Melanopsin Is Expressed in PACAP-Containing Retinal Ganglion Cells of the Human Retinohypothalamic Tract

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Purpose. The putative circadian photoreceptor melanopsin is found in rodents in a subpopulation of intrinsic light-sensitive retinal ganglion cells (RGCs) constituting the retinohypothalamic tract (RHT). The study was conducted to determine whether melanopsin is expressed in the human retina and costored with the neuropeptide pituitary adenylate cyclase activating polypeptide (PACAP), a marker for the RHT, projecting to the suprachiasmatic nucleus (SCN). Furthermore, whether melanopsin expression is conserved in retinas of blind patients with severe retinal degeneration was investigated.

Methods. In situ hybridization and immunohistochemistry was used to demonstrate melanopsin synthesis in human eyes of 17 donors and two postmortem hypothalami containing the SCN. The coexistence of melanopsin and PACAP in elements of the retinohypothalamic tract was studied by dual-labeling immunocytochemistry.

Results. Melanopsin expression was found in a subpopulation of RGCs located in the ganglion cell layer and displaced in the inner nuclear cell layer. Melanopsin-containing cells comprised approximately 0. 8% of all RGCs, with a distinct morphology characterized by two to four dendritic processes constituting a panretinal network. Melanopsin immunoreactivity was primary present at perikaryal boundaries and neuronal processes and to some extent also in the cytoplasm. PACAP and melanopsin were colocalized in the RGCs and PACAP-containing nerve fibers, seemingly innervating the retinorecipient part of the SCN. Melanopsin-expressing RGCs were conserved in retinas of blind patients with severe degeneration of the outer and/or inner layers.

Conclusions. Given the expression of melanopsin in PACAP-containing RGCs of the human RHT, this photoreceptor is a likely first base in the chain of events leading to photoentrainment of both normal and blind people. (*Invest Ophthalmol Vis Sci.* 2004;45:4202–4209) DOI:10.1167/iovs.04-0313

In mammals, photic information is exclusively processed by the retina and reaches the brain through the optic nerve. The eyes are equipped with at least two functionally and anatomically distinct light-detecting systems, the classic image-forming system involving rods and cones and an irradiance detection system.[1] The non–image-forming photoreceptive system synchronizes (entrains) the circadian timing system and regulates pineal melatonin secretion and pupillary constriction[2–4] (for review, see Ref. 5). The retinal projection to the circadian timing system, designated the retinohypothalamic tract (RHT), is part of the non–image-forming projection to the brain.[6–9] In rodents, the RHT costores the two neurotransmitters, glutamate and pituitary adenylate cyclase activating polypeptide (PACAP), which in a complex interplay entrain the circadian clock located in the hypothalamic suprachiasmatic nucleus (SCN) (reviewed in Ref. 10). The non–image-forming irradiance-detection system originates from a subpopulation of light-sensitive retinal ganglion cells (RGCs).[11–13] The recently identified opsin-like molecule melanopsin is likely to constitute the irradiance-detecting photopigment,[14,15] which is expressed in RGCs projecting to the SCN.[6,16–19] This notion is based on studies demonstrating that RHT-projecting RGCs of melanopsin-deficient mice have lost intrinsic photosensitivity[13] and that these mice have impaired light entrainment,[20,21] altered masking behavior,[22] and decreased pupillary light reflex.[13] Furthermore, the spectral sensitivity of the melanopsin-expressing RGCs corresponds to the behavioral action spectrum of photic entrainment.[23–25] Based on action spectrum analysis using light suppression of melatonin as the response parameter, the existence of a similar irradiance-detection system using a short-wave photopigment was recently suggested in humans.[26,27] This could explain why some blind people have retained the ability to entrain circadian rhythms of behavior and physiology to a light–dark cycle.[28–30] In the present study, using in situ hybridization and immunohistochemistry for melanopsin, we demonstrated that a PACAP-containing RHT exists in normal human subjects and that ganglion cells of the human RHT express melanopsin. We also found conserved melanopsin expression in the retina of individuals who have severe retinal degeneration that causes complete or partial blindness.

Methods

Human Brain and Retinal Tissue Preparation

Human Hypothalamus. All material used in the present study was obtained in compliance with the Declaration of Helsinki for research involving human tissue. Human brains from two subjects (one male, age 73, NBB no. S96-048, clinical diagnosis: coronary stenosis; and one female, age 82, NBB no. S95-099, clinical diagnosis: acute myocardial infarct) were obtained from The Netherlands Brain Bank (NBB; coordinator, Rivka Ravid; Amsterdam) by rapid autopsy. The postmortem delay ranged from 4 to 7 hours. Permission for brain autopsy was obtained either from the patient or from partners or relatives. An autopsy was performed according to the protocols of the NBB, which includes measurements of the pH of the cerebral spinal fluid (CSF) to estimate agonal state. The brains were immersion fixed in 10% formalin until embedded in paraffin. A block of hypothalamus containing the SCN was sectioned in 5-μm-thick sections and stained immunohistochemically as described in a later section.

Human Eyes. From 15 human donors, eyeballs were obtained during surgery and fixed immediately. Eyes were obtained from two

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donors 24 hours after death and subsequently fixed. Permission to use the eye tissue was obtained either from the patient or from partners or relatives.

Normal Human Retina. For the study of normal retinal anatomy, nonpathologic areas of the central and peripheral retinas from patients with malignant choroidal melanoma (MCM) were investigated ($n = 10$).

Pathologic Human Retinas. Retinas of patients with the diagnoses of retinitis pigmentosa ($n = 3$), retinal atrophy, secondary glaucoma ($n = 2$), or congenital microphthalmos with secondary glaucoma ($n = 1$) were fixed in phosphate-buffered (0.02 M) 4% formaldehyde (pH 7.0) and paraffin embedded. All patients were virtually blind. Furthermore, two eyes with MCM disclosed partial retinal detachment. These eyes were immersion fixed immediately after removal in either Stefanini's fixative (2% paraformaldehyde and 0.2% picric acid in 0.1 M sodium phosphate buffer [pH 7.2]) or in 4% phosphate-buffered neutral formalin. The retinas were either cut as 12-μm cryostat sections after cryoprotection in 30% sucrose for 24 to 48 hours or were embedded in paraffin and sectioned as 5-μm sections used for in situ hybridization and/or immunohistochemical studies. Two wholemounts of MCM eyes free of tumor were used for immunohistochemical staining.

Cloning of Human Melanopsin cDNA

A 2-kb (nucleotides [nt] 29-2036 according to the cDNA sequence of AF147788) fragment including the full-length coding sequence of the human melanopsin cDNA was cloned by polymerase chain reaction (PCR) run on human retina (QUICK-Clone cDNA; BD Biosciences-Clonetech, Palo Alto, CA). The primers forward, 5′aagcaggggtgctgaggatgg3′, and reverse, 5′aagggaggtctgtgctcggcttca3′ (MWG Biotech, Ebersberg, Germany), and enzyme mix (Expand Long Template; Roche Diagnostics, Mannheim, Germany) were used for the PCR. The product was cloned into a vector (pCTII-TOPO; Invitrogen, Groningen, The Netherlands) and sequenced (MWG Biotech).

In Situ Hybridization Histochemistry

In vitro labeling of cRNA antisense and sense probes was performed as previously described, with ^{33}P-uridine triphosphate (UTP).[31,32] The cDNA template was the earlier-mentioned plasmid containing the melanopsin cDNA. In situ hybridization was performed according to a previously published protocol.[33] In brief, after deparaffination and treatment in acetic anhydride, dehydration in 70% ethanol, and prehybridization (2 hours), retinal sections were hybridized with melanopsin cRNA probes fragmented by incubation in hydrolysis buffer for 50 minutes at 60°C and used in a concentration of 1×10^7 cpm/mL. After hybridization, washing, RNase treatment, and a final wash, radioactively labeled retinas were dried, emulsion dipped (Amersham Biosciences, Little Chalfont, UK) and exposed for 7 to 14 days before being developed. Hybridization was routinely performed in parallel with antisense and sense probes on consecutive retinal sections from the same subject, and no signal was obtained with the sense probes.

Antibodies

Expression and Purification of Cytoplasmic Human Melanopsin. The C-terminal part of human melanopsin was PCR amplified with the primers PR7091, 5′cacccaccccaagtacagggtggc3′, and PR7092, 5′aagggaggtctgtgctcggcttca3′. The PCR product was purified by agarose gel electrophoresis and subcloned in the vector pET100/D-TOPO (Invitrogen) using the *Escherichia coli* TOP10 host as described by the manufacturer. The plasmid pHI7076, encoded a fusion protein with an N-terminal His-G tag and an Express-TAG in frame with the predicted cytoplasmic part of human melanopsin. The predicted amino acid sequence of the fusion protein (melanopsin sequence is italic) was MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDHPF*THPKYRVAIAQHLPCLGVLLGVSRRHSRPYPSYRSTHRSTLISHTSNLSWISIRRRQESLGSESEVGWTHMEAAAVWGAAQQANGRSLYGQGLEDLEAKAPPRPQGHEAETPGKTKGLIPSQDPRM*. The expression *E. coli* host BL21(DE3)* (Invitrogen) was transformed with pHI7076 and grown in Luria-Bertani (LB) medium. Expression of the recombinant fusion proteins was induced by addition of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside). Cells were lysed in 10 mM Tris, 0.1 mM dithiothreitol (DTT), and protein was extracted with Tris-equilibrated phenol (pH 8.0). Protein was precipitated by addition of a 2.5 volume of ethanol. The precipitate was solubilized in 6 M guanidinium chloride, 0.1 M DTT, and 50 mM Tris (pH 8.0). The solubilized protein was passed over a Sephadex G25 column equilibrated with a buffer containing 5 mM mercaptoethanol, 8 M urea, 0.5 M NaCl, 50 mM Tris (pH 8.0; MUNT). Finally the recombinant fusion proteins were captured on a Nickel-nitriloacetic acid column. The His-tagged recombinant protein was eluted with a gradient of imidazole in MUNT. Fusion proteins were eluted at approximately 200 mM imidazole. The presence of the HisG and Express-tag in the N-terminal part of the purified fusion protein was verified by Western blot analysis, as described,[34] using peroxidase labeled anti-HisG and anti-Express monoclonal antibodies (both antibodies obtained from Invitrogen). The recombinant fusion protein was detected as a single anti-HisG and anti-Express epitope reactive band of 18 kDa, corresponding to the calculated molecular mass of the construct. The purified protein gave a single band in denaturing SDS-PAGE when stained (Simply Blue; Invitrogen).

Human Melanopsin Antibodies. Five rabbits were immunized with 50 μg of fusion protein in MUNT buffer. The immunization material was emulsified in three volumes of complete Freund's adjuvant for the first immunization and incomplete adjuvant for subsequent immunizations at 7-day-intervals for the three first immunizations and 4-week intervals thereafter. Serum from one rabbit (no. 5J68, diluted 1:2–10,000) was used for all the described experiments. Absorption of the antibodies with the immunization material dialyzed against PBS with 0.1% Tween abolished all staining. The rabbit anti-human melanopsin antiserum was directed against the C-terminal part of human melanopsin and recognized human but not rodent melanopsin when tested on rodent eye sections (not shown).

PACAP Antibody. A previously characterized mouse monoclonal antibody (MabJHH1) recognizing both PACAP-38 and -27 was used for PACAP immunostaining.[35]

VIP was visualized by our rabbit anti-VIP antibody (291E; diluted 1:1000) described previously,[36] and the neurophysin antibody (diluted 1:1000) was obtained from Dakopatts (A0567; Copenhagen, Denmark). In control experiments, preabsorbtion of the antibodies with their respective antigens or elimination of the primary antibody was performed, which abolished all staining.

Immunohistochemistry

Single and double immunohistochemistry for visualization of melanopsin, PACAP, VIP, and neurophysin was performed as described in detail previously,[16,33,37] using a mixture of biotinylated goat anti-mouse antiserum and/or biotinylated donkey anti-rabbit antiserum and Cy2-conjugated donkey anti-rabbit antiserum (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) and biotinylated tyramide (Tyramide System amplification; DuPont NEN, Boston, MA) and streptavidin-conjugated Texas red or streptavidin conjugated Cy2 (Amersham Bioscience). For detection of antigens in the paraffin-embedded brain and retina tissue, the sections were deparaffinated followed by antigen-retrieval procedures as described by the manufacturer (TechMate 500/1000; Dako) using antigen-retrieval buffer (ChemMate, S203120; Dako) in distilled water.

Photomicrographs

Images were obtained with a camera (model DC200, with accompanying software; Leica, Cambridge, UK) and/or a confocal microscope (model IX70, equipped with Fluoview ver. 2.1.39; Olympus, Birkeroed, Denmark) and appropriate filter settings for detecting CY2 and Texas red. All images were digital and had a depth of field of approximately 1.38, 0.69, 0.35, and 0.23 μm for the $\times$10, $\times$20, $\times$40, and $\times$60 objectives, respectively. Confocal images obtained as stacks of images (0.2 μm thickness) were analyzed on computer (Volocity Imaging

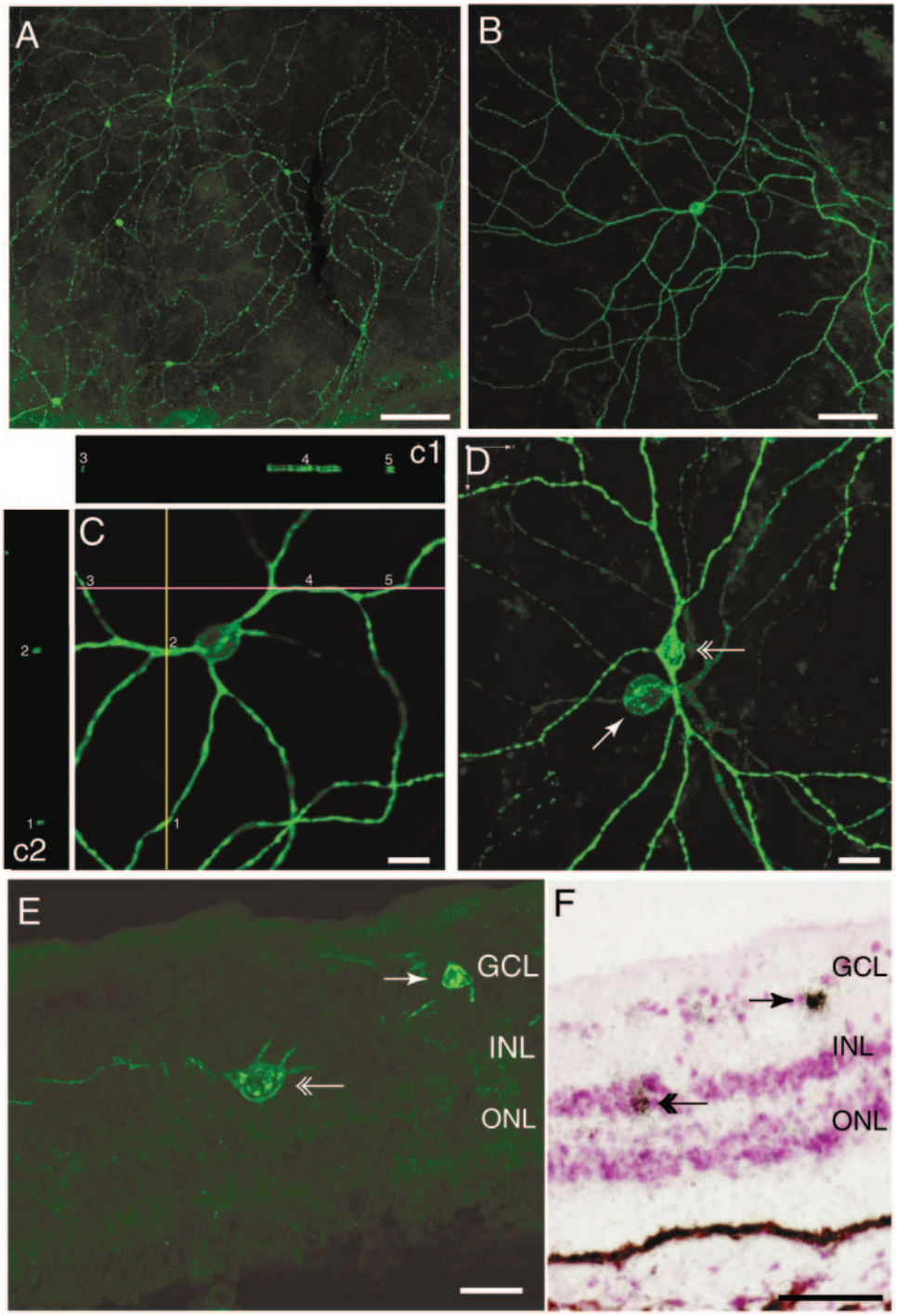


FIGURE 1. Melanopsin expression in the human retina. Confocal photomicrographs (**A–E**) of melanopsin immunoreactivity in a normal part of the retina obtained from an eye removed due to malignant choroidal melanoma. (**A**) Melanopsin is found in a subset of RGCs that have a widespread dendritic network with varicosities. (**B**) A single RGC showing a typical dendritic tree. (**C**) RGC from (**B**) analyzed in an *x-y* and *x-z* plane. This image contains the extended focus of 181 sections of 0.2 μm thickness. (**c1**) A computer generated image in the *x-z* plan corresponding to the *purple line* in (**C**). The numbers 1 to 5 in (**C**) correspond to the same area marked by numbers in (**c1**). Note the localization of melanopsin immunoreactivity in the dendritic membrane. A similar finding is demonstrated in (**c2**), which shows the *y-z* plane corresponding to the *yellow line* in (**C**). In (**D**), two melanopsin-immunoreactive cells located in the GCL (*arrow*) and in the INL (*double arrow*) are demonstrated (see Movie 1 for a three-dimensional animation of the two cells). The localization of melanopsin-containing RGCs in the GCL (*arrow*) and INL (*double arrow*) is also shown in a cross section (**E**). (**F**) In situ hybridization using melanopsin cRNA probe showing the localization of melanopsin mRNA in RGCs in the GCL (*arrow*) and the INL (*double arrow)*. Scale bars: (**A**) 200 μm; (**B**) 100 μm; (**C**, **D**) 23 μm; (**E**) 20 μm, (**F**) 50 μm.

software, ver. 2.6.1; Improvision, Coventry, UK). Images generated were edited for contrast, brightness, and color tone (Photoshop Adobe Systems, Mountain View, CA) and combined into plates (Illustrator; Adobe Systems). Figures were printed on a sublimation printer (Phaser 450 dye; Tektronix, Wilsonville, OR).

RESULTS

Melanopsin and PACAP in Human RGCs

Melanopsin expression in the normal retina was studied by in situ hybridization and immunohistochemistry in retinas obtained from donors with malignant choroidal melanoma. The examined part of the retinas had intact morphology of all sublayers by histologic examination of counter-stained sections. Melanopsin expression was demonstrated in a subset of RGCs evenly distributed throughout the normal retina (Figs. 1A, 1B). Approximately half of the melanopsin-containing perikarya were located in the ganglion cell layer (GCL), whereas the other half were located in the inner nuclear cell layer (INL) adjacent to the border of the inner plexiform layer (IPL; Figs. 1D, 1E). Normal retinas from three subjects (212-03, 1845-03, and 946-02) were used to quantify melanopsin immunoreactive RGCs. In 10 randomly selected areas of each retina, 75 melanopsin-expressing cells were counted (19, 25, and 31 cells/2.8 mm^2 from each subject, respectively). Assuming a total of 1.2 million RGCs in the human retina, which has an area of 1040 mm^2,[38,39] the melanopsin-containing cells represent ~0.8% of the total number of ganglion cells. The mela-

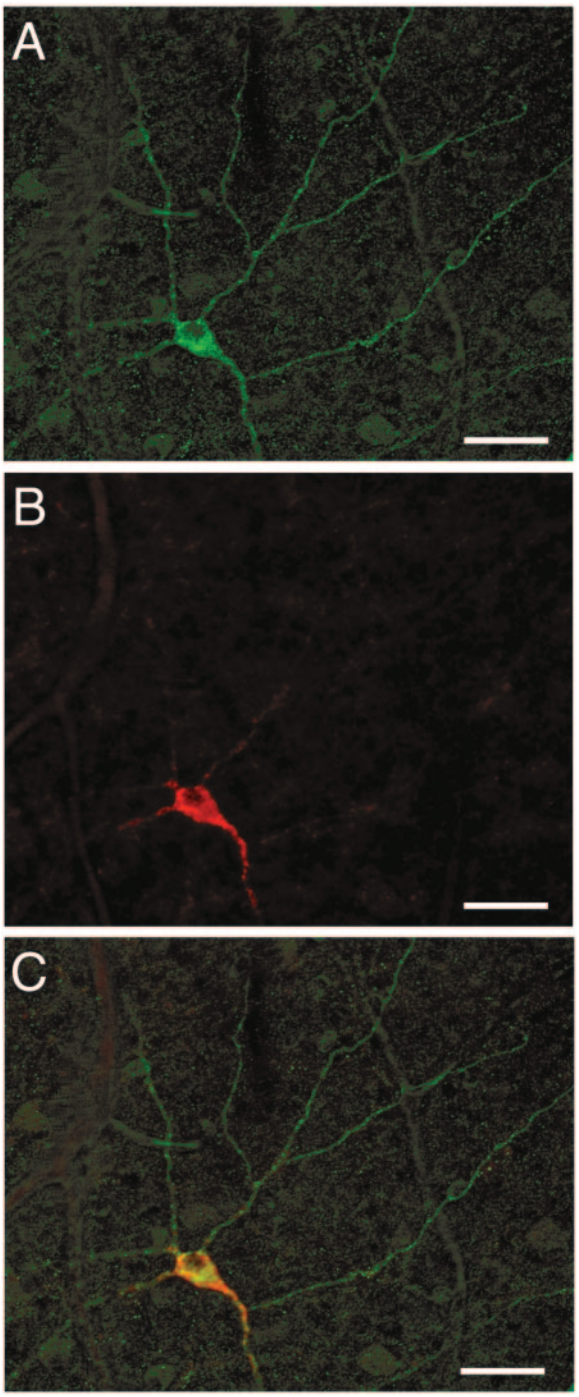


FIGURE 2. Melanopsin-immunoreactive RGCs costore the neuropeptide PACAP. Confocal photomicrographs showing retinal whole-mounts stained for melanopsin (**A**) and PACAP (**B**) and the images merged in (**C**). Only PACAP immunoreactivity was visualized, by using tyramide amplification resulting in less intense melanopsin immunoreactivity than that shown in Figures 1A–E and Figure 5. Scale bars: 50 μm.

nopsin immunoreactivity was primarily located to the surface of the ganglion cell soma and the dendritic processes and to a lesser extent also in the cytoplasm (Figs. 1Cc1, 1Cc2, 1E). Perikarya of individual ganglion cells were round to ovoid, and two clearly defined populations with diameters of either 15 to 20 or 20 to 25 μm were observed (Figs. 1D, 1E). RGCs of both sizes were found in the GCL and INL (Figs. 1D, 1E). The dendritic processes of the melanopsin-containing RGCs in the GCL projected toward the INL where they branched between the IPL and INL. Melanopsin-containing dendrites of RGCs in the INL arborized in the same sublayer (Fig. 1E) (see Movie 1 at

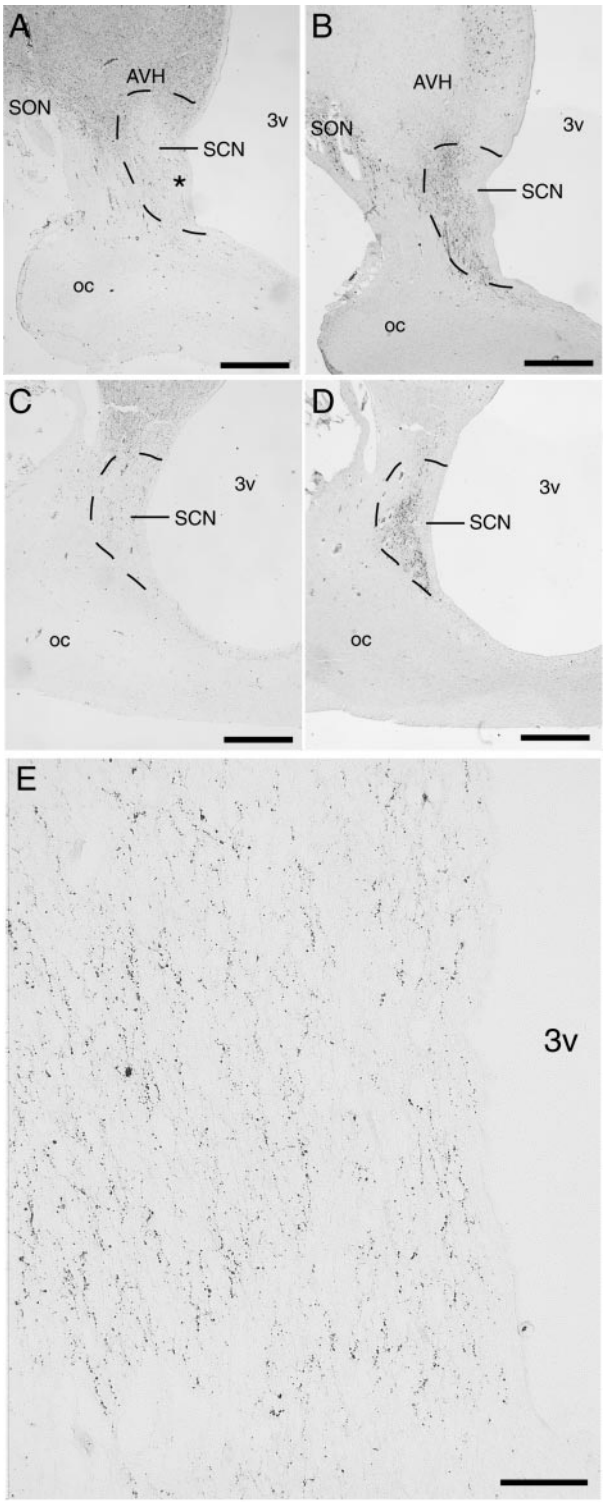


FIGURE 3. PACAP immunoreactivity in the retinorecipient zone of the human suprachiasmatic nucleus (SCN). (**A**, **C**, **E**) Photomicrographs showing PACAP immunoreactivity in nerve fibers of the ventral SCN. The SCN was identified in consecutive sections stained for neurophysin (**B**, **D**). (**E**) High magnification of the area marked (⋆) in (**A**) PACAP was exclusively found in nerve fibers ascending from the optic chiasma. Oc, optic chiasma; 3v, third ventricle; SON, supraoptic nucleus; AVH, anteroventral hypothalamus. Scale bars: (**A–D**) 200 μm; (**E**) 25 μm.

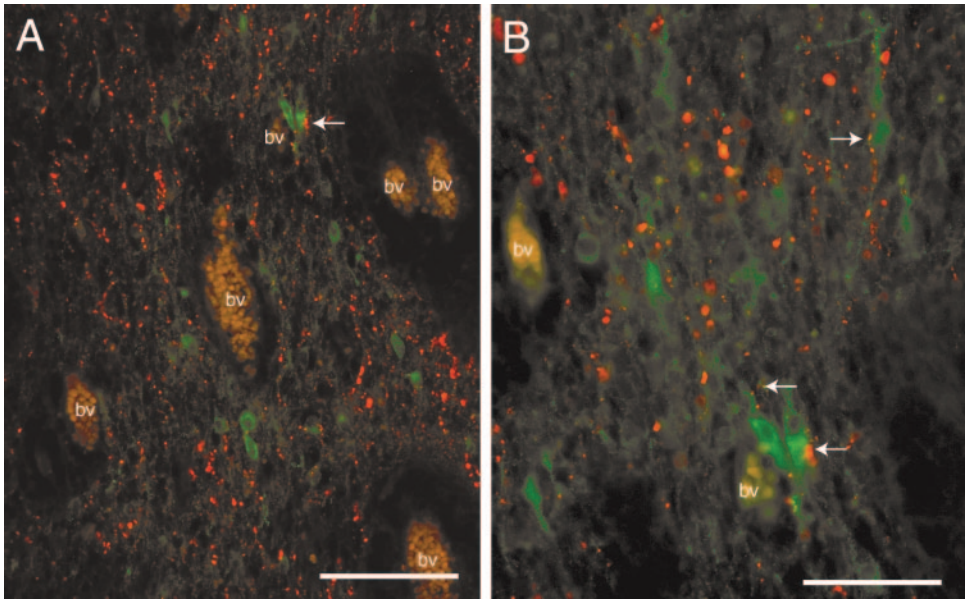


FIGURE 4. Confocal photomicrographs in low (**A**) and high power (**B**) of the human ventral SCN showing PACAP-immunoreactive fibers (*red*) which seem to innervate (*arrows*) VIP-containing neurons (*green*). Bv, blood vessel. Scale bars: (**A**) 100 μm; (**B**) 50 μm.

www.iovs.org/cgi/content/full/45/11/4202/DC1) and the dendritic field covered an area with a diameter of 400 to 800 μm (Figs. 1A, 1B). In situ hybridization using a human melanopsin cRNA probe confirmed the expression of melanopsin in RGCs in both the GCL and the INL (Fig. 1F). By double immunohistochemistry, melanopsin in all RGCs was shown to be colocalized with the neuropeptide PACAP and vice versa (Fig. 2).

PACAP-Immunoreactive Fibers of the Human RHT Are Found in the Retinorecipient SCN

Because PACAP is a marker for RHT projections to the SCN in rodents,[31,40] we examined the retinorecipient zone of the SCN for PACAP-immunoreactive nerve fibers. The human SCN was identified using immunostaining for VIP and neurophysin/vasopressin (NP) which are located in cell bodies receiving retinal projections[41] (see review in Ref. 42). On consecutive and/or double-immunostained sections for VIP and NP, a relatively dense terminal field of PACAP-positive nerve fibers was shown in the retinorecipient zone (ventral part) of the SCN, the size of which gradually decreased from the rostral to caudal part of the nucleus (Fig. 3). The fibers, which mainly ascended from the medial part of the optic chiasma (OC) were delicate with small varicosities (Fig. 3E). PACAP-immunoreactive cell bodies were not found in the SCN. Double immunostaining for PACAP and VIP revealed PACAP-immunoreactive nerve fibers in close apposition to VIP-containing neurons in the ventral SCN (Fig. 4).

Melanopsin in RGCs of Degenerated Retina

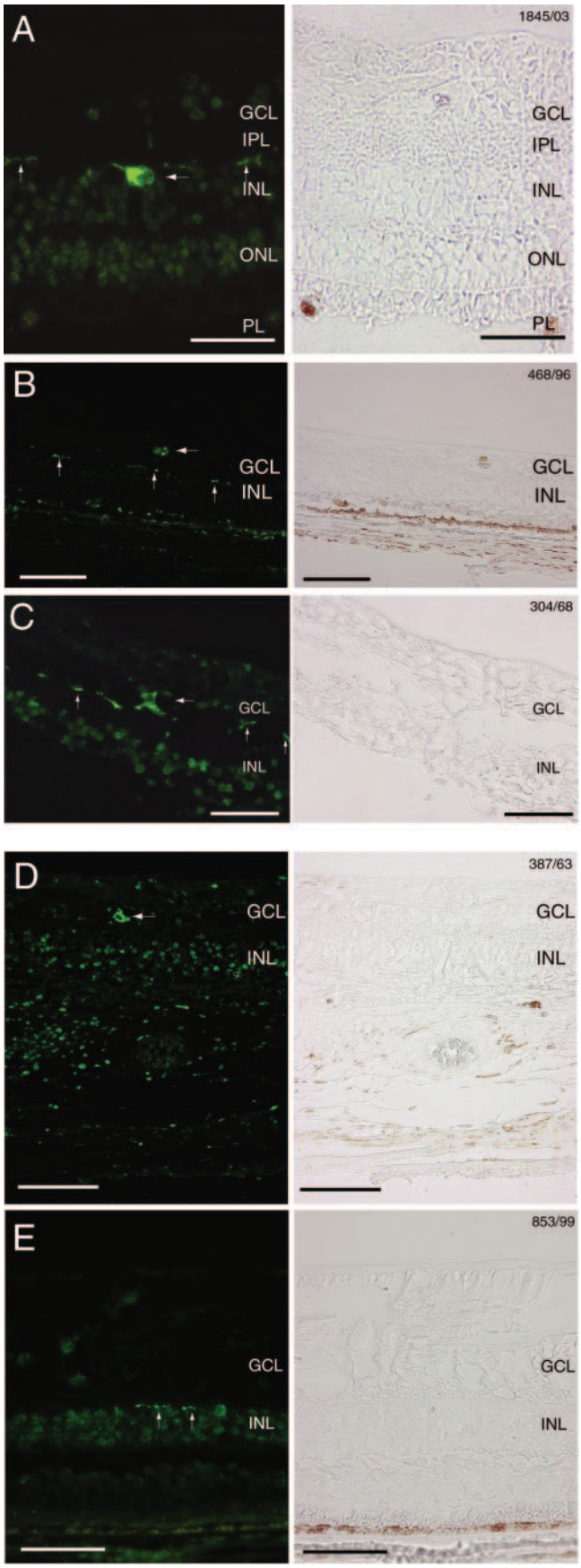
Retinal Detachment. Two donors with malignant choroidal melanoma (1845-03 and 445-02) had retinal detachment. In these areas, the photoreceptor layer (PRL) was partially degenerated, whereas the inner retina was intact (Fig. 5A). In the pathologic areas of these retinas melanopsin immunoreactivity was unaffected, and melanopsin-expressing RGCs were found in both the GCL and the INL, with a normal distribution and appearance of dendritic processes located at the border between the IPL and INL (Fig. 5A).

Retinal Atrophy and Secondary Glaucoma. One donor retina (178-98) with long-lasting glaucoma showed severe atrophy of the GCL. In this patient, melanopsin immunoreactivity could not be detected. In retina from another donor (468-96) with severe atrophy of both the GCL and outer retina, including destruction of the PRL, melanopsin-immunoreactive cells were clearly visible located near the INL (Fig. 5B).

Retinitis Pigmentosa. Severe degeneration involving all layers of the retina was found in donors with retinitis pigmentosa and congenital microphthalmos (Figs. 5C–E). The degenerative changes were not uniform, and in some parts of the retina it was possible to identify the GCL and/or the INL. In all cases the PRL was destroyed. Despite these changes, melanopsin-expressing RGCs and dendritic processes were identified in the inner retina in several donors (853-99, 387-63, 304-68, and 557-64). The dendrites appeared to be located close to the degenerated INL (Figs. 5C–E). In some areas, the retinas were almost completely gliotic, and in these parts no melanopsin was detected.

DISCUSSION

Using immunohistochemistry and in situ hybridization histochemistry, we report for the first time that melanopsin, a recently identified opsin-like molecule, is expressed in a subset of RGCs in the human retina. The distribution pattern is almost similar to that reported in rodent species, but a high proportion of the melanopsin-containing RGCs was displaced in the IPL and INL. The melanopsin-containing RGCs costored the neuropeptide PACAP, and it is likely that melanopsin-expressing RGCs represent the non–image-forming light-detection system recently described in rodents[6–9] and functionally characterized in humans by action spectrum analysis and light-induced melatonin-suppression tests.[26,27] In rats, the melanopsin- and PACAP-containing cells are found to have a topographically distinct retinal distribution with a nearly fivefold higher accumulation of RGCs in the upper part of the retina, a finding that remains functionally unexplained.[16] A similar unequal distribution has not been found in the hamster or the mouse[6,17,43,44] and in the present donor eyes, the distribution of melanopsin-expressing RGCs in the human retina seemed uniform. Our tissue sections were restricted, however, to the normal part of the retina obtained from patients who had eyes removed due to malignant choroidal melanoma, which allowed us to study only part of the retina. A full wholemount of normal retina is necessary to answer finally the



question of regional difference in melanopsin cell densities. The receptive field of melanopsin-expressing RGCs seemed to be even larger in the human retina than in rodents.[6,16–19] This could be due to the smaller relative number of melanopsin-containing RGCs in humans than in the rat (2%) and the mouse (1%).[6] As in rodents, the human melanopsin-containing dendritic processes covered the entire retina, most likely constituting a panretinal photosensitive network. In rats and mice, the melanopsin-expressing cells are intrinsically photosensitive,[23] a property that is lost when melanopsin is genetically eliminated.[13] This observation, together with the behavioral change accompanying genetic deletion of melanopsin[13,20–22] renders melanopsin a likely photoreceptor for irradiance detection. The detection of melanopsin mRNA in the human retina by RT-PCR[15] and the immunohistochemical demonstration of melanopsin expression in nonhuman primate RGCs (Dacey DM, et al. *IOVS* 2002;43:ARVO E-Abstract 3231; Peterson BB, et al. *IOVS* 2002;43:ARVO E-Abstract 5182) is in agreement with the current observation. Thus, the melanopsin-containing system seems to be conserved among mammalian species.

The existence of the RHT in humans was originally demonstrated by visualization of degenerated retinal nerve fibers in the SCN using the paraphenylenediamine method[45] and was later confirmed by tracing studies using DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine)[46] or neurobiotin[41] injected postmortem into the optic nerve. In comparison with the distribution of PACAP immunoreactive nerve fibers in the SCN, it seems most likely that the PACAP fibers in the ventral SCN represent the terminal field of RHT originating from the eye. Our demonstration that the PACAP-immunoreactive nerve fibers seem to innervate the VIP-containing neurons of the ventral SCN adds further support to this notion and is in accord with studies in the rat.[47]

Accumulating data have demonstrated that some blind people are able to entrain circadian rhythms to a light–dark cycle and suppress nighttime secretion of melatonin in response to light stimulation.[28,48] Our findings that melanopsin expression is conserved in retinas of patients with severe retinal diseases could be the anatomic substrate for these functional observations. It remains to be shown, however, that the conserved melanopsin immunoreactivity in the pathologic retinas result in a functional retinohypothalamic tract.

FIGURE 5. Melanopsin was expressed in retinas with severe degeneration of the outer and/or inner layers. Fluorescence photomicrographs of cross-sections of human retinas (and phase-contrast photomicrographs of the same section) showing melanopsin immunoreactivity (*arrow*) in RGCs and dendritic processes in (**A**) a section of retina from a patient with retinal detachment, showing degenerative changes primarily in the photoreceptor layer (PL). (**B**) A melanopsin-containing RGC and dendritic processes (*arrows*) in retina from a patient with retinal atrophy due to secondary glaucoma. (**C**, **D**) Sections of the central (**D**) and peripheral (**C**) retina from two patients with retinitis pigmentosa (RP). Note the complete loss of laminar organization (*arrows*) and complete loss of the PL. (**E**) Section of retina from a patient with congenital microphthalmos. Note the melanopsin-expressing RGC at the border between the INL and IPL and the melanopsin-containing process in the same sublayer (*arrows*). In all sections processed for antigen retrieval, a slight background staining was observed in the INL and outer nuclear layer (ONL). Scale bars: (**A**, **D**, **E**, **G**) 50 μm; (**B**, **C**, **F**, **H**) 100 μm.

Conclusion

Melanopsin, a putative circadian photoreceptor is costored with the neuropeptide PACAP in a subset of RGCs in human retina, the dendrites that form an extensive panretinal network. Melanopsin-containing RGCs constitute the RHT, mediating photic information to the brain including the biological clock in mammals. It is possible that melanopsin is responsible for light entrainment in normal and blind persons.

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