Suppression of Melatonin Secretion in Totally Visually Blind People by Ocular Exposure to White Light

Clinical Characteristics

Joseph T. Hull, PhD,1,2,3 Charles A. Czeisler, PhD, MD,1,2 Steven W. Lockley, PhD1,2

Purpose: Although most totally visually blind individuals exhibit nonentrained circadian rhythms due to an inability of light to entrain the circadian pacemaker, a small proportion retain photic circadian entrainment, melatonin suppression, and other nonimage-forming responses to light. It is thought that these responses to light persist because of the survival of melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs), which project primarily to the circadian pacemaker and are functionally distinct from the rod and cone photoreceptors that mediate vision. We aimed to assess the integrity of nonimage-forming photoreception in totally visually blind patients with a range of ocular disorders.

Design: Within-subject, dark-controlled design.

Participants: A total of 18 totally visually blind individuals (7 females; mean age ± standard deviation = 49.8 ± 11.0 years) with various causes of blindness, including 3 bilaterally enucleated controls.

Methods: Melatonin concentrations were compared during exposure to a 6.5-hour bright white light (7000 lux) with melatonin concentrations measured 24 hours earlier at the corresponding clock times under dim-light (4 lux) conditions.

Main Outcome Measures: Area under the curve (AUC) for melatonin concentration.

Results: Melatonin concentrations were significantly suppressed (defined as ≥33% suppression) during the bright-light condition compared with the dim-light condition in 5 of 15 participants with eyes (retinitis pigmentosa, n = 2; retinopathy of prematurity [ROP], n = 2; bilateral retinal detachments, n = 1). Melatonin concentrations remained unchanged in response to light in the remaining 10 participants with eyes (ROP, n = 3; optic neuritis/neuropathy, n = 2; retinopathy unknown, n = 2; congenital glaucoma, n = 1; congenital rubella syndrome, n = 1; measles retinopathy, n = 1) and in all 3 bilaterally enucleated participants.

Conclusions: These data confirm that light-induced suppression of melatonin remains functionally intact in a minority of totally visually blind individuals with eyes. None of the bilaterally enucleated individuals or those with phthisis bulbi was responsive to light; of the remainder, half were responsive to light. Although inner retinal damage is associated with a high likelihood that nonimage-forming photoreception is absent, the impact of outer retinal damage is more ambiguous, and therefore the assessment of the presence, attenuation, or absence of nonimage-forming light responses in totally blind patients requires careful individual confirmation and cannot simply be assumed from the type of blindness. Ophthalmology 2018;125:1160-1171 © 2018 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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Appropriately timed ocular exposure to light inhibits pineal melatonin secretion in humans.1–3 This response is mediated exclusively by the eyes via the suprachiasmatic nuclei (SCN), the site of the circadian pacemaker, which receives photic input from the retina via the retinohypothalamic tract (RHT).4–5 Efferents from the SCN project to the pineal via the superior cervical ganglion and are required for both the synthesis and light-induced suppression of melatonin. Photic transmission via the RHT-SCN-pineal pathway originates from a subset (~0.5%) of intrinsically photosensitive retinal ganglion cells (ipRGCs) containing the photopigment melanopsin.6,7 These ipRGCs are distributed widely across the retina and project to the ventromedial region of the hypothalamic SCN.8–13

The functional integrity of the retina-RHT-SCN-pineal pathway in visually blind humans can be determined by assessing whether ocular light exposure (LE) can suppress melatonin secretion.14–16 Bilateral lesions of the neural...
pathway between the retina and the SCN, for example, via severing the optic nerve or enucleation, abolish light-induced suppression of melatonin production or photic entrainment of the circadian clock. Consequently, the majority of totally visually blind people (those without conscious light perception) cannot maintain circadian entrainment to the 24-hour light-dark cycle and exhibit non–24-hour sleep-wake rhythm disorder. Classic rod and cone photoreceptors can contribute, but are not required, to mediate melatonin suppression or other “nonimage-forming” responses to light such as circadian phase resetting. The specificity of this nonvisual pathway is underscored by observations that a small fraction of totally visually blind individuals can maintain melatonin suppression and circadian entrainment to light in the absence of light perception and negative neuro-ophthalmologic tests, confirming that the retina-SCN-pineal axis is functionally intact. In addition, wavelength-dependent melatonin suppression has been demonstrated in a blind individual, with substantial suppression during 460 nm blue LE, and no suppression at 555 nm, consistent with a melanopsin-mediated, and not photopic, response. Similar blue-light sensitivity has been shown for pupillary reflex, unconscious “visual” perception and activation of brain areas associated with alertness and mood in such totally visually blind but circadian photoreception intact blind patients.

Given the rarity of totally visually blind patients who retain melatonin suppression, circadian entrainment or pupillary responses to light (only 7 such patients reported in the literature to date; subject codes are from Brigham and Women’s Hospital); Czeisler et al in 1995 (n = 3; nos. 1053, 1155, and 1459); Klerman et al in 2002 (n = 1; no. 1492); Zaidi et al in 2007 (n = 2; no. 22CS and female); Vandewalle et al in 2013 (n = 1, nos. 2811, 1492, and 22CS), we aimed to examine the prevalence of a positive melatonin suppression response in a larger cohort and examine the relationship between the cause of blindness and retention of functional nonimage-forming responses to ocular LE.

Methods

Study Participants

Case histories, ophthalmological examinations, and a melatonin suppression test were performed in 18 healthy, blind patients, including 15 with at least 1 natural eye present (8 male, 7 female) and 3 bilaterally enucleated male patients (Table 1). The test was performed within a broader research study of circadian rhythms in the blind, including assessment of circadian entrainment using field-based techniques. All but 5 of the participants had confirmed non–24-hour rhythms in urinary 6-sulphatoxymelatonin (aMT6s) (24.10–24.79 hours). The other 5 individuals had confirmed 24-hour rhythms in aMT6s (1492, 22CS, 23CM, 2740, 2811). Four of the 18 participants tested were assessed for melatonin suppression on 2 or more occasions (1451, 1492, 22CS, 23CM). Some results have been reported previously in 4 participants (1451, 1492, 22CS, 2811; participant 1451 [with eyes present] was reported as participant no. 9 in Czeisler et al in 1995, as 1451 in Klerman et al in 1998, as and participant no. 2 in Klerman et al in 2002; participant 1492 was reported as participant no. 5 in Klerman et al in 2002, and participant no. 3 in Vandewalle et al in 2013; participant 22CS was reported as the single blind male participant in experiment no. 1 in Zaidi et al in 2007, as the single blind male participant in Gooley et al in 2012, and as participant no. 2 in Vandewalle et al in 2013; participant 2811 was reported as participant no. 1 in Vandewalle et al in 2013). Only the results from the most recent melatonin suppression assessment are included in this report.

Participants were recruited from the community via newspaper, newsletter, and radio advertisements placed through numerous institutions, associations, and groups for visually impaired and blind persons across North America. Each participant provided written informed consent before participating in both the screening procedures and inpatient protocol, and each participant was provided a copy of the text of the research study consent form in Braille, computer text, or audio CD format as requested. Institutional Review Board approval was obtained for all study procedures by the Partners Human Research Committee. The experiment was performed in accordance with the Declaration of Helsinki. Participants were required to be ambulatory, to be taking no prescription medications, and to have no major health, psychological, or neurologic disorders other than complete blindness and sleep disruption associated with their blindness. Participants were healthy based on a comprehensive physical and psychiatric examination, blood and urine tests, and electrocardiogram. Urine toxicology (i.e., for caffeine, nicotine, narcotics) tests confirmed that all participants were drug-free during screening and on admission to the inpatient study. Participants completed the Sleep Disorders Questionnaire. All were negative for risk of sleep apnea; 2 participants (23BP, 26Q5) met the criteria for narcolepsy risk; 2 (22A6, 26Q5) for sleep disruption associated with psychiatric disorders; and 7 (22J2, 27A8, 23BP, 2347, 27Q2, 26Q5, 2306) for nocturnal myoclonus, interpreted as general sleep interruption. Participants reported no history of working night shifts or long-distance travel across 1 or more time zones in the last 3 years before their screening.

Ophthalmologic Testing

On initial screening, participants reported having no conscious light perception. Participants were studied regardless of the cause of blindness (with the exception of cortical blindness), age and rapidity of vision loss, presence or absence of eyes, or self-reported sleep disturbances. An ophthalmologic assessment was performed in all blind participants with eyes before or after the inpatient study. As part of the eye examination, the following descriptions were noted for each eye: history of vision loss, description of appearance of globe/optics, ocular motility, a fundoscopic examination, and pupil reflex to light was examined with a slit lamp with the brightest light of an indirect ophthalmoscope. Visual evoked potential results were available in 9 of the 15 intact blind participants using a flash stimulus (hand-held strobeoscopic lamp) at 2 per second. Electroencephalography was recorded from Fz-Oz, Pz-Oz, Fz-O1, and Fz-O2. Each eye was tested individually in a darkened room during 2 separate trials by covering 1 eye during ocular exposure to the strobe flash stimulus. Sensitivity was 3 uv/cm, and the average peak-to-peak ambient noise level was 2 uv. For the 3 participants who were bilaterally enucleated, cause and history of blindness were obtained by self-report, and a physician confirmed the absence of eyes during the screening physical examination.

Inpatient Protocols

The protocol consisted of assessing melatonin concentrations under dim light conditions during a constant routine (CR) protocol and under bright light conditions during a LE protocol (Fig 1). The CR
<table>
<thead>
<tr>
<th>No.</th>
<th>Sub L.D.</th>
<th>Age, yrs/Sex</th>
<th>Cause of Blindness</th>
<th>Age (yrs) Vision Impaired</th>
<th>Age (yrs) LP Lost</th>
<th>Ocular Examination</th>
<th>View of Anterior Chamber</th>
<th>View of Fundus</th>
<th>Pupil Reflex to Light</th>
<th>Visual Evoked Potentials</th>
<th>Melatonin-Suppression Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22CS</td>
<td>56/M</td>
<td>Retinitis pigmentosa</td>
<td>35</td>
<td>35</td>
<td>OU: external adnexa clear and healthy, posterior subcapsular cataracts, cornea clear, pale discs, atrophy of RPE throughout fundi; OS: dense diffuse bone spicules, asteroid hyalosis, attenuated vessels, exotropia ~25–30 prism D</td>
<td>Yes (OU)</td>
<td>Yes (OU)</td>
<td>Not detectable</td>
<td>Not detectable</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>2811</td>
<td>66/F</td>
<td>Retinitis pigmentosa</td>
<td>20</td>
<td>58</td>
<td>OU: normal conjunctiva, cornea and anterior chamber, few pigmented cells in vitreous, IOL implant (some posterior opacification), aphakia, waxy pallor to optic nerve, bone-spicule pigmentation throughout retina, maculae showed chorioretinal degeneration and vessels severely attenuated, miotic pupils</td>
<td>Yes (OU)</td>
<td>Yes (OU)</td>
<td>Miotic (OU)</td>
<td>Not detectable</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>22J7</td>
<td>56/F</td>
<td>Retinal detachments</td>
<td>10</td>
<td>21</td>
<td>OS: cornea clear with scarring, nonreactive miotic pupil (1 mm), opaque membrane behind pupil OD: exophthalmic, phthisis bulbi, opaque cornea, cataract</td>
<td>Yes (OS); no (OD)</td>
<td>Yes (OS); no (OD)</td>
<td>Miotic (OS); pupil not observable (OD)</td>
<td>Not tested</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>1492</td>
<td>58/M</td>
<td>Retinopathy of prematurity</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>OU: moderate corneal opacities with mature cataracts, corneal clouding</td>
<td>No (OU)</td>
<td>No (OU)</td>
<td>Pupils not observable</td>
<td>Not tested</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>23CM</td>
<td>29/F</td>
<td>Retinopathy of prematurity</td>
<td>&lt;1</td>
<td>14</td>
<td>OS: phthisical, corneal scarring, band keratopathy, posterior synechia of iris to anterior capsule, mature cataract OD: prosthesis, enucleated age 14 due to glaucoma</td>
<td>Barely (OS); n/a (OD)</td>
<td>No (OS); n/a (OD)</td>
<td>Pupil not observable</td>
<td>Not detectable</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>22A6</td>
<td>48/M</td>
<td>Retinopathy of prematurity</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>OS: band keratopathy, corneal opacity OD: diffuse corneal opacity</td>
<td>No (OU)</td>
<td>No (OU)</td>
<td>Pupils not observable</td>
<td>Not tested</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>22J2</td>
<td>53/M</td>
<td>Retinopathy of prematurity</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>OU: band keratopathy OS: leukocoria, total synechia of iris OD: phthisis bulbi, opaque cornea</td>
<td>Barely (OS); no (OD)</td>
<td>No (OU)</td>
<td>Pupils not observable</td>
<td>Not tested</td>
<td>Negative</td>
</tr>
<tr>
<td>No.</td>
<td>Sub L.D.</td>
<td>Age, yrs/Sex</td>
<td>Cause of Blindness</td>
<td>Age (yrs) Impaired</td>
<td>Age (yrs) LP Lost</td>
<td>Ocular Examination</td>
<td>View of Anterior Chamber</td>
<td>View of Fundus</td>
<td>Pupil Reflex to Light</td>
<td>Visual Evoked Potentials</td>
<td>Melatonin-Suppression Result</td>
</tr>
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</tr>
<tr>
<td>8</td>
<td>27A8</td>
<td>56/F</td>
<td>Retinopathy of prematurity</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>OS: anterior synechiae&lt;br&gt;OD: mild phthisis bulbi, dense band, keratopathy, corneal neovascularization</td>
<td>No (OU)</td>
<td>No (OU)</td>
<td>Pupils not observable</td>
<td>Not detectable</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>23BP</td>
<td>33/M</td>
<td>Retinitis/retinopathy (measles)</td>
<td>2</td>
<td>12</td>
<td>OU: phthisis bulbi, dense corneal scarring with vascularization, marked exotropia&lt;br&gt;OD: cornea irregularly thinned nasally</td>
<td>No (OU)</td>
<td>No (OU)</td>
<td>Pupils not observable</td>
<td>Not detectable</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>2740</td>
<td>43/F</td>
<td>Retinopathy (congenital rubella syndrome) and glaucoma (17 yrs)</td>
<td>prenatal</td>
<td>~20</td>
<td>OU: phthisis bulbi, scarred and opacified cornea, dense cataracts, scleromalacia (sclera diffusely thin)</td>
<td>Yes (OU)</td>
<td>No (OU)</td>
<td>Pupils not observable</td>
<td>Not detectable</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>26Q5</td>
<td>39/F</td>
<td>Congenital glaucoma</td>
<td>&lt;1</td>
<td>7–8</td>
<td>No (OU)</td>
<td>No (OU)</td>
<td>Pupils not observable</td>
<td>Not detectable</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2347</td>
<td>68/M</td>
<td>Optic neuritis (spinal meningitis)</td>
<td>2</td>
<td>7–8</td>
<td>OU: phthisis bulbi, corneal surface completely scarred</td>
<td>No (OU)</td>
<td>No (OU)</td>
<td>Pupils not observable</td>
<td>Not tested</td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td>27Q2</td>
<td>43/F</td>
<td>Optic neuropathy w/moderate retinopathy</td>
<td>3</td>
<td>3</td>
<td>Yes (OU)</td>
<td>Yes (OU)</td>
<td>Not detectable</td>
<td>Not tested</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>26Q6</td>
<td>43/M</td>
<td>Cause unknown</td>
<td>15</td>
<td>19</td>
<td>Barely (OU)</td>
<td>No (OU)</td>
<td>Pupils not observable</td>
<td>Not detectable</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2805</td>
<td>63/M</td>
<td>Cause unknown; possibly due to inflammation of retinal/optic nerves (Beriberi disease)</td>
<td>3</td>
<td>56</td>
<td>No (OU)</td>
<td>No (OU)</td>
<td>Pupils not observable</td>
<td>Not detectable</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>
Table 1. (Continued.)

<table>
<thead>
<tr>
<th>No.</th>
<th>Sub I.D.</th>
<th>Age (yrs)/Sex</th>
<th>Cause of Blindness</th>
<th>Ocular Examination</th>
<th>Age (yrs) Vision Impaired</th>
<th>Age (yrs) LP Lost</th>
<th>Visual Field</th>
<th>Visual Evoked Potentials</th>
<th>Melatonin Suppression</th>
<th>Result</th>
<th>Visual Field</th>
<th>Visual Evoked Potentials</th>
<th>Melatonin Suppression</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>1451</td>
<td>40/M</td>
<td>Enucleation due to atrophia bulbi</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Negative</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>22K4</td>
<td>49/M</td>
<td>Enucleation due to ocular trauma</td>
<td>Negative</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Negative</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>2306</td>
<td>53/M</td>
<td>Enucleation due to retinoblastoma</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Negative</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

Note that 1 participant (23CM, no. 5) has 1 eye enucleated and 3 participants (1451, 22K4, and 2306; no. 16, 17, 18, respectively) are bilaterally enucleated. All other participants (n = 14) have 2 natural eyes present.

Constant Routine

Participants were assessed during a CR protocol. From scheduled wake time, participants remained awake in dim light (~0.0087 W/m² [~4 lux]) at 137 cm from the floor in the vertical plane and a maximum of 0.048 W/m² (~15 lux) at 187 cm from the floor in the horizontal plane) and semirecumbent in bed with the head of the bed raised at a 45° angle for up to 40 hours. Food and fluid were given in small isocaloric meals (150 mEq Na⁺/100 mEq K⁺ [±20%] controlled nutrient; 2000–2500 ml fluid isocaloric; basal energy expenditure × 1.4 activity for calculated diet) every hour starting 2 hours after scheduled wake time. Constant posture and wakefulness were maintained and verified by technicians who sat with the participant at all times. At the end of the CR, the head of the bed was lowered and participants were given 8-hour recovery sleep opportunity in darkness.

Bright Light Exposure

After a post-CR 8-hour recovery sleep opportunity, participants were scheduled to a 16-hour wake episode (LE day). Light exposure was required to be timed to coincide with release of melatonin. In an entrained individual, melatonin release would occur at night, and therefore scheduling of LE would be straightforward. In individuals with non–24-hour rhythms, however, ensuring that the light occurs when melatonin is released requires additional calculations. On the basis of previous experience, the dim light melatonin onset (DLMO) was expected to occur 6.75 hours before the fitted core body temperature (CBT) minimum (see “Assessment of CBT”). The study sleep–wake schedule was designed on the basis of the CBT rhythm of each individual such that the 6.5-hour bright LE (Lights On) was scheduled to start 4.75 hours after scheduled wake time, which was timed to coincide with the expected DLMO. This ensured that the LE occurred at a time of melatonin release. The duration of the bright LE for 1 participant (23BP, no. 9) was 10.75 hours (between 4.75 and 15.50 hours after scheduled wake time) as the result of CBT minimum calculation error.

Participants were seated 1.5 hours before the initiation of a 6.5-hour LE and remained seated for 9 hours. During the bright LE, participants remained seated and were instructed to keep their eyelids open. Participants wore clear U-Vex goggles during the bright LE to block any residual ultraviolet light (U-Vex, Boston, MA). All room contents were covered with white bed sheets. Participants were instructed by the technician to maintain a fixed or free gaze at predetermined, equal intervals during the bright LE (18 minutes fixed, 18 minutes free, 6 minutes fixed, 6 minutes free, 6 minutes fixed, 6 minutes free, repeated hourly). During the fixed gaze intervals, participants were instructed to orient their face forward in the same direction that they were sitting with their chin slightly elevated upward and to refrain from turning their head to the left or right.
to the right or up or down. During the free-gaze interval of the bright LE, participants were allowed to move their heads in any direction. The technician sitting in the room during the LE session was instructed not to discuss light levels with the participant.

Lighting Conditions

Ambient room lighting was generated using standard, broad-spectrum ceiling-mounted fluorescent lamps (4100K, Philips Lighting, the Netherlands) controlled with digital ballasts and transmitted through an ultraviolet-stable filter (Lextran 9030 with prismatic lens; GE Plastics, Pittsfield, MA). Daily light intensity readings were taken and recorded by technicians approximately 2 hours after scheduled wake time to verify predetermined light settings in the room for CR (days 3–4, 4–5 or 34–35) and LE (days 5, 6, or 36). A separate light reading was taken at the center of the room with the light meter fixed to a pole 183 cm from the floor pointing at wall of the square room (vertical plane). In addition, a technician sitting in the room during the bright LE measured light intensity levels after (1) every other fixed gaze interval with a light meter attached to a pole 137 cm from the floor pointing toward the wall placed approximately 64 cm in front the participant’s face and (2) after each fixed and free gaze interval measured from the participant’s forehead toward the approximate direction of the participant’s fixed gaze and free gaze. Light intensity levels (lux) were measured with an IL-1400 photometer (International Light, Inc, Newbury, MA). The average (+ standard deviation) light intensity recorded from pole readings during CR was 2.7±0.8 lux and before, during, and after the bright LE was 2.7±0.7, 6807.9±1324.1, and 3.9±1.3 lux, respectively. Average bright light levels varied above or below the participant mean lux levels among participants but remained consistent throughout the bright LE.

Assessment of Core Body Temperature

Core body temperature (CBT) was recorded continuously every minute during CR using a disposable rectal thermistor (Yellow Springs Instrument Company, Yellow Springs, OH). The thermistor was inserted approximately 10 cm (marked by tape) into the rectum and only removed for bowel movements and showers. A real-time, online data acquisition system was used to monitor CBT collection by via the rectal thermistor. Technicians periodically asked participants to check that the thermistor was inserted no less than the marked-off 10 cm length.

The CBT data were used to assess endogenous circadian phase during CR and used to calculate the appropriate timing of the bright LE. Nonlinear least squares harmonic-regression analysis was used to fit a dual-harmonic model with correlated noise to each participant’s CBT data by the investigator during the first 24 hours of the CR. The CBT data from the first 5 hours of CR were excluded from the analysis to eliminate evoked effects produced by the transition from sleep to wake after the preceding 8-hour sleep opportunity. The projected timing of the onset of melatonin secretion (the target time for initiating the bright LE) was estimated to occur 6.75 hours before the participant’s fitted CBT minimum.

Assessment of Plasma Melatonin

Blood samples were collected every 30 to 60 minutes during CR and every 10 to 60 minutes during LE to measure pineal melatonin levels (n = 15). Blood was sampled every 10 minutes for 3 hours starting 1.5 hours before the room light intensity increased for the experimental LE to capture any acute suppression of pineal melatonin after the light intensity increase. Blood sampling was reduced to every 20 minutes 1.5 hours after the room light intensity increased and continued for the remainder of the LE and 1.5 hours after the room light intensity was decreased to the dim light background setting. An indwelling 18- to 20-gauge intravenous catheter was inserted into the participant’s forearm vein on day 2. The catheter was connected to a 12-foot small-lumen tube, which could be passed through a porthole to allow blood sampling outside the participant’s room during CR and LE. Between samples, a solution of 0.45% saline with 5000 IU/L of heparin was infused at a rate of 40 to 51 ml/hour depending on the sampling rate to maintain patency. Approximately 1 ml of blood was aliquoted into a 3-ml Vacutainer containing the anticoagulant ethylene diaminetetraacetic acid for each sample taken. Samples were placed on ice for less than 45 minutes before being spun for 10 minutes in a centrifuge at +4°C at 2200 to 2800 revolutions per minute. Plasma was transferred into a 2-ml aliquot tube and frozen at −20°C. All plasma and saliva samples were assayed for melatonin at Pharmasan, Inc (Osceola, WI), using a commercial radioimmunoassay kit (Elias USA, Inc, Osceola, WI) that uses I-125-melatonin as a tracer and reported as pg/ml. Sensitivity of detection for plasma melatonin was 0.7 pg/ml.

Assessment of Salivary Melatonin

Participants were asked to fill at least half of a 7-ml plastic sample tube within 5 minutes every 60 minutes during CR and LE for melatonin assay. Salivary melatonin was only analyzed and reported here for 3 participants (nos. 7, 8, and 10), because blood samples could not obtained. For 1 participant (no. 7), saliva

![Figure 1. Example of study protocol (no. 14; 26Q6) for assessing of melatonin concentrations over approximately 3 days. Melatonin concentrations (solid black line) are plotted by pg/ml (y-axis) and by clocktime (x-axis) under dim light (<4 lux; light gray shading) for 38 hours during constant routine (CR) and for 4.75 hours before and after the 6.5-hour light exposure (LE) (~7000 lux; white area; no shading). An 8-hour sleep opportunity under darkness (0 lux; dark gray shading) separated the CR and LE days.](image-url)
samples were obtained every 30 minutes during LE. Samples were placed on ice for less than 45 minutes before being frozen at −20°C. Sensitivity of detection for salivary melatonin was 0.2 pg/ml.

**Analysis**

**Melatonin Suppression.** Plasma and salivary melatonin concentrations during the bright LE were compared with melatonin levels at the corresponding clock times 24 hours earlier during dim LE on CR day except in 1 case (27Q2, no. 13), in whom data from 48 hours earlier were used due to a spontaneous phase change before LE that skewed the suppression assessment. The area under the curve (AUC) for melatonin concentrations for CR (CR_AUC) and LE (LE_AUC) during corresponding clock times relative to the bright LE (Lights On to Lights Off) was calculated by summation of the areas between time points using the trapezoidal method. Calculation of suppression was defined during LE or CR, respectively. The magnitude of melatonin suppression was defined as the percent difference (% suppression) between the CR_AUC and LE_AUC plasma melatonin concentrations during the corresponding clock times of the LE. Participants had to reach a threshold of 33% suppression to be considered a positive responder. Percent suppression was determined by dividing the difference between the CR_AUC and LE_AUC plasma melatonin levels by CR_AUC: 

\[
\%\text{Suppression} = \frac{\text{CR}_\text{AUC} - \text{LE}_\text{AUC}}{\text{CR}_\text{AUC}} \times 100
\]

**Dim Light Melatonin Onset.** The 25% dim light melatonin onset (DLMO 25%) was calculated for melatonin profiles on CR and LE days. The DLMO was defined as the interpolated time point at which plasma melatonin levels exceeded 25% of peak to trough of CR melatonin profile. The amplitude of the melatonin profile during CR was calculated by fitting a 3-harmonic regression model to melatonin data during the CR, excluding the first 5 hours and the last 30 minutes of data during CR. An estimate of DLMO 25% was excluded if there was 3 hours of missing data between 2 consecutive data points.

**Results**

**Ophthalmologic Evaluation**

Details of each participant's neuro-ophthalmologic evaluation are provided in Table 1. The ophthalmologic examination revealed multiple etiologies of blindness, although a definitive cause of blindness could not be obtained in 2 participants (26Q5 and 2805, nos. 14 and 15, respectively), because the participant did not know nor could it be determined during the exam (i.e., fundus not observable). The causes of blindness were retinopathy of prematurity (ROP) (n = 5), retinitis pigmentosa (n = 2), congenital glaucoma (n = 1), optic neuritis/neuropathy (n = 2), congenital rubella syndrome (n = 1), retinal detachment (n = 1), retinitis/retnopathy (related to measles; n = 1), and etiology unknown (n = 2). An observation of the fundus could only be performed in 4 of the 5 participants with whom a clear, definitive view of the anterior chamber could be made in at least 1 eye. In addition, miotic pupil(s) (n = 2) or "obstruction" within the anterior chamber (n = 11), such as corneal opacity or clouding, precluded any assessment of the pupil reflex to light in 13 ocular-intact blind participants. Pupil constriction was not detectable in either eye in response to the brightest light of an indirect ophthalmoscope in a standard clinical exam in the remaining 4 participants in which at least 1 pupil was observable. There were only 3 participants (22CS, 2811, and 26Q5U [nos. 1, 2, and 11, respectively]) who exhibited voluntary eye movements with full ductions and versions. No detectable activation of the posterior visual cortex was observed with the flash stimulus during visual evoked potential results testing in each of the 9 blind patients tested. A negative electroretinogram was previously reported for participant 1492 (no. 4).

**Melatonin Suppression Test**

Melatonin concentrations decreased by more than 33% during the 6.5-hour LE in 5 blind participants (82.8%, 79.2%, 72.4%, 83.5%, 41.1% for nos. 1–5, respectively) (Fig 2), with an average ± standard deviation of 71.6% ± 17.5% (n = 5), similar to that observed in sighted participants. Of these, 2 participants were diagnosed with ROP, 2 participants were diagnosed with retinitis pigmentosa, and 1 participant had complete bilateral retinal detachments. The magnitude of suppression was attenuated in 1 participant (no. 5) exhibiting nearly half the percent suppression than the other 4 participants.

In 2 of 5 participants who exhibited melatonin suppression (nos. 1 and 2), the LE started within 1 hour after DLMO and suppressed melatonin to below DLMO levels within 0.53 and 0.62 hours, respectively. Suppression remained constant throughout the LE (Fig 2). In the remaining 3 suppressed participants (nos. 3–5), the LE began before predicted DLMO by 1.71, 0.65, and 1.29 hours, respectively. In these patients, melatonin levels were substantially suppressed but showed evidence of recovery during the LE, reaching DLMO 4.60, 5.21, and 1.95 hours after Lights On, respectively (Fig 2). All 5 participants exhibited rapid elevation of melatonin levels after Lights Off (Fig 2).

Melatonin concentrations did not decrease by more than 33% during the bright LE in 10 of 15 participants with eyes (nos. 6–15; average ± standard deviation] suppression −7.6% ± 23.8%, range −49.9–14.0%) or in the 3 bilaterally enucleated participants (nos. 16–18; −9.9% ± 17.7%, range −22.4%–10.3%) (Fig 2). When examined relative to the presence of phthisis bulbi, none of the 5 patients with bilateral phthisis bulbi demonstrated suppression (nos. 9–12, 14); 5 of the remaining 10 participants without bilateral phthisis bulbi did exhibit melatonin suppression (Fig 2).

Four of the 5 individuals who exhibited melatonin suppression had exhibited a normally phased 24-hour rhythm in urinary aMT6s before the study. The fifth participant with melatonin suppression (2217V) had exhibited a non–24-hour rhythm but chose to wear opaque scleral shells for cosmetic purposes, presumably leading to a lack of photic entrainment under normal conditions. She removed these scleral shells for the melatonin suppression study and exhibited a positive response. Only 1 participant who did not exhibit melatonin suppression (2740) exhibited 24-hour urinary aMT6s rhythms before study, presumably due to entrainment by nonphotic time cues.

**Discussion**

These results confirm and expand previous reports that light-induced melatonin suppression is observed in a minority of totally visually blind individuals. Of those participants with intact eyes who did not have bilateral phthisis bulbi, however, half exhibited melatonin suppression, Of the 5 participants who responded to light, 1 had a phthisical eye and exhibited half the melatonin suppression response as the responders whose globes had not begun to shrink. Given that...
Figure 2. Melatonin suppression test of individual participants. Melatonin concentrations during constant routine (CR) (solid black line) are superimposed over the melatonin concentrations across the corresponding 16 hours during light exposure (LE) day (filled diagonal lines) relative to time since start of LE (x-axis; 0 hours) on LE day for each participant. Melatonin concentrations were measured under constant dim light (<4 lux) during CR. On LE day, participants received a 6.5-hour (10.75 hours for 23BP) experimental LE (~7000 lux; white area, no shading) with exposure to dim light (4 lux, gray shaded area) for 4.75 hours before and after the experimental LE. All figures for each participant depict plasma melatonin, except for 3 participants (nos. 7, 8, and 10) for whom salivary melatonin is shown.
none of the participants studied with bilateral phthisis bulbi, or any of those with bilateral enucleation, have exhibited photic melatonin suppression (Tables 1 and S1, available at www.aaojournal.org), it would appear that intact globes are required for presentation of this response.

Unlike animal models in which selective abolition of rod/cone development via targeted gene-knockouts was used to assess photic input via ipRGCs to the circadian pacemaker (e.g., 22), studies in humans are confined to assessing the resilience of this pathway with less knowledge of the specific damage to the retina or optic nerve. The extent of the damage may differ between etiologies of blindness, as well as within a disease, however, and we have observed entrainment of circadian rhythms in visually impaired patients with only 1 eye.19 In this case, the participant’s remaining (phthisical) eye had other physical abnormalities with the eye, including corneal scarring, a cataract, and band keratopathy (Table 1), which may have further attenuated the intensity and spectral quality of the light entering the eye and induced a submaximal response.35 Similar difficulties, including cataracts, that prevented observation of the fundus were also apparent in 2 other participants (22J7 and 1492; nos. 3 and 4, respectively); however, but full melatonin suppression responses were observed, and therefore evaluating the quality of light reaching the retina is not straightforward. As noted, assumptions about the circadian photoresponse status cannot be assumed from the disorder type or presence of cataract and must be measured and confirmed on an individual level.

Studies have attempted to examine selective disruption of the visual system on nonsurgical responses. A number of studies examining damage to the inner retina, including the ganglion cell layer, have shown mixed results.36–39 For example, one study has reported impaired melatonin suppression responses in patients with optic neuropathy due to glaucoma.36 The salivary melatonin suppression response to 60 minutes of 600-lux white light was reportedly absent in grouped analyses of 9 patients with bilateral advanced primary open-angle glaucoma, but who retained visual light perception, compared with normally sighted controls who exhibited an approximately 50% suppression of melatonin. Likewise, postillumination pupillary responses (maintenance of pupill constriction after a light stimulus is turned off) was reported to be impaired in advanced glaucoma patients in response to blue, but not red light, suggesting that ipRGC function, implicated in maintaining postillumination pupillary responses, was suboptimal.38,39 Conversely, blue light—induced melatonin suppression responses have been reported to be preserved in some visually impaired (light perception or better) patients with neurodegenerative optic neuropathies that selectively affect retinal ganglion cells, namely, Leber’s hereditary optic neuropathy and dominant optic atrophy.40 Although disruption to ganglion cells would be expected to include the melanopsin-containing ipRGCs, and therefore disrupt circadian photoresponse, it appears that this was not the case and that the ipRGCs were preserved.

There are a number of limitations to these previous investigations.36,37 First, given the high inter-individual variation between individual melatonin levels,41 uncorrected grouped data are inappropriate for determining melatonin suppression (Fig 2). When examining unusual populations whose responses to light may be borderline, it is possible that some individuals exhibit melatonin suppression but that their responses are masked by (1) negative responses of other individuals and (2) the large interindividual levels of absolute melatonin in the group data. In addition, there are interindividual differences in the timing of melatonin secretion (i.e., DLMO) across the majority of the visually blind population and within an individual at any given time. Assessments are ideally performed at the same circadian time for each individual (e.g., relative to DLMO), rather
than clock time. A dark or dim light control condition is also vital to ensure against false positive results that might result from “drift” or changes in circadian phase.

Moreover, the studies on postillumination pupillary responses may not be long enough to induce a stable response in individuals with impaired visual function. We have recently shown that the time course of pupillary constriction and recovery is substantially different in a totally visually blind man with intact light-induced pupillary constriction compared with sighted controls and that these differences require longer exposure durations to be measured. Such temporal differences have important consequences: In the first confirmation of nonvisual responses in totally visually blind humans, pupillary constriction was reportedly negative in individuals with intact melatonin suppression and circadian entrainment responses that may simply have been due to use of a standard clinical, rapid ophthalmoscope or “penlight”-type pupil examination that was too short to detect sustained pupillary constriction mediated by the nonimage-forming photoreception system. We have recently shown in a totally visually blind man who had a negative clinical pupil exam in the current study that 7 seconds of LE are needed to initiate constriction of the pupil and that approximately 30 seconds of darkness are needed to fully redden the pupil post-LE, several orders of magnitude slower than fully sighted individuals. The clinical implications are that carefully controlled tests of melatonin suppression and pupil constriction responses must be performed in patients considering enucleation a visually blind eye for cosmetic reasons to assess whether nonvisual light responses remain intact, as removal of the eyes will likely induce nonentrained circadian rhythms and non-24-hour sleep–wake rhythm disorder.

The number of totally visually blind people investigated for intact nonvisual responses to light with at least 1 eye remains very low, totaling 26 in all published studies. On the basis of data from the 25 participants studied by our group, photic suppression of melatonin is preserved in approximately one third (8/25) of those who are totally blind with at least 1 eye (Czeisler et al, 1995; Table S1, available at www.aaojournal.org; Klerman et al, 1998). Closer inspection of the ocular diagnoses for 22 of the 25 participants with available data (Tables 1 and S1) show that half of the patients (8/16) without bilateral phthisis bulbi exhibited light-induced melatonin suppression. None of the 6 patients with bilateral phthisis bulbi exhibited melatonin suppression. Furthermore, all of those who did have their melatonin suppression test protocols performed in patients considering enucleation a visually blind eye for cosmetic reasons to assess whether nonvisual light responses remain intact, as removal of the eyes will likely induce nonentrained circadian rhythms and non-24-hour sleep–wake rhythm disorder.

In conclusion, our findings confirm that a substantial fraction of blind individuals who have no light perception still exhibit melatonin suppression responses to ocular LE, presumably through intact ipRGC function. Clinical ophthalmologic examinations can be used to guide clinical care. In addition to diagnosis of bilateral phthisis bulbi and enucleation, carefully performed, extended-duration pupillary constriction tests may be helpful in identifying patients in whom nonimage-forming retinal responses are preserved. The melatonin suppression test protocol remains a reliable method for confirming the functional integrity of the retina-RHT-SCN-pineal axis and nonvisual light-responsive blind individuals.

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Author Contributions:

Conception and design: Hull, Czeisler, Lockley
Data collection: Hull, Lockley
Analysis and interpretation: Hull, Czeisler, Lockley
Obtained funding: Czeisler, Lockley
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Abbreviations and Acronyms:

aMT6s = urinary 6-sulphatoxymelatonin; AUC = area under the curve; CBT = core body temperature; CR = constant routine; DLMO = dim light onset of melatonin; ipRGC = intrinsically photosensitive retinal ganglion cell; LE = light exposure; RHT = retino-hypothalamic tract; ROP = retinopathy of prematurity; SCN = suprachiasmatic nuclei.

Correspondence:

Steven W. Lockley, PhD, Division of Sleep and Circadian Disorders, Brigham and Women’s Hospital, Harvard Medical School, 221 Longwood Avenue, Boston, MA 02115. E-mail: slockley@hms.harvard.edu.