

# Melanopsin System Dysfunction in Smith-Magenis Syndrome Patients

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**PURPOSE.** Smith-Magenis syndrome (SMS) causes sleep disturbance that is related to an abnormal melatonin profile. It is not clear how the genomic disorder leads to a disturbed synchronization of the sleep/wake rhythm in SMS patients. To evaluate the integrity of the intrinsically photosensitive retinal ganglion cell (ipRGC)/melanopsin system, the transducers of the light-inhibitory effect on pineal melatonin synthesis, we recorded pupillary light responses (PLR) in SMS patients.

**METHODS.** Subjects were SMS patients ( $n = 5$ ), with molecular diagnosis and melatonin levels measured for 24 hours and healthy controls ( $n = 4$ ). Visual stimuli were 1-second red light flashes (640 nm; insignificant direct ipRGC activation), followed by a 470-nm blue light, near the melanopsin peak absorption region (direct ipRGC activation). Blue flashes produce a sustained pupillary constriction (ipRGC driven) followed by baseline return, while red flashes produce faster recovery.

**RESULTS.** Pupillary light responses to 640-nm red flash were normal in SMS patients. In response to 470-nm blue flash, SMS patients had altered sustained responses shown by faster recovery to baseline. SMS patients showed impairment in the expected melatonin production suppression during the day, confirming previous reports.

**CONCLUSIONS.** SMS patients show dysfunction in the sustained component of the PLR to blue light. It could explain their well-known abnormal melatonin profile and elevated circulating melatonin levels during the day. Synchronization of daily melatonin profile and its photoinhibition are dependent on the activation of melanopsin. This retinal dysfunction might be related to a deficit in melanopsin-based photoreception, but a deficit in rod function is also possible.

**Keywords:** retina, ipRGC, melanopsin, retinohypothalamic pathway, pupillary light reflex, pupillometry, Smith-Magenis syndrome

Smith-Magenis syndrome (SMS) is a genomic disorder associated with a common deletion interval of 3.5 to 5.0 Mb of an interstitial region of chromosome 17, band p11.2. Among the several genes included in this region, retinoic acid induced 1 (*RAI1*) gene atypical deletions and heterozygous point mutations are associated with the phenotype.<sup>1–4</sup> The clinical phenotype includes craniofacial anomalies, intellectual disability, self-injurious and aggressive behavior, as well as severe sleep disturbances, described as short sleep, nocturnal awakenings, difficulty falling asleep at night, and daytime sleepiness.<sup>5,6</sup> Sleep disturbances in SMS patients are usually correlated with an abnormal melatonin profile, with high melatonin levels during the day and low levels at night.<sup>7–9</sup> This behavior of melatonin production, highly frequent in SMS patients, is not expected since melatonin production is blocked

by environmental light, especially light in the blue spectral range.<sup>10,11</sup>

Pineal melatonin is produced under strict control of the circadian timing system, and its production is synchronized by the light/dark environmental cycle. Melatonin is most abundantly synthesized during the dark phase regardless of the behavioral distribution of the daily activity of the considered mammalian species.<sup>10</sup> Elevated levels of circulating melatonin are associated with the dark phase of the light/dark cycle provided there is no light in the environment, since light during the night inhibits pineal melatonin synthesis, mainly through the activation of a highly specialized retinal melanopsin system.<sup>11–17</sup>

The neural system that mediates light entrainment of circadian rhythms, melatonin photoinhibition, and pupillary



TABLE 1. Demographic and Clinical Data of the Subjects

Subjects	Age, y	Sex	Molecular Diagnosis	Clinical Symptoms	Treatment
SMS 1	7	M	3.7 Mb interstitial deletion in 17p11.2	Development delay, sleep disturbance, irritability, cardiopathy	Risperidone, fluoxetine, imipramine, melatonin
SMS 2	14	F	3.7 Mb interstitial deletion in 17p11.2	Development delay, irritability	Carbamazepine, risperidone
SMS 3	26	F	3.7 Mb deletion in 17p11.2	Development delay, sleep disturbance, neurogenic bladder, dysmorphic features	Amlodipine
SMS 4	10	F	Interstitial deletion in 17p11.2	Development delay, sleep disturbance, compulsive feeding	Risperidone, topiramate, imipramine, melatonin, metformin
SMS 5	7	M	Interstitial deletion in 17p11.2	Development delay	Risperidone, sertraline
Average	12.8				
Standard deviation	7.9				
Control 1	17	F	-	-	-
Control 2	17	F	-	-	-
Control 3	17	F	-	-	-
Control 4	17	F	-	-	-
Average	17.0				
Standard deviation	0.0				

Mb, megabase.

responses to light, besides several other nonimage-forming visual functions, originates in the intrinsically photosensitive retinal ganglion cells (ipRGCs). These retinal ganglion cells express a photopigment called melanopsin that enables them to be directly activated by light.<sup>11-14,17-20</sup> The ipRGC axons leave the retina as part of the optic nerve and project to central structures that regulate the circadian rhythm such as the suprachiasmatic nucleus, the subparaventricular zone, the ventrolateral preoptic area, and the intergeniculate leaflet.<sup>15,21</sup>

It is well established that both exogenous and endogenous mechanisms might affect the circadian rhythm generation and/or synchronization; therefore, the alteration of one or both mechanisms might cause perturbations of the circadian internal order (chronodisruption),<sup>22</sup> leading to sleep disturbances.

The spectral sensitivity of melanopsin peaks in the blue spectral range, at ~480 nm.<sup>23</sup> The ipRGCs control the pupillary response to light. The initial transient peak constriction in response to light is attributed to ipRGCs stimulated by rods and cones, while the sustained component of the pupillary response to light has been attributed to the direct activation of the ipRGCs by the light.<sup>24,25</sup>

The integrity of the retinal melanopsin system may be assessed by the pupillary light reflex (PLR), which measures the constriction and subsequent dilation of the pupil to a change in light stimulation. The PLR to flashes of red light (which falls away from the peak of the melanopsin absorption spectrum) and of blue light, that maximally activates ipRGCs, provide a measure of the melanopsin contribution to the PLR. Thus, the PLR is an important noninvasive tool that allows the measurement of the functionality of the retinal melanopsin system.<sup>26-30</sup>

We speculate that the SMS patients' sleep disorders might be the result of a dysfunctional ipRGC/melanopsin retinal system, since their sleep/wake disturbances are usually associated with an abnormal melatonin production profile, displaced to daytime, and resistant to photoinhibition. This assumption finds support in several demonstrations of the association between sleep disturbances and alteration in ipRGC activity assessed through the PLR<sup>27-31</sup> and in the demonstration that the lack of one *RAII* allele (the primary

gene responsible for most features of SMS, including the inverted circadian rhythm of melatonin<sup>32,33</sup>) affects the nonvisual light-signaling dependent behavior.<sup>34</sup>

In order to evaluate the functionality of this retinal system, we tested SMS patients using the PLR protocol,<sup>26</sup> particularly its sustained component that is controlled by the ipRGC/melanopsin system.<sup>24,25</sup>

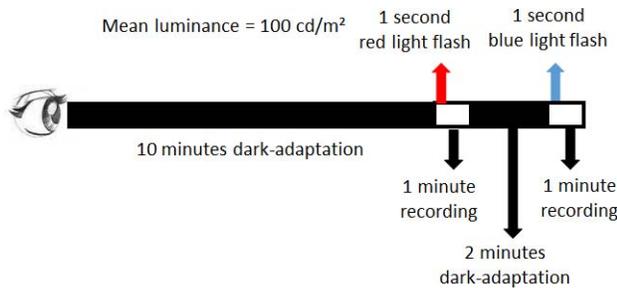
## MATERIALS AND METHODS

### Participants

PLRs were recorded from five SMS patients (aged 7 to 26 years old; average = 13 ± 8 years old) and four healthy volunteers (all subjects were 17 years old). SMS patients were selected in the Child Neurology Outpatient Clinic of the Clinics Hospital of the University of São Paulo. All parents received appropriate information about the nature and possible consequences of the study and signed a written informed consent. The research followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committees of the Institute of Psychology (CAAE 56608616.1.0000.5561) and of the Clinics Hospital of the University of São Paulo (CEP 16761). All subjects underwent complete ophthalmologic examination. Inclusion criteria for SMS patients were defined as molecular diagnosis, with 17p11.2 deletion demonstrated with MLPA (multiplex ligation-dependent probe amplification) technique, normal ophthalmologic examination, and ability to understand the task. For control subjects the exclusion criteria were presence of ophthalmologic or central nervous system diseases. Table 1 provides the SMS subjects' demographic data. Sleep/wake behavior was recorded for 1 month with sleep logs for all SMS subjects.

### Pupillary Light Response Protocol

PLR was measured monocularly at the stimulated eye while the other eye was covered. PLR was measured with the RETiport system with a light stimulator (Super Color Ganzfeld Q450SC; Roland Consult, Brandenburg, Germany). A dark adaptation period of 10 minutes preceded the light stimulation. PLRs were



**FIGURE 1.** The PLR protocol started with 10 minutes of dark adaptation followed by a 1-second red flash. After 2 minutes of dark adaptation, the blue flash was presented. Pupil size was recorded during 1 minute, beginning 5 seconds before each light flash presentation.

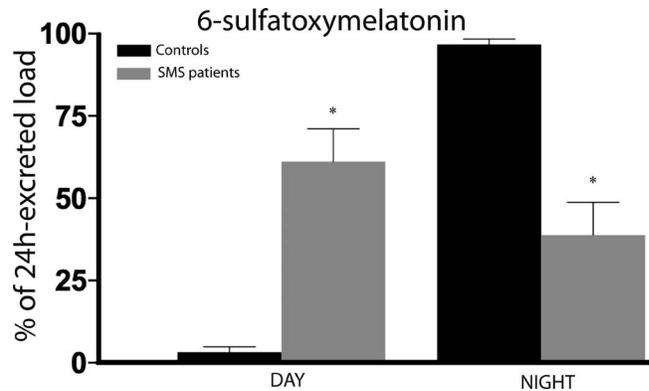
recorded in response to 1-second light flashes using two different wavelengths: red light (peak wavelength  $\pm$  full width at half maximum:  $638 \pm 9$  nm), which falls away from the peak of the melanopsin absorption spectrum (insignificant direct activation of the ipRGCs), followed by blue light ( $469 \pm 11$  nm), which is close to the peak absorption of melanopsin (direct activation of the ipRGCs). A 2-minute interval between the flashes was observed. Photopic luminance was set to 100 candelas per square meter ( $\text{cd}/\text{m}^2$ ) for both red and blue lights. One luminance level was used in order to make the protocol the briefest possible, since the behavioral disturbances of the patients did not allow us to perform extensive measurements. The choice of  $100 \text{ cd}/\text{m}^2$  was based on a previous study of Park et al.,<sup>26</sup> showing clear differences between the sustained responses to blue and red light flashes at this luminance level. In addition, previous studies from our group showed that luminances higher than  $100 \text{ cd}/\text{m}^2$  do not significantly change the magnitude of the sustained response.<sup>27,29,30</sup> Figure 1 is a diagram of the protocol, showing the time course of the measurements, parameters of the light flashes, and the intervals between them. Recordings were repeated if blink artifacts coincided with the peak response or happened to occur between 5 and 7 seconds after flash presentation. If repetition was necessary, the protocol was performed again on another day.

### Urinary 6-Sulfatoxymelatonin

Urinary 6-sulfatoxymelatonin levels were assessed in the SMS patients and matched controls using ELISA (IBL International, Hamburg, Germany), according to the manufacturer's instructions. Urine samples were collected from all subjects during 24 hours in three different containers, according to the corresponding period: morning (7:00 AM to 1:00 PM), afternoon (1:00 PM to 7:00 PM), and night (7:00 PM to 7:00 AM). The final analysis was done computing day (7:00 AM to 7:00 PM, morning + afternoon) versus night (7:00 PM to 7:00 AM) in both SMS patients and control subjects, and 6-sulfatoxymelatonin was measured as a day and night percentage of the 24-hour excreted load. Samples were homogenized, had their volumes assessed, and were kept under  $-80^\circ\text{C}$  prior to the assay.

### Data Analysis

We analyzed the PLR peak response (time and amplitude of the smallest diameter after the light stimulation) and the median amplitude of the sustained component (average amplitudes between 5 and 7 seconds after the flash onset) to both red and blue lights. The sustained response of the PLR had previously



**FIGURE 2.** SMS patients' ( $N = 5$ ) and controls' ( $N = 4$ ) urinary 6-sulfatoxymelatonin levels during the day (7:00 AM to 7:00 PM) and at night (7:00 PM to 7:00 AM). Values are expressed as percentage of the 24-hour excreted load ( $\mu\text{g}/\text{h}$ ).

been estimated using a longer flash presentation as the average pupil constriction between 15 and 30 seconds after light offset.<sup>24</sup> Here we followed parameters previously standardized for a 1-second flash presentation: the sustained pupil response was measured at 6 seconds after flash offset.<sup>26</sup> However, we used a broader window (5–7 seconds) in order to avoid artifacts that could influence the results if only 1 second was considered to calculate this component. The urinary 6-sulfatoxymelatonin values are expressed in percentage of the 24-hour mean for each patient, and the mean  $\pm$  standard error of mean ( $n = 5$  patients and  $n = 4$  controls) for each period of time was analyzed by 1-way ANOVA.

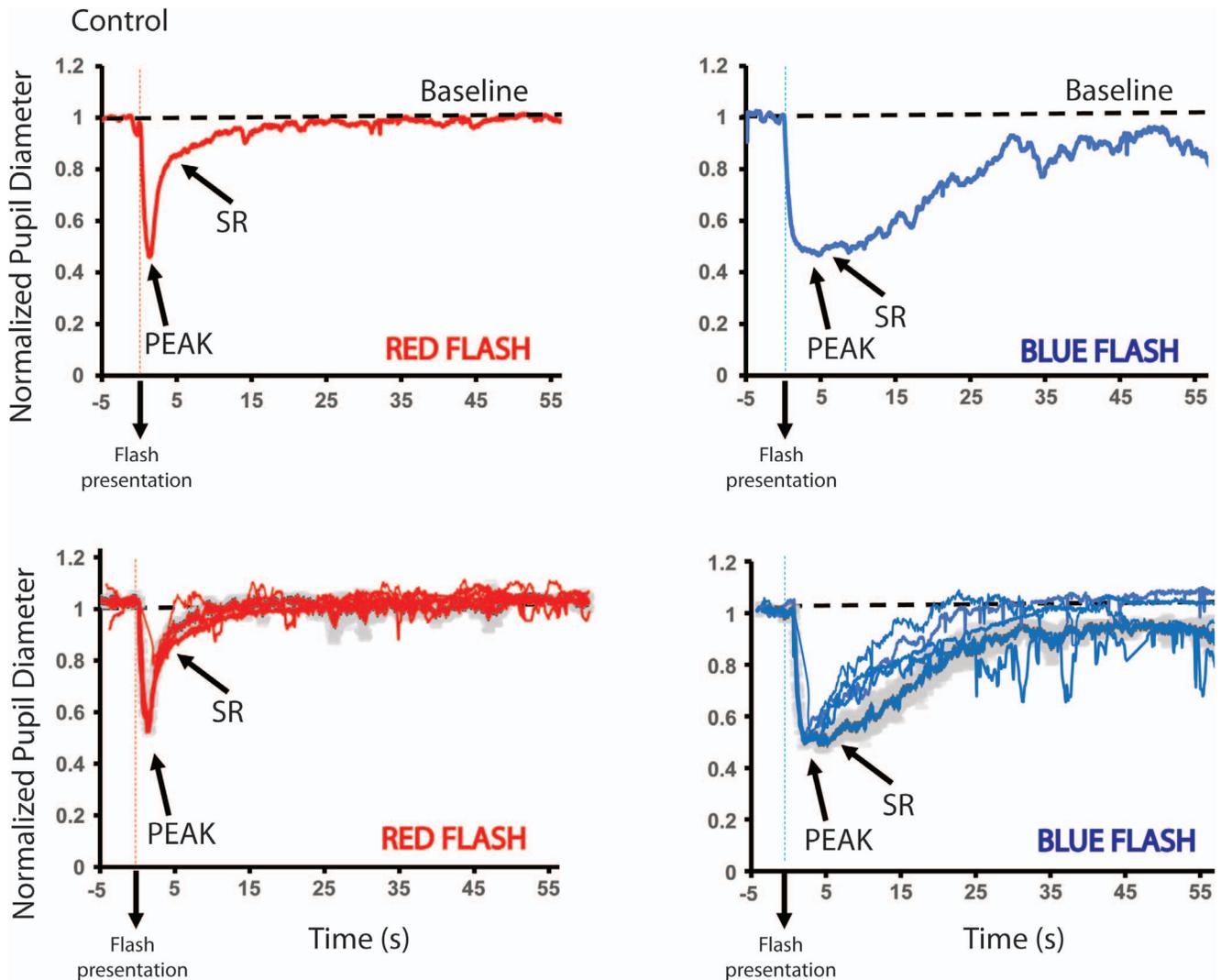
### RESULTS

Sleep onset for SMS patients ranged from 8 PM to 11 PM and wake-up time varied from 4 AM to 8 AM, with sleep onset being stable for each patient and more variability observed for wake-up time. Diurnal naps lasting approximately 1 hour were frequently reported for patients 2, 3, and 4, occurring at 8 AM for patient 2 (this patient usually wakes up at 5 AM), at 2 PM for patient 3, and at 8 AM and 2 PM for patient 4. The other two patients had sporadic diurnal naps. All patients, except subject 1, reported one night awakening, usually lasting approximately 30 minutes, in the beginning or in the end of the main sleep episode, but families were already instructed to keep lights off in these moments.

As shown in Figure 2, control subjects showed the expected daily 6-sulfatoxymelatonin profile, with higher levels during the night and lower levels during the day. On the contrary, SMS patients presented the previously described inversion,<sup>4</sup> with higher percentage during the day and lower percentage during the night.

PLRs from a representative control (upper panel) are shown in Figure 3 for both parameters analyzed: peak response and sustained component. As previously mentioned, the response to a blue light flash shows a conspicuous sustained component, which is attributed to the function of the ipRGCs.

The PLRs of the SMS patients ( $n = 5$  for the red flash, and  $n = 4$  for the blue flash) are shown in Figure 3 (lower panel) together with the average responses of the controls (thicker traces) and the respective standard errors (shaded area). For the red flash, the responses of the SMS patients overlap the average ( $\pm$ SE) response of the control subjects. On the other hand, SMS patients showed altered sustained components of the PLR for the blue flash compared to control subjects.



**FIGURE 3.** Normalized pupil diameter of a representative control for the red (*left*) and for the blue (*right*) flash presentation (*upper panel*). *Lower panel* shows the average responses of control subjects in *thick traces* ( $n = 4$  eyes) and individual responses of the SMS patients ( $n = 5$  for the red flash, and  $n = 4$  for the blue flash) in *thin traces*. The *shaded area* is the standard error of the control group. *Left graph* shows the responses to the red flash and *right graph* to the blue flash. The *black dotted lines* represent the baseline recording prior to flash presentation. PLR recorded from the 638-nm red light stimulation (*left*) and from the 469-nm blue light stimulation (*right*). Components analyzed: peak response (minimum amplitude of normalized pupil diameter and time to peak after the flash) and sustained response. The sustained response was calculated from the average normalized pupil diameter between 5 and 7 seconds from time 0, which was the flash onset. Flashes were presented for 1 second, 5 seconds after recording started (*red and blue dotted lines*).

The peak constriction of the pupil was expressed with reference to the normalized baseline pupil diameter. For the red flash stimulation, this value was  $0.53 \pm 0.07$  for the control group and  $0.56 \pm 0.12$  for the SMS patients. The peak response of the control subjects occurred  $1.40 \pm 0.12$  and the SMS patients  $1.52 \pm 0.32$  seconds after the flash onset. The sustained response after the red flash stimulation was  $0.90 \pm 0.05$  for the control subjects and  $0.89 \pm 0.06$  for the SMS patients.

For the blue flash, the normalized pupil diameter at the peak constriction was  $0.47 \pm 0.04$  for the control subjects and  $0.48 \pm 0.04$  for the SMS patients. The time to reach the peak was  $2.30 \pm 0.70$  seconds after the flash onset for the control group and  $1.88 \pm 0.30$  seconds for the SMS patients. The sustained response (normalized pupil diameter between 5 and 7 seconds after the flash) was  $0.51 \pm 0.04$  for the control group and  $0.64 \pm 0.03$  for the SMS patients.

Figure 4 shows the average  $\pm$  standard deviation of the control subjects and the SMS patients for the three parameters analyzed: peak constriction (left graphs), time to peak (middle graphs), and sustained response (right graphs) for the red flash (upper graphs) and for the blue flash (lower graphs). Table 2 shows the individual results for the red (left column) and for the blue (right column) flash.

## DISCUSSION

We showed, using the PLR, that SMS patients have a decreased ipRGCs activation compared to healthy subjects. This was indicated by their sustained response to a 469-nm blue light stimulation, showing a faster recovery toward the baseline pupil diameter in the dark. These new findings point to an abnormal functioning of the retinal-melanopsin system and

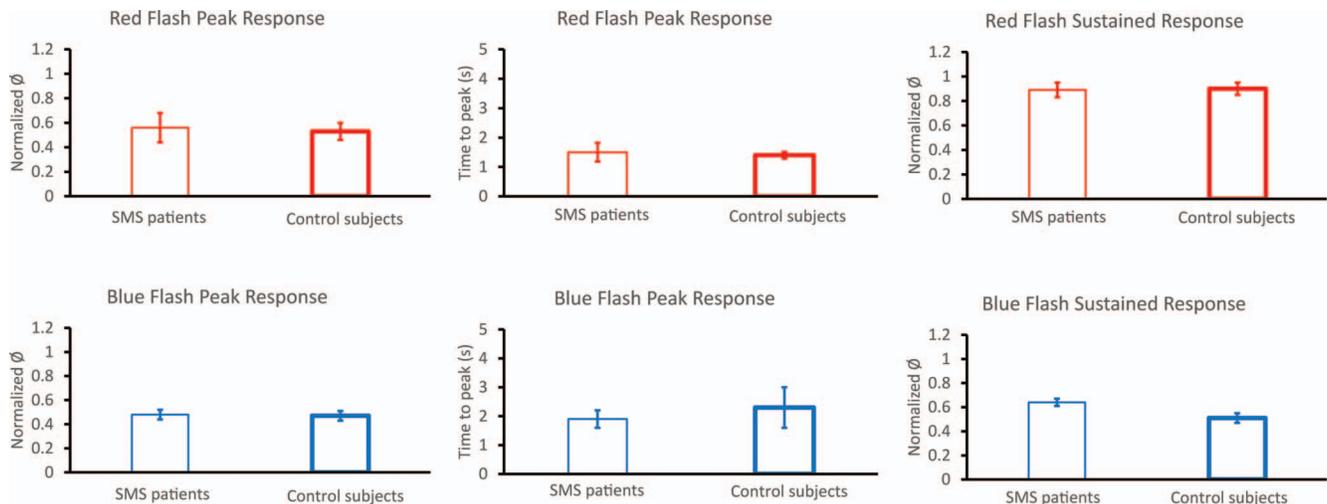


FIGURE 4. Average ± 1 SD of control subjects and SMS patients for the three parameters analyzed: peak response amplitude as normalized pupil diameter (left graphs), time to peak in seconds (middle graphs), and sustained response (right graphs) for the red flash (upper graphs) and for the blue flash (lower graphs).

might explain, at least partially, the anomaly of the daily melatonin profile in SMS patients.

The altered melanopsin response of our patients, evidenced in their PLR changes to blue light, might be associated with their sleep disturbances, since the ipRGCs, or melanopsin-expressing RGCs, project both to the suprachiasmatic nucleus (SCN) of the hypothalamus, involved in the regulation of the circadian rhythm, and to the olivary pretectal nucleus (OPN), involved in the PLR, as well as other nonvisual areas.<sup>21,35,36</sup> Previous studies have attributed a reduction in the sustained response of the PLR to a decreased activation of the ipRGCs,<sup>26-30</sup> although it is still not known whether the same type of ipRGCs in the human retina projects to both the SCN and the OPN.<sup>37</sup> This reduction in response might be present in other functions mediated by the ipRGCs and has been linked by several authors to a disruption in sleeping pattern.<sup>28-31</sup> On the other hand, patients with neuroretinal disorders that seem to spare the ipRGCs, such as Leber's hereditary optic neuropathy, have not shown signals of sleep disturbances.<sup>27</sup>

Reduced scotopic ERG responses, with no anatomic or molecular retinal alteration, were found in the RAI<sup>1+/-</sup>, the

mouse model of SMS, indicating a possible photoreceptor cause of light entrainment dysfunction.<sup>34</sup> Considering that rods also send sustained signals to the ipRGCs, which contribute to the PLR, and that rod responses become increasingly prolonged as the stimulus intensity increases,<sup>38</sup> a deficit in rod function is also possible.

Park et al.<sup>26</sup> have shown that in human PLRs recorded from dark-adapted eyes using low stimulus intensity, the sustained components to red and blue light were quite similar. The sustained pupil responses became different between red (640 ± 10 nm) and blue (467 ± 17 nm) light only above approximately 1 log cd/m<sup>2</sup>. In the present study, we used similar wavelengths (red light = 638 ± 9 nm, and blue light = 469 ± 11 nm) used by Park et al.,<sup>26</sup> and the luminance of the blue flash (100 cd/m<sup>2</sup>) was much above 1 log cd/m<sup>2</sup>. Moreover, measurements performed in humans and nonhuman primates<sup>24</sup> support the hypothesis that the sustained constriction of the pupil after blue light offset depends on ipRGCs/melanopsin activation.

Considering the activation of the photoreceptors by the blue flash, one might also consider that other cellular signals, such as ON-bipolar and amacrine cells, are involved in the

TABLE 2. Individual Results of the Red (Left Column) and the Blue (Right Column) PLRs

	Eye	Peak Response: Normalized Ø/Time, s		Sustained Response*	
		Red	Blue	Red	Blue
SMS 1	OS	0.76/2.0	0.53/2.3	0.98	0.68
SMS 2	OS	0.50/1.5	0.46/1.8	0.91	0.62
SMS 3	OS	0.52/1.5	0.45/1.8	0.85	0.63
SMS 4	OD	0.55/1.5	0.48/1.6	0.85	0.61
SMS 5†	OD	0.47/1.1	-	0.85	-
Average		0.56/1.52	0.48/1.88	0.89	0.64
Standard deviation		0.12/0.32	0.04/0.30	0.06	0.03
Control 1	OD	0.49/1.3	0.46/1.5	0.86	0.47
Control 2	OD	0.46/1.3	0.43/1.5	0.86	0.57
Control 3	OS	0.55/1.5	0.48/2.6	0.95	0.49
Control 4	OS	0.62/1.5	0.52/2.8	0.92	0.52
Average		0.53/1.40	0.47/2.30	0.90	0.51
Standard deviation		0.07/0.12	0.04/0.70	0.05	0.04

Ø, diameter.

\* Sustained response = normalized pupil diameter between 5 and 7 seconds.

† Patient SMS 5 was not able to complete the examination.

activation of ipRGCs and therefore might play a role in the PLR as well.<sup>39,40</sup> Although the changes were found only in the sustained response to the blue flash pointing to a disturbance of the melanopsin/ipRGC system in SMS patients, further studies are necessary to investigate the integrity of these downstream retinal mechanisms in SMS patients.

Other hypothesis is the reduction in the number of ipRGCs (or the number of active ipRGCs) in the retina of SMS patients. This condition was previously reported in patients with glaucoma.<sup>41,42</sup> Gracitelli et al.,<sup>42</sup> for instance, found a positive association between ipRGC/melanopsin dysfunction and nerve fiber layer thickness. However, glaucoma patients showed changes not only in the sustained response but also in the peak response of the PLR to blue light and to red light as well. Since the SMS patients show disturbances only in the sustained response to blue light, the results are more suggestive of an alteration of the melanopsin expression or function than to a decrease in the amount of the ipRGCs.

If melanopsin expression or function is disturbed in SMS patients, as we propose, one might speculate its possible cause. One possibility is that it might be related to the genetic alteration that causes SMS. The alteration of the *RAI1* gene, which plays an important role in the development of the central nervous system and controls the activity of other genes, such as the clock genes, is well described in SMS patients.<sup>1,2,43</sup> However, evidence of interaction between the *RAI1* gene and the opsin 4 (*OPN4*, the gene responsible for the melanopsin expression), or even alteration of the *OPN4* itself in SMS patients, has not been investigated, to our knowledge.

Another possibility that could explain the sustained response disturbances in SMS patients is a dysfunctional mechanism of the melanopsin regeneration. It has been shown that melanopsin can regenerate using external<sup>44</sup> and intrinsic photoregenerative mechanisms.<sup>24,45-47</sup> Moreover, PLRs are affected if regeneration of melanopsin is disturbed.<sup>44</sup> Further investigations might consider phototransduction as well as photoregenerative mechanisms as a possible cause of melanopsin dysfunction in SMS patients.

The results of the 6-sulfatoxymelatonin in SMS patients showed impairment in their expected daily melatonin production profile. These results are consistent with the lack of photoinhibition, leading, in some cases, to a phase-shifting in the production of melatonin and in all cases high circulating levels during the day. It is well known that the regular daily pattern of melatonin production by the pineal gland contributes to circadian synchronization in most vertebrates.<sup>48</sup> It is noteworthy that the daily melatonin profile alteration in SMS patients remains highly reproducible from day to day in these individuals.<sup>49</sup> These abnormalities are found in the great majority of patients, more than 95% as shown by Potocki et al.<sup>7</sup> (18 out of 19 patients), De Leersnyder et al.<sup>8</sup> (26 out of 27 patients), Nováková et al.<sup>9</sup> (3 out of 5), among others, and is usually associated with sleep disturbances in these patients.<sup>6</sup> In addition, an apparent abnormal daily profile of clock genes, particularly *Per2*, has been recently described in SMS patients.<sup>9</sup> However, it should be stressed that it is not possible to postulate a generalized circadian rhythm disturbance as a cause of altered melatonin profile since De Leersnyder et al.<sup>8</sup> showed that all SMS-studied patients, even though there is circadian disruption of melatonin profile and sleep/wake cycle, presented with no alteration in the expected circadian pattern of cortisol, growth hormone, and prolactin secretion or body temperature.<sup>8</sup> Moreover, a putative decline in the robustness of the circadian clock rhythm, as stated by Nováková et al.,<sup>9</sup> would explain the phase-shift of melatonin production observed in SMS patients, but it does not explain why the disturbed circadian melatonin profile is not photoinhibited by the daily

indoor light or sunlight. As stated by De Leersnyder,<sup>4</sup> even though there is an anomalous melatonin rhythm, it was reproducible day after day and follows a regular 24-hour period secretion. This suggests a dysfunction in the phase relationship between the light/dark environmental cycle and the circadian clock rather than to a circadian time-generation dysfunction.<sup>4</sup> As stated previously, the retinal ipRGC melanopsin system and its central projections are part of the neural system controlling the daily melatonin profile and its synchronization to the light/dark cycle.

The integrity of the ipRGCs/melanopsin system is fundamental for sending light information necessary for the entrainment of circadian melatonin rhythm and for several other functions of the nonimage-forming visual system. In addition, it guarantees that via the well-known photoinhibition phenomenon, the nocturnal melatonin profile is restricted to and follows the exact duration of the night darkness; it is, in fact, one of the most stable biological signals to time the physiological changes necessary for daily and seasonal adaptations.<sup>16</sup> The daily nocturnal production of melatonin is a critical signal for the synchronization of peripheral clocks by the SCN,<sup>49-51</sup> and it is essential for the integrity of the internal circadian timing system. The daily melatonin signal is important to time daily sleep/wakefulness,<sup>52-54</sup> activity/rest,<sup>48</sup> and energy metabolism,<sup>55</sup> among several other functions.<sup>56-58</sup> Therefore, in addition to a dependent direct genetic mechanism as a likely cause of the alterations observed in SMS,<sup>59-61</sup> the consequent disturbed melatonin profile aggravates and potentiates sleep/wakefulness and behavioral and metabolic symptoms as seen in SMS patients. As a consequence, the therapeutic correction of the melatonin profile has been used to alleviate several of these symptoms, as well as to aid in proper sleep.<sup>4,62,63</sup>

In this way, the reduced ipRGC response that we demonstrated to be present in SMS patients should be considered one of the pathogenetic components of the well-described circadian, metabolic, sleep, and daily melatonin profile disturbances of the Smith-Magenis syndrome.

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