38. Estevez ME, Fergusson PM, Barth MC, Borgehuis BG, Chan E, et al. Form and function of the rd1 cell, an intrinsically photosensitive retinal ganglion cell type contributing to geniculocortical vision. J Neurosci 32: 13608–13620.

39. Baver SB, Pickard GE, Sollars PJ (2008) Two types of melanopsin retinal ganglion cell differentially innervate the hypothalamic suprachiasmatic nucleus and the olivary pretectal nucleus. Eur J Neurosci 27: 1763–1778.

40. Chen SK, Bailea TC, Hattar S (2011) Photorearrangement and pupillary light reflex are mediated by distinct populations of ipRGCs. Nature 476: 92–95.

41. Kawasaki A, Herbst K, Sander B, Mäde D (2009) Selective wavelength pupillometry in Leber hereditary optic neuropathy. Clin Experiment Ophthalmol 38: 322–324.

42. Bremmer FD, Shallo-Hoffmann J, Riedel-Eva P, Smith SE (1999) Comparing pupill function with visual function in patients with Leber’s hereditary optic neuropathy. Invest Ophthalmol Vis Sci 40: 2529–2534.

43. Wakakura M, Yokoe J (1995) Evidence for preserved direct pupillary light response in Leber’s hereditary optic neuropathy. Br J Ophthalmol 79: 442–446.

44. Li RS, Chen BY, Tay DK, Chan HH, Pu ML, et al. (2008) Melanopsin-expressing retinal ganglion cells are more injury-resistant in a chronic ocular hypertension model. Invest Ophthalmol Vis Sci 47: 2951–2958.

45. Robinson GA, Madison RD (2004) Axotomized mouse retinal ganglion cells containing melanopsin show enhanced survival, but not enhanced axon regrowth into a peripheral nerve graft. Vision Res 44: 2667–2674.

46. Belyk MA, Smeraksi CA, Provencio I, Sollars PJ, Pickard GE (2003) Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. J Comp Neurol 460: 390–397.

47. Hamihal J, Hindersson P, Knudsen SM, Georg B, Fahrenkrug J (2002) The photopigment melanopsin is exclusively present in pituitary adenylate cyclase activating polypeptide-containing retinal ganglion cells of the retinohypothalamic tract. J Neurosci 22: RC191.

48. Seki T, Itoh H, Nakamachi T, Endo K, Wada Y, et al. (2011) Suppression of rat retinal ganglion cell death by PACAP following transient ischemia induced by high intraocular pressure. J Mol Neurosci 48: 30–38.

49. Seki T, Nakatani M, Taki C, Shinohara Y, Ozawa M, et al. (2006) Melanopsin photopigment capable of driving pupillary constriction in mice. Nature 444: 203–207.

50. Tamas A, Gabriel R, Racz B, Denes V, Kiss P, et al. (2004) Effects of pituitary adenylate cyclase activating polypeptide in retinal degeneration induced by monosodium-glutamate. Neurosci Lett 372: 110–113.