Supporting Information

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SI Methods

Subjects. Participants were healthy, young subjects [n = 17; 9 female; age, 20–26 y (median, 22 y); body mass index, 18.8–29.4 kg/m^2 (median, 21.7 kg/m^2)] (Table S1). All participants were nonsmokers, moderate caffeine and alcohol consumers [i.e., ≤2 caffeine U/d; ≤9 alcohol U/wk; 1 unit is equivalent to a half pint (220 mL) of beer or 1 (25 mL) measure of spirits or 1 glass (125 mL) of wine] and were not taking medication. None had worked on night shifts during the year preceding the experiment or traveled across more than one time zone during the last 2 mo. Extreme morning and evening types, as assessed by the Horne–Östberg Questionnaire (1), were excluded (scores <30 or >70). None complained of excessive daytime sleepiness as assessed by the Epworth Sleepiness Scale (2) (scores <11) or of sleep disturbances as determined by the Pittsburgh Sleep Quality Index Questionnaire (3) (score ≤7). All participants had normal scores on the 21-item Beck Anxiety Inventory (4) and the 21-item Beck Depression Inventory II (5) (scores <14). They were right-handed as indicated by the Edinburgh Inventory (6). Absence of color-blindness was assessed by the 38-plate edition of Ishihara's Test for Color-Blindness (Kanehara Shupman).

At least 1 wk before the experiment, volunteers were familiarized to the MR environment during a short 15-min MRI session, during which a structural image of the brain was acquired.

To record one to three volunteers on the same day at approximately the same circadian time, volunteers were requested to follow one of three sleep schedules differing by 1 h: 2300 hours to 0700 hours ± 30 min, 0000 hours to 0800 hours ± 30 min, and 0100 hours to 0900 hours ± 30 min for subjects starting the experiment at 0830 hours, 0930 hours, and 1030 hours, respectively (Fig. 1 in main text). Volunteers were requested to refrain from all caffeine- and alcohol-containing beverages and from intense physical activity for 3 d before participating in the study.

Experimental Protocol. Three drops of tropicamidum 0.5% (Tropicol) were administered in the subjects' eyes 20 min before entering the scanner to inhibit pupillary constriction (arrow on Fig. 1 in main text).

During the data acquisition period, all of the subjects interacted with the same investigator, who used a standardized set of sentences between each session. This protocol was implemented to minimize variation in motivational state [e.g., encouragement by an investigator that may modify brain responses (7)]. No feedback was given on performance.

Auditory Stimuli. Stimuli were delivered using COGENT 2000 (http://www.vislab.ucl.ac.uk/Cogent/) implemented in MATLAB (Mathworks) on a 2.8-GHz Xeon Dell personal computer and were transmitted to the subjects using MR CONTROL amplifier and headphones (MR Confon). The first session was preceded by a short sound calibration session during which the volume level was adjusted for each subject so as to ensure optimal auditory perception during scanning.

Task 1: Main Task. Stimuli onset asynchrony was pseudorandomly set to range between 3 and 11 s (mean interstimulus interval, 4.8 s). Each of the 262 stimuli was used only once in task 1. Angry (131) and neutral (131) stimuli were pseudorandomly distributed over the entire session and evenly assigned to each illumination condition (blue light, green light, and the darkness period in between).

Task 2: Voice Localizer. Mean of the fundamental frequencies (F0) and distribution of energy through time (amplitude envelope, EN) have a critical role in conveying emotional information in voices. To confirm that differential emotional effects on brain activity in task 1 were driven by vocal prosody rather than being related to these low-level acoustic features, six types of stimuli were presented during task 2: angry tokens (50 stimuli of the first session), neutral tokens (50 stimuli of the first session), EN of angry tokens (50), EN of neutral tokens (50), F0 of angry tokens (50), and F0 of neutral tokens (50). Stimuli (750 ms) were presented in 15-s blocks of 10 stimuli (stimuli onset asynchrony, 1.5 s) of the same type separated by 4- to 7-s rest periods (mean rest duration, 5.6 s). Five blocks of 1-back task for each type of stimuli were pseudorandomly spread over the entire session (one key press for present and another for not present; 15% of the stimuli were repeated).

Light Exposure. Light was produced by a bright white light source (PL900; Dolan-Jenner Industries), filtered by narrow interference band-pass filters (FWHM, 10 nm; Edmund Optic) to produce the two monochromatic illuminations. A filter wheel (AB301-T; Spectral Products) was computer controlled to change light wavelength by switching band-pass filters. The light was transmitted by a metal-free optic fiber from the source to two small diffusers placed in front of the subjects' eyes. The diffusers were designed for the purpose of this type of study and ensured a uniform illumination. Light was administered through a 4 × 5.5-cm frame placed 3 cm away from the eye. Spectra of each monochromatic light were checked at the level of the diffusers (AvaSpec-2048; Avantes), and the 480-nm and 532-nm band-pass filters used produced light with a maximum radiance at, respectively, 472.8 nm and 527.3 nm. In the MR scanner, one wavelength was always followed by the other, and the first wavelength used was counterbalanced over subjects. The first light exposure occurred ≈3 h after habitual wakeup time (i.e., during the biological day when melatonin secretion is low; ref 8). Irradiance could not be measured directly in the magnet, but the light source was calibrated and irradiances estimated to be 7×10^{12} and 3×10^{13} photons per cm^2 per s (840-C power meter; Newport) after prereceptoral lens absorption for the different wavelengths was taken into account (9). The total amount of blue light received during the experiment was well below the blue-light hazard threshold (10).

fMRI Acquisitions. In each fMRI session, the four initial scans were discarded to allow for magnetic saturation effects. Head movements were minimized using a vacuum cushion. Structural brain images were acquired during the habituation session and consisted of a T1-weighted 3D modified driven equilibrium Fourier transform (MDEFT) (11) (repetition time, 7.92 ms; echo time, 2.4 ms; time of inversion, 910 ms; flip angle, 15°; field of view, 256 × 224 mm^2; matrix size, 256 × 224; voxel size, 1 × 1 × 1 mm^3).

fMRI Analyses. Functional volumes were spatially normalized [standard Statistical Parametric Mapping (SPM)5 parameters] to an echo planar imaging template conforming to the Montréal Neurological Institute space and spatially smoothed with a Gaussian kernel of 8 mm at FWHM.

The analysis of fMRI data, based on a mixed-effects model, was conducted in two steps, accounting respectively for individual-level fixed effects and group-level random effects. For task 1, changes in brain regional responses were estimated using a general linear model in which auditory stimuli, as well as light onset and light

offset, were modeled as events convolved with a canonical hemodynamic response function. Ten regressors modeled the different prosody (angry, neutral) in each light condition (blue, green, dark) and photon density (lower, higher), and 12 regressors modeled onset and offset of the different wavelengths and photon densities. A parametric modulation by time was added to each regressor to track any linear change of the amplitude of brain responses to the auditory stimuli, light onset, and light offset across time. For task 2, each block was modeled using boxcar functions, convolved with a canonical hemodynamic response function. Six regressors modeled the six different block types. Because melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGC) do not cease firing at light offset (12), brain responses to light offsets are unlikely to represent a nonclassical response to light. The regressors derived from light offsets, for task 1, and realignment of the functional volumes, for both sessions, were thus considered as covariates of no interest. Analyses were first conducted irrespective of irradiance changes. A second set of (unconclusive) analyses included irradiance as a factor (*SI Results*). High-pass filtering was implemented in the matrix design using a cutoff period of 256 s in task 1 and of 128 s in task 2 to remove low-frequency drifts from the time series. Serial correlations in the fMRI signal were estimated using an autoregressive (order 1) plus white-noise model and a restricted maximum likelihood algorithm.

The summary statistic images resulting from the contrasts computed at the fixed-effect level were further smoothed (6-mm FWHM Gaussian kernel) and before entering in the random-effects analysis. The resulting set of voxel values for each contrast constituted maps of the t statistics thresholded at $P_{uncorrected} = 0.001$. Statistical inferences were performed after correction for multiple comparisons at a threshold of $P_{corrected} = 0.05$. Corrections for multiple comparisons were computed on the entire brain volume (family-wise error method) or on small spherical volumes around a priori locations of activation (small volume correction; 10-mm radius). Activations were expected in structures involved in the processing of emotional auditory stimuli, in arousal regulation, or in nonclassical effects of light on brain activity [as reported in our previous investigation (13–15)]. Brain areas to which the melanopsin-expressing ipRGC project or functionally linked to the suprachiasmatic nucleus were also considered as a priori locations of activation (see below for literature used). When used, inclusive and exclusive masks were thresholded at $P = 0.001$ and $P = 0.05$, respectively.

To investigate a possible effect of the sex of the participants, two-sample t tests were conducted at the random-effects level with sex as group factor (male, female) on the contrasts seeking for wavelength effects (blue vs. green) on brain responses to anger prosody of task 1.

Psychophysiological interaction (PPI) analyses were computed to identify any brain regions showing a significant change in functional connectivity with the left or right superior temporal gyrus/sulcus (STG/S) as a function of light exposure (blue vs. green) during the processing of anger or neutral prosody. In each individual, time-series of activity from the left and right STG/S were extracted from the local maxima detected within 10 mm of the STG/S peaks from the group analysis obtained when contrasting blue vs. green light for anger prosody stimuli. New linear models were prepared for both PPI analyses (for left and right STG/S) at the individual level, using three regressors. One regressor represented the contrast blue vs. green light for anger or neutral prosody stimuli (this regressor was positive under blue light exposure and negative for green light). The second regressor was the time-series of activity from the left or the right STG/S. The third regressor represented the interaction of interest between the first (psychological) and the second (physiological) regressors. To build this regressor, the underlying neuronal activity was first estimated by a parametric empirical Bayes formulation, combined with the psychological factor, and subsequently convolved with the hemodynamic response function (16). The model also included movement parameters used as covariates of no interest. A significant psychophysiological interaction indicated a change in the regression coefficients between any reported brain area and the left or right STG/S that was related to the negative or neutral auditory stimuli and more so under blue light than under green light exposure. Next, individual summary statistic images obtained at the first-level (fixed-effects) analysis were spatially smoothed and entered in a second-level (random-effects) analysis using one-sample t tests. Inferences were conducted as for the main-effect analysis. Because the PPI including the time-series of activity of the right STG/S did not reveal any significant difference of the light condition, we carried out four separate analyses testing for changes in the regression coefficients between the right STG/S and any reported brain area that was related to the negative or neutral auditory stimuli in one light condition but not the other (blue or green). The latter analyses were carried out as described except for the first regressor, which represented the main-effect blue or green light for anger or neutral prosody stimuli (this regressor was positive under blue light exposure or under green light).

A priori location of interest for fMRI multiple comparison corrections over small spherical volumes [x, y, z].

Frontal cortex: inferior frontal gyrus/sulcus [3, 39, −12 mm] (17).
Temporal cortex: superior temporal gyrus/sulcus [62, −30, 6 mm; 60, −15, 0 mm; 68, −20, 4 mm; 63, −13, −1 mm; 58, 6, −10 mm; −60, −24, 0 mm; −45, −15, −6 mm; −59, −12, 1 mm; −62, −14, 0 mm; −60, −2, −9 mm] (7, 17, 18).
Limbic areas: amygdala [16, −4, −18 mm;] (15, 19); hippocampus [−28, −24, −14 mm; 28, −24, −14 mm] (15).
Subcortical areas: hypothalamus [6, −6, −12, mm; 8, 0, −10 mm; −6, −12, −12 mm] (20–22).

Other Statistical Analyses. All other statistical analyses were computed with Statistica 6.1 (StatSoft). Repeated-measures ANOVAs with light condition (blue and green) and prosody (angry and neutral) as within-subject factors were computed on accuracy and reaction times for the gender discrimination task in fMRI tests and on emotional prosody ratings for the judgment task performed outside the scanner after the two fMRI sessions.

SI Results

Demographics. Subject characteristics are provided in Table S1.

Behavior. ***Accuracy.*** The gender discrimination task was well executed by all participants (accuracy >87% on average for all condition), indicating that the stimuli were well perceived. Repeated-measures ANOVA on accuracy with prosody (anger, neutral) and light condition (blue, green) as within-subject factors revealed no main effects of prosody [$F(1,16) = 1.93$; $P = 0.18$], no main effect of light condition [$F(1,16) = 0.002$; $P = 0.96$], and no interaction between prosody and light condition [$F(1,16) = 2.11$; $P = 0.17$; Fig. 2*A* in main text]. These results indicate that accuracy during this simple task was not influenced by the emotional or illumination conditions.

Reaction times. Subjects were instructed to privilege accuracy to reaction times and to wait for the sound to be over before responding. Reaction times were computed starting at the onset of the sounds, which were 750 ms long.

Repeated-measures ANOVA on reaction times during the gender decision task with prosody (anger, neutral) and light condition (blue, green) as within-subject factors revealed a main effect of prosody, with slower reaction times for anger stimuli [$F(1,16) = 24.69$; $P = 0.0001$] but no main effect of light condition [$F(1,16) = 0.1184$; $P = 0.74$] nor interaction between prosody and light condition [$F(1,16) = 1.1$; $P = 0.31$; Fig. 2*B* in main text].

These results demonstrate that the emotion of the auditory stimuli was well perceived by the participants in accordance with the literature (7) and that reaction times were not influenced by the light condition.

Emotional valence evaluation. In addition to verifying whether the difference in the emotional condition was well perceived by the participant, we wondered whether the light condition under which each voice stimuli was heard for the first time (during task 1) would influence the emotional valence attributed to the voice stimuli in task 3 (subjects were exposed to 50 voice stimuli of task 1 during task 2). Repeated-measures ANOVA on emotional valence scores attributed to the stimuli after the fMRI session with prosody (anger, neutral) and light condition (blue, green) in which these stimuli were presented during task 1 as within-subject factors confirmed a significant main effect of prosody, with more negative values for anger stimulation [$F(1,16) = 93.69$; $P < 10^{-6}$] but no main effect of light condition [$F(1,16) = 0.36$; $P = 0.56$] nor interaction between prosody and light conditions [$F(1,16) = 0.75$; $P = 0.4$; Fig. 2*C* in main text]. This result further confirmed that emotionality conveyed by the voice stimuli was unambiguously perceived by the participants. The light condition under which the stimuli were first heard during task 1 did not influence subsequent emotion judgment.

Functional MRI. ***Effect of sex.*** None of the two-sample *t* tests with sex as group factor (male, female) and testing for wavelength (blue, green) effects on brain responses related to anger prosody in task 1 showed any significant difference between the groups at the whole-brain level (even for a threshold of $P_{corrected} = 0.9$) or in a priori regions of interest (threshold: $P_{uncorrected} = 0.001$).

Note on possible influence of irradiance level on effects reported in main text. In addition to our main objective, which was to investigate the impact of the wavelength of the ambient light context, this experiment attempted to investigate irradiance impact on brain activity using two ambient light levels for each wavelength (3×10^{13} and 7×10^{12} photons per cm^2 per s). Results of the analyses including irradiance as a factor are reported here for completeness but cannot be considered as reliable, for two main reasons.

First, light sensitivity of the classical photopic luminance visual and nonclassical system follows a log function, and an irradiance change of only half a log unit, such as in the present experiment, does not represent a very substantial variation. Technical limitations at the time of the experiment did not allow for the administration of higher irradiance levels than the level used (3×10^{13} photons per cm^2 per s). In addition, recent research implies an important role for rods in the nonclassical impact of light in rodents at higher irradiance levels than previously thought (23, 24). This may suggest that in humans very low light levels could have a significant nonclassical impact on brain activity. At the time the research was planned and undertaken (2006–2007), we did not have that information. Because the sensitivity of melanopsin-expressing ganglion cells is relatively low (23), we were careful not to use irradiances that would be too low for detecting a significant impact of only 1 min of light exposure on ongoing nonclassical brain responses. We therefore chose to use a lower irradiance level of 7×10^{12} photons per cm^2 per s, which is only half the higher level used in this experiment.

The irradiance levels we used, differing by half a log unit, could be suitable to detect an impact of irradiance on melatonin suppression (25). However, it is unknown whether it would be the case using only 1-min exposures. In addition, the dose–response relationship for the nonclassical impact of light might differ with the aspect considered. For instance, pupil constriction seems to follow a different dose–response relationship in rodents (26).

Second, including the irradiance factor in the analyses reduces by a factor of 2 the number of trials included in each condition, which creates a statistical power issue. The sample size of the present study ($n = 17$) is important for a neuroimaging study, and further increasing it would not substantially increase its power. Indeed, SPM is known to become overly conservative below 12 observations, but in contrast, it is a common empirical finding that above 15 volunteers the addition of further observations does not substantially increase sensitivity. Results of a separate experiment including 27 subjects and using the same irradiance levels as in the present study but with a short-duration working memory task are also inconclusive and confirm the failure of the present approach to resolve the irradiance issue. This issue could only be resolved by increasing the number of trials per conditions. This could not be done in the present study without compromising the emotional aspect of the task because habituation occurs quickly within the emotional system (27).

Investigating the impact of irradiance of an illumination on ongoing brain activity requires designing a novel protocol in which the number of trials in each condition is increased using a task showing little habituation and irradiance levels differing by more that one log unit (or even more). We are currently conducting this type of research using unique equipment allowing higher irradiance levels.

Table S2 lists the differences in brain activity observed when including irradiance level as a factor in the analyses. Analyses of the irradiance factor yielded no clear results. The results could be taken to indicate that green light has a lower impact than blue light irrespective of the irradiance level, supporting the involvement of the nonclassical photoreception system at both irradiances. This seems consistent with the results of the other study that we reanalyzed. Finally, on the basis of these results, one may speculate that, with ambient blue light exposure, low irradiance has a higher impact than high irradiance, perhaps supporting an involvement of rods at lower light levels.

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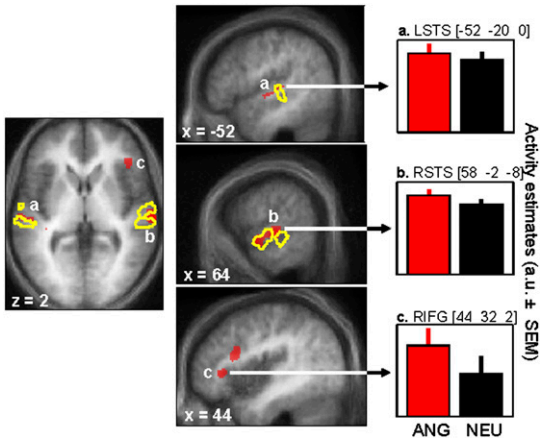

Fig. S1. Significant differences between the brain responses to anger and neutral prosody during the gender discrimination task (task 1). (*Left*) Statistical results of task 1 overlaid on the population mean structural image ($P_{uncorrected} < 0.001$). The yellow area corresponds to the voice-selective area in the temporal cortex identified in the voice localizer session (task 2) ($P < 0.05$ family wise error corrected for multiple comparisons over the entire brain volume). (*Right*) Mean activity estimates [arbitrary units (a.u.) ± SEM] of brain activity associated with anger (ANG, red) and neutral (NEU, black) prosody. (*A*) Left superior temporal gyrus/sulcus; (*B*) right superior temporal gyrus/sulcus; (*C*) right inferior frontal gyrus/sulcus.

Table S1. Subjects characteristics

N	17
Age (y)	22.3 ± 1.6
Body mass index (kg/m^2)	22.3 ± 2.7
Sex (male/female)	8/9
Sleep disturbanc	3.6 ± 1.7
Daytime propensity to fall asleep	5.1 ± 2.6
Chronotype	49.8 ± 5.7
Anxiety level	4.1 ± 3.7
Mood	3.6 ± 4
Right-handed participants	17
Ethnicity	Caucasian (all)

Values are number or mean ± SD. Sleep disturbance was determined by the Pittsburgh Sleep Quality Index Questionnaire (3). Daytime propensity to fall asleep was assessed (during daytime nonstimulating situations) by the Epworth Sleepiness Scale (2). Chronotype was assessed by the Horne–Östberg Questionnaire (1). Anxiety level was measured on the 21-item Beck Anxiety Inventory (4). Mood was assessed using the 21-item Beck Depression Inventory II (5). The Edinburgh Inventory (6) was administered to verify that the participants were right-handed.

Table S2. Impact of (low and high) irradiance level on brain activity

Brain area	Side	*x*, *y*, *z*	*Z* score	*P* value
Brain response to light onsets				
Blue light onset low > green light onset low, modulated by time				
Amygdala	R	18, −4, −20	3.27	0.041
Blue light onset high > green light onset low, modulated by time				
Amygdala	R	14, −8, −32	3.72	0.012
Brain response to anger prosody stimului				
Anger × (blue low > green low)*				
Superior temporal sulcus	L	−56, −10, −18	3.28	0.030
Hippocampus	L	−28, −28, −12	3.60	0.012
Anger × (blue low > green high)†				
Superior temporal sulcus	L	−60, −24, −16	3.75	0.009
Hippocampus	R	36, −20, −18	3.16	0.043
Psychophysiological interaction with the left STG/S				
Anger × (blue low > green low)				
Amygdala	L	−18, −14, −26	3.13	0.046

For the sake of concision, only contrasts with significant results are reported (i.e., all other possible contrasts were tested but yielded no significant results).

*Clusters not affected by an exclusive mask ($P = 0.05$) of the [neutral × (blue low > green low)] contrast, indicating that these effects were specific to the emotional (angry prosody) stimuli.

†Clusters not affected by an exclusive mask ($P = 0.05$) of the [neutral × (blue low > green high)] contrast, indicating that these effects were specific to the emotional (angry prosody) stimuli.