

# Melatonin modulates visual function and cell viability in the mouse retina via the MT1 melatonin receptor

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A clear demonstration of the role of melatonin and its receptors in specific retinal functions is lacking. The present study investigated the distribution of MT1 receptors within the retina, and the scotopic and photopic electroretinograms (ERG) and retinal morphology in wild-type (WT) and MT1 receptor-deficient mice. MT1 receptor transcripts were localized in photoreceptor cells and in some inner retinal neurons. A diurnal rhythm in the dark-adapted ERG responses was observed in WT mice, with higher a- and b-wave amplitudes at night, but this rhythm was absent in mice lacking MT1 receptors. Injection of melatonin during the day decreased the scotopic response threshold and the amplitude of the a- and b-waves in the WT mice, but not in the MT1<sup>-/-</sup> mice. The effects of MT1 receptor deficiency on retinal morphology was investigated at three different ages (3, 12, and 18 months). No differences between MT1<sup>-/-</sup> and WT mice were observed at 3 months of age, whereas at 12 months MT1<sup>-/-</sup> mice have a significant reduction in the number of photoreceptor nuclei in the outer nuclear layer compared with WT controls. No differences were observed in the number of cells in inner nuclear layer or in ganglion cells at 12 months of age. At 18 months, the loss of photoreceptor nuclei in the outer nuclear layer was further accentuated and the number of ganglion cells was also significantly lower than that of controls. These data demonstrate the functional significance of melatonin and MT1 receptors in the mammalian retina and create the basis for future studies on the therapeutic use of melatonin in retinal degeneration.

electroretinogram | neuroprotection | visual sensitivity | glaucoma

Melatonin plays an important role in many aspects of mammalian neurobiology by acting via two types of G protein-coupled receptors (MT1 and MT2) that are negatively coupled with adenylyl cyclase (1). Many investigations have shown that retinal photoreceptors of vertebrates synthesize melatonin under the direct control of a circadian clock (2, 3). In a few cases, the circadian pacemaker driving rhythmic melatonin synthesis has been localized to the photoreceptors themselves (2, 4, 5). In the retina melatonin levels are high during the night and low during the day and exposure to light during the night induces a rapid and dramatic decrease in the levels of melatonin (6, 7). Retinal melatonin synthesis is directly controlled by the circadian clock via E-box mediated transcription (8) and via the gating of the cAMP signaling cascade (9).

Several studies have reported that melatonin modulates retinal functions and accumulating experimental evidence indicates an involvement of this hormone in retinal pathologies (reviewed in refs. 10 and 11).

The role played by specific melatonin receptors in the retina is not well defined. Melatonin receptors are expressed by several retinal cell types, including the photoreceptors (12, 13), suggesting that melatonin receptors may be involved in the regulation of photoreceptor physiology. Indeed, previous studies have reported that melatonin influences the membrane conductance of dark adapted frog photoreceptors (14), acts directly on the rod photoreceptors to increase dark adaptation (15), and potentiates rod signals to ON type bipolar cells in fish retina (16). Melatonin is also

involved in the modulation of the electroretinogram of the green iguana (17), chicken (18), and, possibly, man (19).

No previous study has investigated the functional roles of specific melatonin receptors in the mouse retina. This lack of data are because the vast majority of mouse strains do not produce melatonin (20) and many that do so carry a mutation that leads to the rapid degeneration of photoreceptors (e.g., C3H *rd1*). In the present study, we have crossed mice with targeted deletion of the *MT1* melatonin receptor gene (MT1<sup>-/-</sup> mice) (21) onto the C3H<sup>+/+</sup> background (20). The latter make melatonin but do not develop retinal degeneration (22, 23). We then investigated the effect of disruption of the *MT1* gene on retinal structure and function.

## Results

**Distribution of MT1 Receptors mRNA in the Mouse Retina.** *MT1* transcripts were detected in the outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL) by fluorescent in situ hybridization. *MT1* mRNA was abundant in the ONL, the retinal layer containing the nuclei of the photoreceptor cells, and the INL, where the cell bodies of horizontal, bipolar, amacrine, and Müller cells are located (Fig. 1A). In contrast, the *MT1* transcript level was low in the GCL (Fig. 1A). No significant signal was detected in sections treated with the sense probe (Fig. 1B). To corroborate the results obtained by in situ hybridization with an independent technique, we performed RT-PCR with cells obtained from the various retinal layers using laser capture microdissection (LCM) (3, 8). A typical microdissection of a retinal section is shown in Fig. 1C. *MT1* mRNA was amplified from cells captured in the ONL and INL; a faint band was also observed in GCL (Fig. 1D).

**Dark-Adapted Electroretinogram.** The electroretinogram (ERG) is a powerful tool to noninvasively study the response of the retina to a flash of light. The major components of the ERG are the a-wave and the b-wave, which represent the activation of the photoreceptors and the depolarizing second order neurons (on bipolar cells) respectively. The amplitudes and implicit times of the a-wave and b-wave are used to quantify the retinal light response (24, 25). Fig. 2 shows typical traces for the dark-adapted ERG recorded with our experimental protocol under different luminance levels. At the lowest luminance levels this ERG reflects the activity of the rod pathway, whereas at the higher intensities, both the cone and the rod systems may contribute to the response. Consistent with previous reports (24), the ERG waveform showed a clear dependence on the luminance levels; the amplitude of the a- and b-wave

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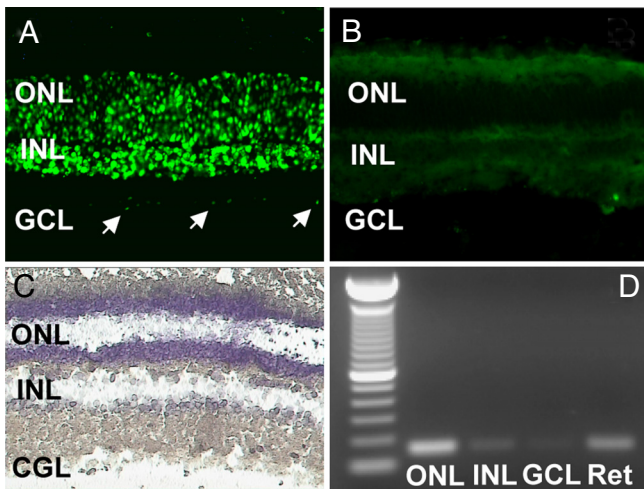
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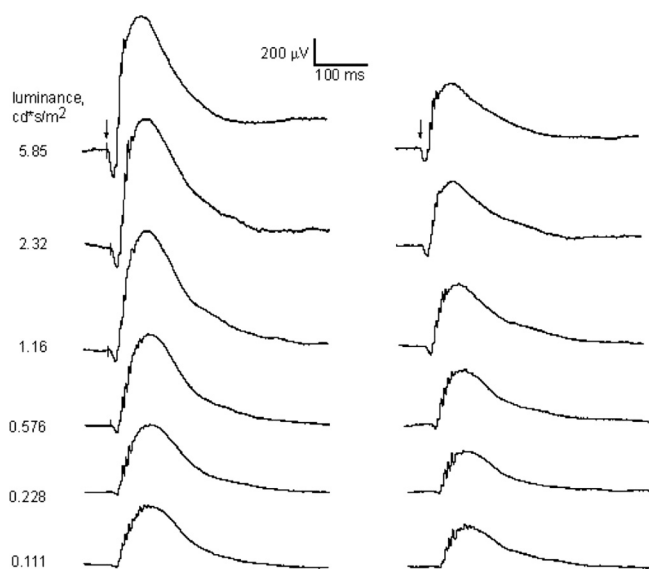
This article contains supporting information online at [www.pnas.org/cgi/content/full/0904400106DCSupplemental](http://www.pnas.org/cgi/content/full/0904400106DCSupplemental).



**Fig. 1.** Localization of *MT1* transcripts in the retina determined by in situ hybridization using a fluorescein-labeled probe. (A) The antisense probe detected a clear signal in the outer nuclear layer (ONL) and inner retinal layer (INL); a faint signal was also present in some ganglion cells (GCL, arrows). (B) No signal was detected using the sense probe. (C) Laser capture microdissection (LCM) of individual cell layers from the mouse retina. (D) RT-PCR generated amplicons of *MT1* receptor mRNA in the outer nuclear layer (ONL), in the inner nuclear layer (INL), and in the ganglion cell layer (GCL) collected with LCM. The RT-PCR products had the predicted size (150 bp).

significantly increased with the increase in the luminance levels, whereas the implicit times decreased. In C3H/f<sup>+/+</sup> wild-type mice, the amplitudes of the a- and b-wave were significantly greater at midnight than at midday (two-way ANOVA,  $P < 0.001$ ) (Fig. 3), but not in C3H f<sup>+/+</sup> MT1<sup>-/-</sup> mice (two-way ANOVA,  $P > 0.1$ , Fig. 3). The implicit times of the a- and b-wave also showed a clear dependence on the time of the day (shorter implicit time at midnight than at midday), but the genotype did not significantly affect the implicit time (two-way ANOVA,  $P > 0.05$ ) (Fig. 3).

**Effects of Melatonin Injection on the Dark-Adapted ERGs.** We tested whether i.p. injection of melatonin (1 mg/kg) during the day (ZT6)



**Fig. 2.** Representative trace of dark-adapted ERG in C3H/f<sup>+/+</sup> (Left) and C3H/f<sup>+/+</sup> MT1<sup>-/-</sup> (Right) at the seven different luminance levels recorded at night (ZT18). A clear reduction in the amplitude of the a- and b-waves is present in C3H/f<sup>+/+</sup> MT1<sup>-/-</sup>.

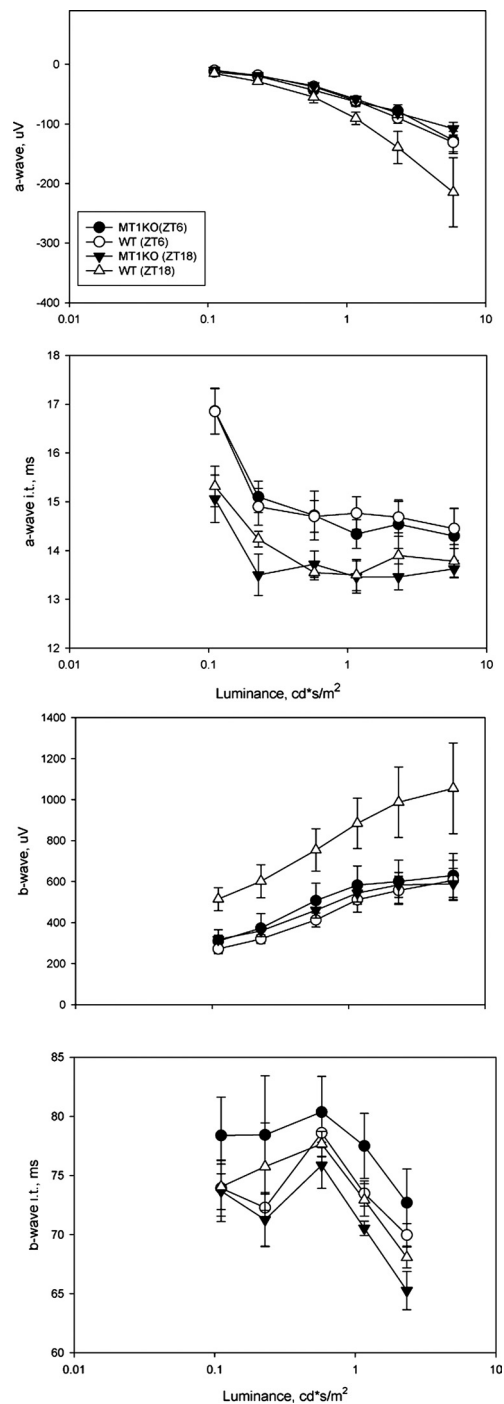
would affect the dark-adapted ERG. C3H/f<sup>+/+</sup> and C3H/f<sup>+/+</sup> +MT1<sup>-/-</sup> mice were injected with melatonin and dark adapted for 1 h before recording the ERG. We found that melatonin injected during the daytime significantly increased the amplitude of the a- and b-wave in C3H/f<sup>+/+</sup> mice compared with saline injected control mice (two-way ANOVA,  $P < 0.01$ ) (Fig. 4). Melatonin injection did not produce any changes in the ERG of MT1<sup>-/-</sup> mice (two-way ANOVA,  $P > 0.05$ , Fig. 4). The implicit times were not affected by melatonin in either genotype (two-way ANOVA,  $P > 0.05$  in all cases). We then tested whether the scotopic threshold response was also affected by melatonin injection using a protocol similar to those used in previous studies (24, 25). As observed above, melatonin injection in C3H/f<sup>+/+</sup> significantly enhanced the amplitude of the b-wave with respect to the values measured in vehicle injected mice (two-way ANOVA,  $P < 0.001$ , Fig. 5). C3H/f<sup>+/+</sup> mice injected with melatonin had a lower scotopic response threshold ( $-4.6 \log \text{cd}^* \text{s/m}^2$ ) than vehicle injected ( $-3.6 \log \text{cd}^* \text{s/m}^2$ ). Melatonin injection did not affect the scotopic response threshold in C3H/f<sup>+/+</sup> +MT1<sup>-/-</sup> (two-way ANOVA,  $P > 0.1$ ) (Fig. 5) further suggesting that the effect of melatonin on the modulation of visual sensitivity is mediated by MT1 receptors. Similar data were obtained when the experiment was performed in the middle of the night (ZT18, two-way ANOVA,  $P < 0.05$ ) (Fig. S1).

**Effect of Melatonin Injection on the Photopic ERGs.** The effects of melatonin injection on the photopic ERGs were investigated using a protocol similar to that used by Cameron et al. (24). ERGs from C3H/f<sup>+/+</sup> and MT1<sup>-/-</sup> mice were recorded after 2.5, 5, 10, and 15 min of rod saturating background light exposure. In C3H/f<sup>+/+</sup> mice, melatonin injection increased the amplitude of the b-wave whereas no significant effect was observed in C3H/f<sup>+/+</sup> MT1<sup>-/-</sup> (two-way ANOVA,  $P < 0.001$  and  $P > 0.1$  respectively, Fig. 6). The implicit time of the b-wave was slightly but significantly reduced in C3H/f<sup>+/+</sup> (two-way ANOVA,  $P < 0.05$ ) (Fig. 6).

Melatonin injection at ZT 18 did not affect the photopic ERGs (b-wave amplitude and implicit time) in C3H/f<sup>+/+</sup> or in C3H/f<sup>+/+</sup> +MT1<sup>-/-</sup> (two-way ANOVA,  $P > 0.1$ ) (Fig. S2).

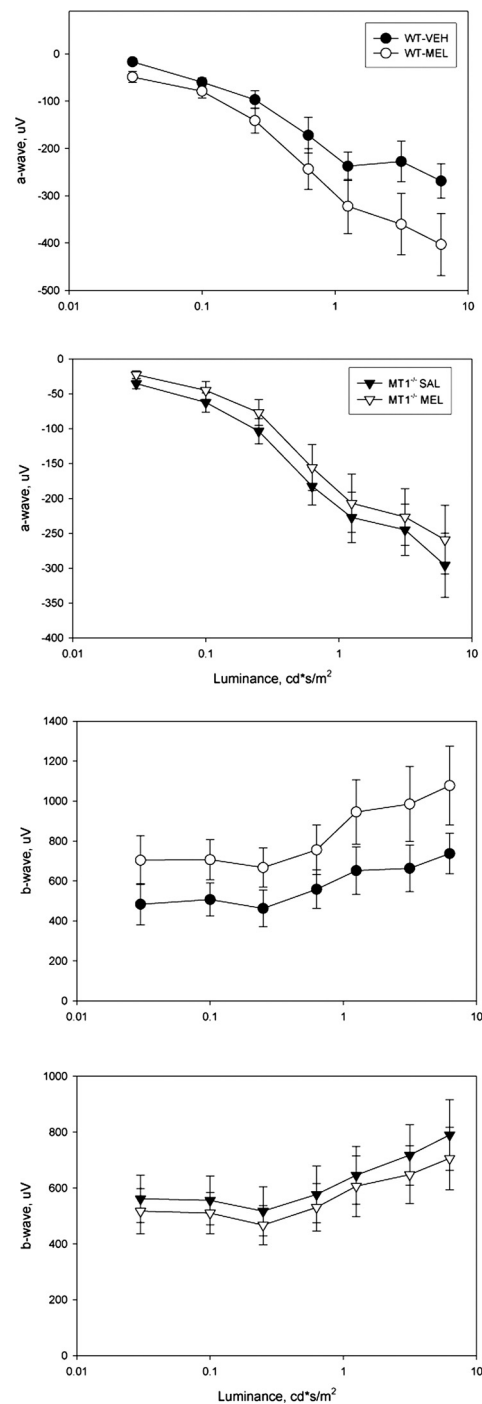
**Effect MT1 Removal on Retinal Circuitry.** No major alterations in retinal morphology were evident between C3H/f<sup>+/+</sup> and C3H/f<sup>+/+</sup> +MT1<sup>-/-</sup> at 3 months of age. Major retinal cell types, including rods and cones (Fig. 7 A, B, and E), horizontal cells (Fig. 7 F and G), rod and cone bipolar cells (Fig. 7 C and D), various types of amacrine cells (Fig. 7 F and G), such as TH-positive amacrine cells (Fig. 7 I), and ganglion cells (Fig. 7 G), and Müller glia and astrocytes (Fig. 7 A and B), were visualized with specific antibodies (Table S1) and appeared normal in morphology, number, pattern of stratification, and overall architecture, conforming to published literature (26). Synaptic markers, and namely mGluR6, PSD95, Bassoon, Kinesin II and synaptophysin were normally distributed in the two plexiform layers of both genotypes (Fig. 7 A–D, F, H, and I). Local signs of focal rearrangements (i.e., formation of rosettes, a common finding in retinal reorganization) or signs of glial reactivity in Müller cells and astrocytes, also frequently reported in a variety of mutants and KO mice (27) were absolutely undetected in the strains under use.

**Effect of MT1 Removal on Retinal Cell Viability During Aging.** A detailed morphometric investigation of the retina was performed using light microscopy in C3H/f<sup>+/+</sup> and the C3H/f<sup>+/+</sup> MT1<sup>-/-</sup> mice at three different ages (Figs. 8 and 9). The statistical analysis indicated a significant effect of the age and strain for many of the parameters examined (two-way ANOVA,  $P < 0.05$ ). No significant differences were observed at 3 months of age between the C3H/f<sup>+/+</sup> and C3H/f<sup>+/+</sup> +MT1<sup>-/-</sup> in any of the morphometric parameters investigated (two-way ANOVA,  $P > 0.1$  in all cases) (Fig. 9). At 12 months of age, we observed a significant reduction in the number of photoreceptor cells (Holm-Sidak test,  $P < 0.05$ ) in C3H/f<sup>+/+</sup> MT1<sup>-/-</sup> with respect to



**Fig. 3.** Quantification of dark-adapted ERG responses to flashes of light recorded in the middle of the day (ZT6) and in the middle of the night (ZT18). Mice (3–4 months old) were dark-adapted for at least 30 min before the recordings were performed. Data are presented as mean  $\pm$  SEM;  $n = 4$ –6 for each time point and genotype. Two-way ANOVA indicated a statistically significant contribution of the time of day for the a-wave ( $P < 0.05$ ) and b-wave ( $P < 0.001$ ) amplitudes and for genotype ( $P < 0.005$ , a-wave;  $P < 0.001$ , b-wave). The implicit time of the a-wave and b-wave was also affected by the time of the day ( $P < 0.001$  in both cases), but not by the genotype ( $P > 0.1$  and  $P > 0.1$ , respectively).

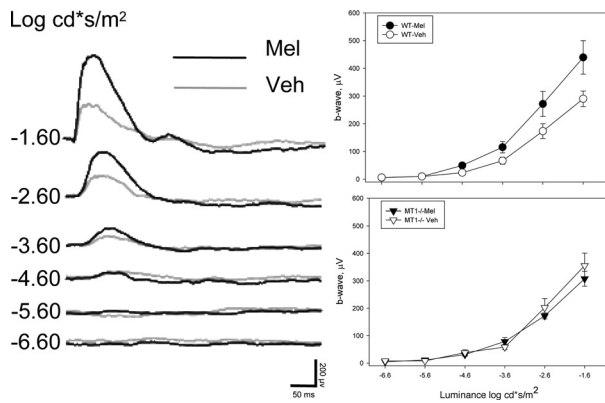
C3H/ $f^{+/+}$  at the same age. All other parameters were not significantly different (two-way ANOVA,  $P > 0.1$  in all cases). At 18 months of age, the differences between the C3H/ $f^{+/+}$  and the C3H/ $f^{+/+}$ MT1 $^{-/-}$  retinas were quite remarkable. The length of the inner segments (IS), outer segments (OS), and the number of photoreceptor cells and cells in the



**Fig. 4.** Quantification of dark-adapted ERG responses to flashes (0.11–5.85  $\text{cd}^*\text{s}/\text{m}^2$ ) of light recorded after 1 h of dark adaptation and i.p. injection of melatonin (1 mg/kg) or vehicle in the middle of the day (ZT6). Data are presented as mean  $\pm$  SEM;  $n = 4$ –6 for each time point and genotype. In C3H/ $f^{+/+}$  mice melatonin injection induced a significant increase in the amplitude of the a-wave and b-wave (two-way ANOVA,  $P < 0.001$  in both cases). In C3H/ $f^{+/+}$ MT1 $^{-/-}$  mice melatonin injection did not affect the amplitude of the a-wave or b-wave (two-way ANOVA,  $P > 0.1$ , a-wave; and  $P > 0.1$ , b-wave). Mice were 3–4 months old at the time of the experiment.

GCL of C3H/ $f^{+/+}$ MT1 $^{-/-}$  retinas were significantly reduced with respect to the values measured at 3 months of age and with respect to the values in age matched C3H/ $f^{+/+}$  mice (Holm-Sidak test,  $P < 0.05$  in all cases). No significant differences were observed in the number of cells in the inner nuclear layer (two-way ANOVA,  $P > 0.1$ ) (Fig. S3).





**Fig. 5.** Representative traces of responses in 3- to 4-month-old C3H/f<sup>+/+</sup> mice injected with melatonin or vehicle (*Left*). Quantification of the dark-adapted response of the b-wave to flashes of light ( $-6.6$  to  $-1.6$  log cd\*s/m<sup>2</sup>; 0.11 5 cd\*s/m<sup>2</sup>) recorded after 1 h of dark adaptation and intraperitoneal injection of melatonin (1 mg/kg) or vehicle in the middle of the day (*Right*). Data are presented as mean  $\pm$  SEM;  $n = 5-8$  for each time point and genotype. In C3H/f<sup>+/+</sup> mice, melatonin injection induced a significant reduction in the scotopic threshold response and increased the amplitude of the b-wave (two-way ANOVA,  $P < 0.001$ ). In C3H/f<sup>+/+</sup>MT1<sup>-/-</sup> mice, melatonin injection did not affect the scotopic threshold response or the amplitude of the b-wave (two-way ANOVA,  $P > 0.1$ ).

## Discussion

The data obtained in the current study indicate that *MT1* receptors are widely distributed within the murine retina (Fig. 1). The distribution of *MT1* mRNA that we have observed is consistent with data reported in the literature in other mammalian species (12, 13).

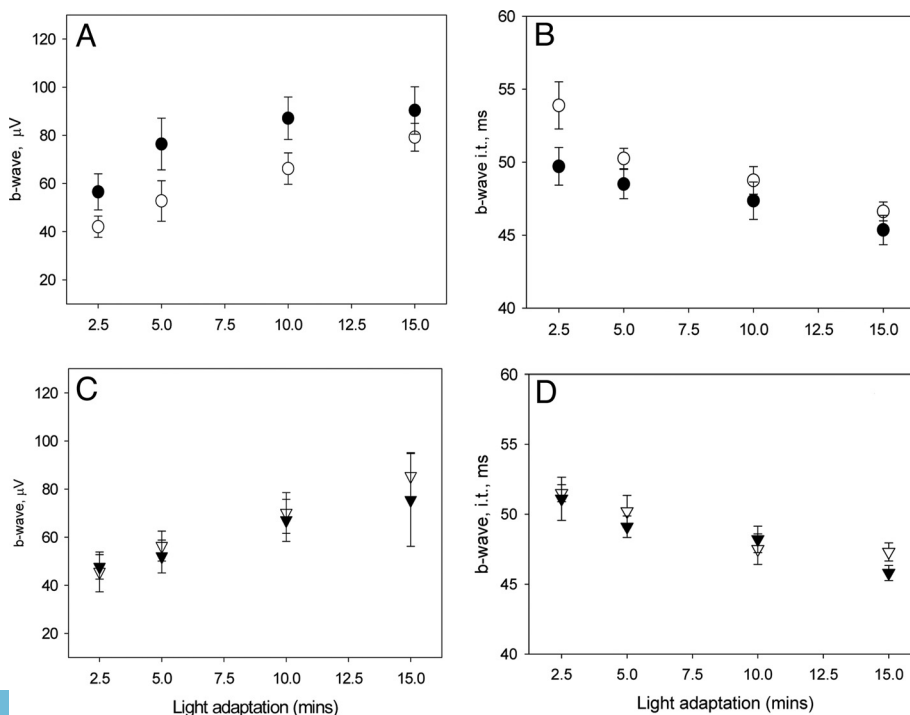
The fact that *MT1* transcripts are localized on photoreceptors may suggest that melatonin is involved in the modulation of visual function, and consistent with this hypothesis, our data indicate that melatonin and *MT1* receptors are involved in the modulation of visual sensitivity. In C3H/f<sup>+/+</sup> mice, the dark-adapted ERG had significantly larger amplitudes of the a- and b-waves at night (when melatonin levels are high) than during the middle of the day (Fig.

3). This diurnal difference was not present in the C3H/f<sup>+/+</sup>MT1<sup>-/-</sup> animals, suggesting that the observed increase in the amplitude is due to the action of melatonin via *MT1* melatonin receptors. Although it is possible that the difference in the amplitude of the a-wave and b-waves measured in the middle of the day and in the middle of the night in C3H/f<sup>+/+</sup> mice may be due in part to the difference in the length of the dark adaptation (30 min vs. 6 h), we believe that this contribution is negligible since dark adaptation processes are largely complete within 30 min (28). This conclusion is supported by the analysis of melatonin injections on ERG responses after a short period of dark adaptation at midday.

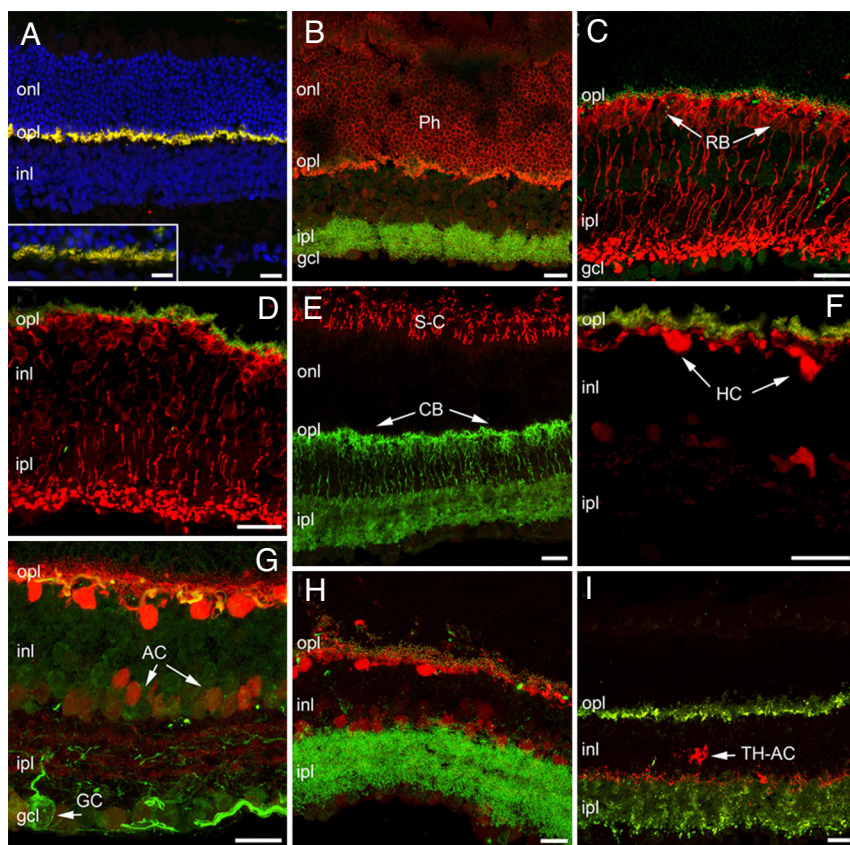
Administration of exogenous melatonin (1 mg/kg) during the daytime increased the amplitude of the a- and b-wave to levels similar to those observed during the night in C3H/f<sup>+/+</sup> but not in MT1<sup>-/-</sup> mice, providing direct evidence that melatonin modulates ERG responses of the mouse retina (Figs. 3 and 4). Additional evidence that melatonin increases the light sensitivity of the retina comes from the observation that melatonin injection lowers the scotopic threshold (Fig. 5). These results are consistent with previous reports in the green iguana, chicken, and man (17–19) and expand those findings by demonstrating that melatonin acts via *MT1* receptors. Our data also indicate that melatonin can affect the amplitude of the photopic ERG response in an *MT1* receptor-dependent manner (Fig. 6). It is not known whether these effects on ERG responses are due to the action of melatonin on photoreceptors, inner retinal circuitry, or both. However, the enhanced a-wave amplitudes in dark adapted mice after melatonin injection implicate a photoreceptor site of action for at least part of the response.

A previous study has reported that *MT1* receptor mRNA expression in the rat retina peaked at embryonic day 16 and then decreased reaching the adult level at postnatal day 14, suggesting these receptors may have a role in retinal development (29). Our data do not support such a conclusion since in young (3-month-old) C3H/f<sup>+/+</sup>MT1<sup>-/-</sup> the pattern of stratification and overall architecture of the retina is similar to that of age matched C3H/f<sup>+/+</sup> and conforming to published literature (26).

Experimental evidence suggests a possible influence of melatonin in photoreceptor degeneration. In albino rats, administration of



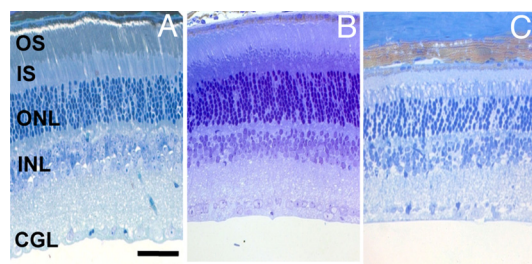
**Fig. 6.** Quantification of light-adapted ERG responses to flashes of light recorded after 1 h of dark adaptation and i.p. injection of melatonin (1 mg/kg) in the middle of the day (ZT6). Data are presented as mean  $\pm$  SEM;  $n = 4-8$  for each time point and genotype. In C3H/f<sup>+/+</sup> mice (A and B) melatonin injection induced a significant increase in the amplitude of the b-wave (two-way ANOVA,  $P < 0.001$  in both cases) and a reduction in the implicit time (two-way ANOVA,  $P < 0.05$ ). In C3H/f<sup>+/+</sup>MT1<sup>-/-</sup> mice (C and D) melatonin injection did not affect the amplitude of the b-wave or the implicit time (two-way ANOVA,  $P > 0.1$ , b-wave;  $P > 0.1$ , implicit time). Mice were 3–4 months old at the time of the experiment.



**Fig. 7.** Immunofluorescence staining of vertical retinal sections from C3H/f<sup>+/+</sup>MT1<sup>-/-</sup> mice at 3 months, showing a normal complement of cell types and retinal lamination. (A) TO-TO-3 nuclear staining (blue) shows normal retinal layering. mGluR6 (red) and PSD95 (green-yellow) are normally distributed in the outer plexiform layer (opl), as also shown at higher magnification (*Inset*). (B) Recoverin staining (red) of photoreceptor (Ph) outer segments and cell bodies. Bassoon staining (green) shows the characteristic pattern of semicircular, punctate structures in the opl, associated to photoreceptor synaptic ribbons. (C) Protein kinase C $\alpha$  antibodies (red) show rod bipolar cells (RB) with a normal complement of kinesin II-positive puncta (green) decorating their dendritic tips. (D) RB cells (labeled red by PKC $\alpha$  antibodies are postsynaptic to rod spherules, labeled green by PSD95. (E) Antibody against 5-cone opsin (red) reveals a normal pattern of cones (dorsal retinal quadrant); Go $\alpha$  (green) shows the morphology of ON-bipolar cells, including cone bipolars (CB). (F) Calbindin staining (red) of horizontal cells (HCs). A normal complement of amacrine cells (AC) are also more weakly stained. PSD95-positive photoreceptor terminals in the opl are appropriately apposed to horizontal cell dendrites. (G) Calbindin staining of HC cell bodies (red) combined with associated neurofilament staining of their axonal arborizations (green). Neurofilament labels ganglion cells in their entirety. One large-size ganglion cell is indicated (GC). (H) Calbindin staining (red) associated to bassoon show that dendrites of HCs are appropriately decorated by ribbons at their presynaptic sites in the opl. Some dendritic sprouting, also observed in the C3H/f<sup>+/+</sup> retina, is visible in the opl. (I) Antibodies against tyrosine hydroxylase show a population of dopaminergic amacrine cells (TH-ACs). Their typical pattern of stratification, mostly visible in sublamina 1 of the ipl and in the opl, is indistinguishable from the C3H/f<sup>+/+</sup> counterpart. Synaptophysin vesicle staining (green) shows a normal distribution in photoreceptor terminals in the opl and throughout the ipl, as expected. (Scale bars, 20  $\mu$ m.)

exogenous melatonin increases light-induced photoreceptor degeneration (30) and intraocular injection of luzindole, a melatonin receptor antagonist, decreases light damage (25). Although melatonin appears to enhance light damage, it may have neuroprotective effects in other models of photoreceptor degeneration. In mice carrying a genetic defect that causes the degeneration of the rod photoreceptors (*rds/rds*), daily injections of melatonin significantly delayed photoreceptors loss, suggesting that melatonin could be of potential use for treatment of this form of retinal degeneration (31). Our data indicate that melatonin via MT1 receptors may indeed increase photoreceptor viability during aging (Figs. 8 and 9).

A role for melatonin in protecting against the development of glaucoma and in lowering the intraocular pressure has been also proposed (10–11). Indeed, our data showing that in C3H/f<sup>+/+</sup>MT1<sup>-/-</sup> mice have a significant reduction in the number of ganglion cells (Figs. 8 and 9) with respect to age matched C3H/f<sup>+/+</sup> mice reinforce the idea that melatonin acting via MT1 receptors protects ganglion cells from degeneration.

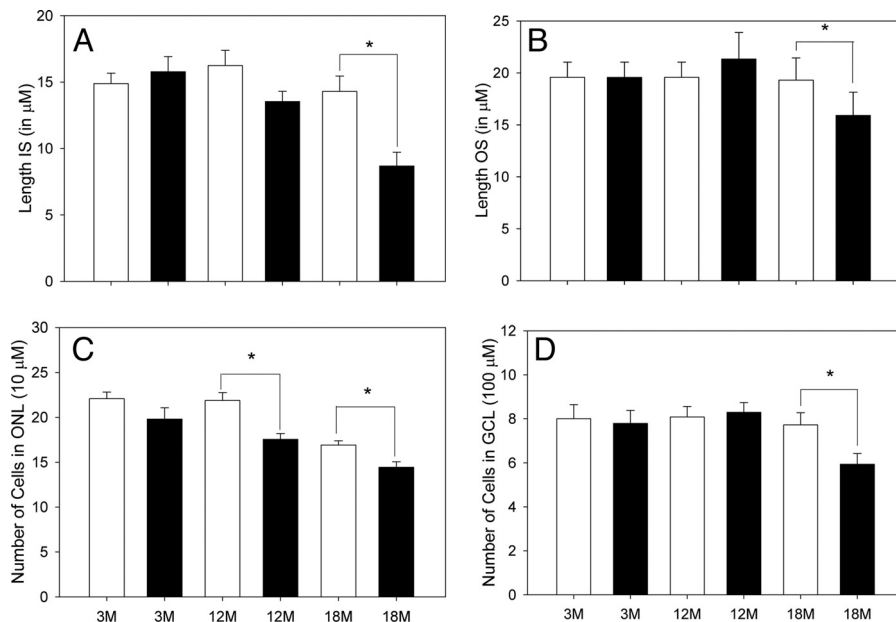


**Fig. 8.** Photomicrographs of retinas from C3H/f<sup>+/+</sup>MT1<sup>-/-</sup> mice. Mice were 3 (A), 12 (B), and 18 (C) months of age. (Scale bars, 50  $\mu$ m.)

Melatonin may also protect photoreceptors and ganglion cells during aging by acting as antioxidant as well via its action on MT1 receptors. Indeed, previous studies have shown a possible role for retinal melatonin as a free radical scavenger within photoreceptors. In isolated photoreceptors from frog retina, melatonin was approximately 100 times more potent than vitamin E in inhibiting light-induced oxidative processes (32) and melatonin can reduce the lipid peroxidation induced by nitric oxide in rat retinal homogenates (33). However, supraphysiological concentrations of melatonin may be needed for the antioxidant actions, and MT1 receptor activation appears to play a dominant role in maintaining cell viability. Indeed recent clinical data indicate that melatonin levels are significantly lower in patients affected by age-related macular degeneration (AMD) thus suggesting a possible role of melatonin in the etiology of AMD (34). Our data indicate that activation of MT1 receptors via specific agonists may provide a tool to treat and possibly prevent AMD. The mechanisms whereby MT1 receptor activation delays age-related retinal cell death are yet to be determined.

In conclusion, our data indicate that melatonin, via MT1 receptors, plays an important role in the modulation of retinal function at night, optimizing the performance of the visual system in this nocturnal animal. Because both a-wave and b-wave amplitudes are affected, this modulation may be initiated at the level of the photoreceptor cells, consistent with the expression of MT1 mRNA by these primary sensory neurons. This fine tuning of the visual system performance can provide a clear evolutionary advantage for a nocturnal animal under natural conditions. Our study also indicates that melatonin and MT1 receptors increase photoreceptor and ganglion cell viability during aging. These data provide direct experimental evidence in support of the functional significance of melatonin and MT1 receptors in the mammalian retina. They also form the basis for future studies on the therapeutic use of melatonin in retinal diseases associated with decreased retinal sensitivity to light and degeneration of retinal cells, such as that in age-related macular degeneration or glaucoma.





**Fig. 9.** Morphometric analysis of retinas of C3H/f<sup>+/+</sup> (white bars) and C3H/f<sup>+/+</sup>MT1<sup>-/-</sup> (black bars) at the three different ages. In panels A–D are shown the results obtained by measuring the length of the rod outer segment (OS) and inner segment (IS), counting the total number of photoreceptor nuclei (ONL) and ganglion cells in the central superior retina. A significant change in the length of the OS, IS, and in the number of cells in the ONL and GCL is present between C3H/f<sup>+/+</sup> and C3H/f<sup>+/+</sup>MT1<sup>-/-</sup> at 18 months of age. In both genotypes, the number of cells in the ONL showed a significant reduction with aging (two-way ANOVA,  $P < 0.05$ ). Each bar, the mean  $\pm$  SEM;  $n = 4–6$ . \*,  $P < 0.05$  (Holm-Sidak test).

## Methods

**Animals.** C3H MT1<sup>-/-</sup> knock-out mice homozygous for the *rd1* mutation, generously donated by Drs. Reppert and Weaver (University of Massachusetts Medical School), were back-crossed with C3H/f<sup>+/+</sup> mice in which the *rd1* mutation has been removed to produce C3H/f<sup>+/+</sup>MT1<sup>-/-</sup>.

**In Situ Hybridization and Immunocytochemistry (ICCH).** Details about the procedures used for in situ hybridization and ICCH are described in detail in published work (26, 27). For additional details, see [SI Methods](#) and [Table S1](#).

**Laser Capture Microdissection (LCM) of Retinal Layers and RT-PCR Analysis.** Tissue preparation and RT-PCR were performed as described (3, 9).

**Electroretinogram (ERG).** Dark adapted electroretinogram was performed as described in previously published work (24, 25) and detailed in [SI](#).

**Retinal Morphology.** Mice were euthanized, and before eye nucleation the superior cornea was marked with a hot needle.

**Supporting Information.** For more methods see [SI Text](#).

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