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Effect of Spectral Transmittance through Red-Tinted Rodent Cages on Circadian Metabolism and Physiology in Nude Rats

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Light entrains normal circadian rhythms of physiology and metabolism in all mammals. Previous studies from our laboratory demonstrated that spectral transmittance (color) of light passing through cages affects these responses in rats. Here, we addressed the hypothesis that red tint alters the circadian nocturnal melatonin signal and circadian oscillation of other metabolic and physiologic functions. Female nude rats (Hsd:RH-*Foxn1*^{rnu}; *n* = 12 per group) were maintained on a 12:12-h light (300 lx; 123.0 μW/cm^2; lights on 0600):dark regimen in standard polycarbonate translucent clear or red-tinted cages. After 1 wk, rats underwent 6 low-volume blood draws via cardiocentesis over a 4-wk period. Plasma melatonin levels were low during the light phase (1.0 ± 0.2 pg/mL) in rats in both types of cages but were significantly lower in red-tinted (105.0 ± 2.4 pg/mL) compared with clear (154.8 ± 3.8 pg/mL) cages during the dark. Normal circadian rhythm of plasma total fatty acid was identical between groups. Although phase relationships of circadian rhythms in glucose, lactic acid, pO_2, and pCO_2 were identical between groups, the levels of these analytes were lower in rats in red-tinted compared with clear cages. Circadian rhythms of plasma corticosterone, insulin, and leptin were altered in terms of phasing, amplitude, and duration in rats in red-tinted compared with clear cages. These findings indicate that spectral transmittance through red-colored cages significantly affects circadian regulation of neuroendocrine, metabolic, and physiologic parameters, potentially influencing both laboratory animal health and wellbeing and scientific outcomes.

Abbreviations: CCT, correlated color temperature; SCN, suprachiasmatic nuclei; TFA, total fatty acid.

Light is a potent biologic force essential to life on our planet. Circadian rhythms of behavior, physiology, and metabolism for all mammals, including laboratory animals, are entrained and regulated by the daily light–dark cycle.[1-3,5,7-12,28,33,68] These chronobiologic rhythms include locomotor activity,[2,3,23,25,27,50,60] feeding and drinking,[2,3,23,41] sleep–wake cycle,[2,3,5,23,25] hormone secretion,[2,17-19,22,31,42,58,59,65] body temperature,[23,66] intermediary metabolism,[6,16-18,24,41-43,44,68] immune function,[13,46] and cardiovascular[43] and neurologic,[49] functions and are essential to health and wellbeing. Alterations in intensity, duration, and spectral transmittance (quality or wavelength; perceived as color) of light at a particular time of the day can upset many of these diurnal rhythms.[7-12,16] Our previous investigations[5,15-18] showed that adherence to appropriate lighting and lighting protocols, as outlined in the *Guide for the Care and Use of Laboratory Animals*,[40] is not only beneficial but essential to the health and wellbeing of laboratory animals and experimental outcomes. We found that a small light leak of as little as 0.2 lx (0.08 μW/cm^2) during an otherwise normal dark phase (12 h) in our animal rooms elicited disruptions in circadian rhythms of plasma measures of endocrine physiology and metabolism in rats.[15-18] Circadian patterns of plasma melatonin, total fatty acid (TFA), glucose, lactic acid, corticosterone, pO_2, and CO_2 levels were altered markedly. Using a novel tissue-isolated human tumor model[4] and tumor perfusion technique[19] developed in our laboratory, we further demonstrated that light exposure at night suppresses normal nighttime melatonin production by the pineal gland, leading to changes in host metabolism and accelerated human cancer growth.[4,5] This early work provided in vivo laboratory experimental evidence demonstrating in humans that light exposure at night, as occurs in the night-shift worker population, increases the risk of breast cancer.[21,33,63]

More recent studies[16] revealed that the spectral transmittance passing through standard laboratory rodent cages significantly influences temporal coordination of circadian patterns of these same plasma measures of endocrine physiology and metabolism in pigmented, nude female rats. Animals were maintained in standard translucent clear, amber, or blue rodent cages and were assessed for plasma melatonin, total fatty acid, glucose, lactic acid, corticosterone, insulin, leptin, pO_2 and CO_2 levels at 6 circadian time points. Intensity and duration of light was maintained the same for all groups, but rats housed in amber- and blue-tinted cages were exposed to more light in the visible spectrum of either longer or shorter wavelengths, respectively, compared with animals maintained in the clear cages exposed to broad-spectrum visible light (380 to 750 nm). Results revealed major differences in circadian patterns of plasma measures of endocrine physiology and metabolism in rats maintained in the standard amber- and blue-tinted cages compared with those maintained in translucent clear cages.[16]

Our sense of vision, that is, our awareness of color, shapes, brightness and motion, occurs as a result of light detected by the retinal rod and cone photoreceptors in the eyes activating the neural pathway of the primary optic tract.[10,31,52] Below the level of consciousness, however, by means of the alternate

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neural pathway of the retinohypothalamic tract, we experience the most profound effects of light in our daily lives.[2,3,9,10,14,31,34,35,47,54] The neural pathway of the retinohypothalamic tract projects to the master circadian pacemaker residing in the superchiasmatic nuclei (SCN) of the hypothalamus, which regulates near-24-h rhythms, including sleep–wake cycle, body temperature, daily feeding, and secretory hormone patterns. In a process termed phototransduction, photic information is transmitted via the retinohypothalamic tract to the SCN, which signal the pineal gland via a polysnaptic pathway to produce the hormone melatonin (N-acetyl-5-methoxytryptamine) primarily during darkness.[59,60] The nocturnal rhythmic melatonin signal contributes to the temporal coordination of most of the aforementioned normal behavioral and physiologic functions. In this manner, ocular light exposure both entrains the circadian melatonin rhythm and may change the duration of the nighttime melatonin peak relative to the photoperiod. Generally, the most powerful wavelengths for circadian regulation of these responses in all mammals reside in the spectral region between 450 and 500 nm (perceived as blue part of the visible spectrum).[11] We also now know that longer wavelength light in the spectral region above 550 nm (perceived as yellow-red) of sufficiently high intensity and duration can acutely suppress melatonin and cause phase shifts or entrain circadian rhythms.[8,26,31,32,36,49-51,58,69]

In our previous investigations,[16] we examined the effect of different standard laboratory animal cage colors, specifically the principal wavelengths of visible and near-visible light they transmit to laboratory animals during the light phase, on circadian regulation physiology and metabolism. That initial study[17] revealed that temporal coordination of circadian rhythms of physiology and metabolism can be altered significantly in pigmented, nude female rats housed in either blue polycarbonate or amber polysulfone compared with polycarbonate translucent clear standard laboratory rodent cages.

For many years, red safety lamps (wavelengths above 650 nm) have been used during dark-phase laboratory animal light exposure and animal room rodent racks, lights, and observation windows have been covered with red-tint film primarily for, but not limited to, nighttime observation.[50-52] Indeed, even sodium yellow lamps have sometimes used during the animal dark phase.[53] This practice is based on the belief that the use of such lighting during both light and dark phases in animal rooms is less stressful, more healthful, and esthetically more appealing to both animals and animal care givers alike.[25] However, concern arose regarding the potential effect on physiology and metabolism influencing the health and wellbeing of laboratory animals maintained 24/7 during both light and dark phases in such environments. Previous work[9,31] now included in the current 2011 edition of *The Guide*[40] provided a clearer understanding of the effects on animals maintained in environments where they are exposed to monochromatic or polychromatic light whose spectral quality is predominantly above 600 nm in the red-appearing portion of the visible spectrum. The reasoning underlying this observation is based on early studies in humans and rodents showing that, although spectral transmittance of light in the yellow-orange– and red-appearing portions of the visual spectrum could influence circadian and neuroendocrine responses associated with the melatonin rhythm, the effects were somewhat weak.[7,10,11,69] Originally it was believed that the scotopic rod system of the eye, which contains the chromophore rhodopsin and is primarily responsible for night vision, is relatively insensitive to red-appearing light at wavelengths longer than 600 nm. An abundance of largely overlooked work during this same time period, however, provided considerable evidence that rodents do, indeed, respond to red-appearing light during both light and dark phases.[50,51,55,56,67,69] The end result today is that many investigators and animal care personnel assume that the use of monochromatic light above 600 nm for observation purposes, particularly during but not limited to nighttime, elicits little or no circadian-disruptive effect on normal circadian physiology and behavior. Arguably, adequate scientific justification for continual light- and dark-phase use of red-appearing lights, examining potential neuroendocrine and neurobehavioral metabolic and physiologic parameters in these laboratory animals, is sparse.

In the current study, we examined the hypothesis that spectral transmittance of polychromatic light passing through red-tinted rodent cages perturbs not only circadian melatonin production but the temporal coordination of normal metabolic and physiologic activities in pigmented female nude rats commonly used in cancer research. Our approach was to house these animals in translucent polycarbonate red-tinted cages compared with standard translucent clear polycarbonate laboratory rodent cages currently in common use. Caging differed only in coloration or tint (for example, red tint), thereby exposing rats during the light phase to different spectral transmittances of light; intensity and duration of light exposure remained constant for all rats.

Materials and Methods

Reagents. HPLC-grade chloroform, ethyl ether, methanol, glacial acetic acid, heptane, hexane, were purchased from Fisher Chemical (Pittsburgh, PA). Fatty acid and rapeseed oil methyl ester standards and boron trifluoride–methanol, potassium chloride, sodium chloride, sodium hydroxide, perchloric and trichloroacetic acids were purchased from Sigma Scientific (St Louis, MO). Ultrapure water was purchased from Cayman Chemical (catalog no. 400000, Ann Arbor, MI).

Animals, housing conditions, and diet. Female adult, pigmented, homozygous, athymic, inbred nude rats (Hsd:RH-*Foxn1*^{rnu}; age, 3 to 4 wk) were purchased from Harlan (Indianapolis, IN) and were certified by the vendor to be free of all known rodent bacterial, viral, and parasitic pathogens. Rats were maintained in an AAALAC-accredited facility in accordance with the *Guide*.[40] All procedures for animal use were approved by the Tulane University IACUC.

Rats were maintained in cages containing hardwood maple bedding (catalog no. 7090, Sanichips, Harlan Teklad, Madison, WI; 2 bedding changes weekly). To ensure that all rats remained infection-free from both bacterial and viral agents, serum samples from sentinel animals housed only on the combined soiled bedding from other study cages in the same housing unit were tested quarterly and during the course of this study by multiplex fluorescent immunoassay for rat corona virus, Sendai virus, pneumonia virus of mice, sialodacryoadenitis virus, Kilham rat virus, Toolan H1 virus, reovirus type 3, *Mycoplasma pulmoni,* lymphocytic choriomeningitis virus, mouse adenovirus 1 and 2, Hantaan virus, *Encephalitozoon cuniculi,* cilia-associated respiratory bacillus, parvovirus NS1, rat parvoviruses, and rat murine virus, rat theilovirus (IDEXX Research Animal Diagnostic Laboratory, Columbia, MO) and for external and internal parasites. All blood samples analyzed during the course of this study tested negative for the aforementioned bacterial and viral agents. Rats had free access to food (no. 5053 Irradiated Laboratory Rodent Diet, Purina, Richmond, IN) and acidified water. Quadruplicate determinations of this diet (100 g) contained 4.1 g total fatty acid, composed of 1.05% myristic (C14:0), 15.94% palmitic (C16:0), 1.47% palmitoleic (C16:1n7), 3.97% stearic

(C18:0), 22.22% oleic (C18:1n9), 54.55% linoleic (C18:2n6), and 0.26% arachidonic (C20:4n6) acids. Minor amounts of other fatty acids comprised 0.54%. Conjugated linoleic acids and trans fatty acids were not found. More than 90% of the total fatty acids (TFA) was in the form of triglycerides; more than 5% was in the form of free fatty acids.

Caging, lighting regimens, and spectral transmittance measurements. After a 1-wk acclimation period, rats were randomized into 2 of 6 animals each (3 per cage) and placed in translucent laboratory rodent cages (10.5 in. × 19 in. × 8 in.) that were either clear (control) or red-tinted (experimental). Rodent cages used in this study were purchased (polycarbonate translucent clear, catalog no. R20PC, Ancare, Bellmore, NY) or provided (polycarbonate translucent red-tinted, catalog no. 18780M, Lab Products, Seaford, DE). All cages were maintained with identical stainless steel lids (catalog number 10SS, Ancare) for holding food and water covered by polysulfone translucent clear microfilter tops (catalog no. N10MBT, Ancare). The SPF rats were maintained in environmentally controlled rooms (25 °C; 50% to 55% humidity) with diurnal lighting (12:12-h light:dark cycle; lights on, 0600). Animal rooms were lighted with a series of 3 overhead ballast–lamp systems each containing 4 cool-white fluorescent lamps each (model no. F32T8TL741, Alto Collection 32 W, Philips, Somerset, NJ); animal rooms were completely devoid of light contamination during the dark phase.[4,5,16-18]

Daily during the course of this experiment, the animal room was monitored for normal light-phase lighting intensity at 1 m above the floor in the center of the room (123.0 μW/cm^2; 300 lx) and outside, from within, and at the front of the animal cages (at rodent eye level). Irradiance measures used a silicon-diode detector head (model no. SEL033, International Light Technologies, Peabody, MA) with a wide-angle input optic (model no. W6849), and a filter (model no. F23104) provided a flat response across the visible spectrum. Illuminance measures used a silicon diode detector head (model no. SEL033) with a wide-angle input optic (model no. W10069) and a filter (model no. Y23104) to provide a photopic illuminance response. The meter and associated optics were calibrated annually and during the course of this study. All calibrations were based on the standard values of the US National Institute of Standards and Technology. Each day and at the same time (0800), prior to light intensity measurements for that day, all cages on the rack shelves (red cages on upper shelf; clear cages directly below on lower shelf; stainless steel shelf covering cage bonnet and stainless steel lid to occlude light entry through top of cage) were rotated one position to the right (placed at an identical, premeasured distance apart) in the same horizontal plane; the cage at position 2 (last position at right on the shelf) was moved to position 1 (first position at left on the shelf). Although there were no significant differences in light intensity as measured outside of and from within the front of each cage at each of 4 positions, the daily cage shift further ensured uniformity of intensity of ocular light exposure and accounted for the effects of any unforeseen subtle differences, such as light intensity or spectral transmittance perturbations, due to position on the rack shelf.

To minimize the potentially confounding effects of cage clouding or aging as a result of cage cleaning during the course of this study, only new cages were used. Cages were cleaned and sanitized by using a cage and rack washer–disinfector system (model no. GEW 112222, Getinge, Rochester, NY) with a high-detergency, low-alkalinity compound (catalog no. 18030F, Pharmacal, Naugatauk, CT) and a phosphoric acid washing detergent (catalog no. 08430F, Pharmacal) followed by sufficient fresh water rinsing at 82 °C (180 °F) for 3 min (total cycle time, 30 min) to reduce potential soap film buildup and clouding; although the cages were not autoclaved, the procedure described here resulted in thorough sanitation. Cages then were tested microbiologically by using a luminometer (model no. 001, novaLUM, Charm Sciences, Lawrence, MA). All cages underwent 4 cleaning treatments during the course of the study; the cleaning treatment did not introduce noticeable pitting, glazing, or cracking, which would affect light transmittance. Cage light intensity measurements made daily over the 6-wk course of this study showed no variation as a result of the cleaning procedure.

Under current convention, when discussing human and laboratory animal environments, the term 'luminous flux' (lx) is used to indicate the amount of light falling on a surface that stimulates the mammalian eye during daytime, or the perceived brightness to the eye (photometric values). Measures of luminous flux are appropriate for human daytime vision but are not appropriate for quantifying light stimuli that regulate circadian, neuroendocrine, or neurobehavioral physiology in animals or humans.[8,9,29,49,37] Consequently, radiometric values of irradiance (μW/cm^2) were measured in the cages by using the same equipment and system. Given these standards, the light stimuli reported here are presented in terms of lx and μW/cm^2 for ease of understanding.

Measures of spectral transmittance through cages were taken as previously described[16] in a test room whose measurements were nearly identical to those of the animal room and that had identical cool-white fluorescent lighting but no animals in the cages at time of testing. Briefly, correlated color temperature in degrees Kelvin (CCT), a measure of the color appearance of light transmitted into the cages as it relates to temperature in degrees Kelvin, and photon flux (photons/cm^2/s), the measure of light photons landing on a given area (cm^2),[39] were measured (model CL200A, Chroma Meter, Konica–Minolta, Tokyo, Japan). Spectral transmittances were quantified by using a hand-held spectroradiometer (FieldSpec, ASD, Boulder, CO). The 2 cage types (clear and red) were held in place, upside down, to cover the cosine receptor foreoptic attachment used for irradiance measurements. The optical sensor on the meter was centered inside each cage and oriented in each of 4 horizontal directions of the room while spectral power measurements were recorded. In addition, the sensor was directed upward, directly toward the overhead fluorescent lamps lighting the room, with a distance of 1.4 m to the light source. Spectral power distributions were recorded when the meter was pointing directly at the overhead fluorescent lighting source; this measurement was the most stable among the 5 measurements taken for each cage and, therefore, was used for comparison between cages.

Pearson correlations (Origin 8, OriginLab, Northampton, MA) were performed to determine similarities and differences of the spectral power distributions between cages. Based on irradiance measures, the correlation coefficient of the spectral power distributions from 380 to 760 nm was determined. As a more detailed method of analysis, the spectral power distribution was divided into 50-nm bins (that is, 400 to 700 nm) for the clear and red cages; Pearson correlations then were determined between cage conditions and combined with initial measurements.

Arterial blood collection. After 2 wk of the described lighting regimens, rats underwent a series of 6 low-volume blood draws via cardiocentesis to collect left ventricular arterial blood, as described previously,[4,5,16-19,61,62] over a period of 30 d. Briefly, blood collections on all 6 rats in each group were designated at 4-h intervals to comprise the 24-h feeding period (that is, 0400, 0800, 1200, 1600, 2000, and 2400). For instance, all rats in both

groups were tested at the same time point, that is, 0400; the next time point for sampling, that is 0800, was 5 d later; each animal was tested only once every 5 d to eliminate the effects on feeding, stress, and potential mortality for a combined total of 72 whole-blood samples (6 animals/group × 2 groups × 6 time points/animal). Each animal was lightly anesthetized by CO_2 inhalation by placement for 10 to 15 s into a 10 in. × 8 in. × 8 in. acrylic gas anesthetizing chamber (catlog no. AB2, Braintree Scientific, Braintree, MA) through which CO_2 and air was passed to approximate a 70% CO_2, 30% air environment; on first sign of unconsciousness (loss of righting reflex) and while still spontaneously breathing, the rat was removed from the chamber and placed in supine position while breathing room air unassisted in preparation for cardiocentesis; 1-mL samples were taken from the left ventricle by cardiocentesis (less than 5% total blood volume) via tuberculin syringe (25 gauge, 3/8 in.; Becton–Dickinson, Franklin Lakes, NJ) moistened with sodium heparin (1000 U/mL; Elkin-Sinn, Cherry Hill, NJ), as described previously.[16-19] Blood sampling during the dark phase (that is, 2000, 2400, 0400) was done under a safelight red lamp (120 V, 15 W; catalog no. 152 1517, model B, Kodak, Rochester, NY) to preserve the nocturnal melatonin surge.[4,5,16-19,38] Animal red-lamp exposure at eye level during the brief 45-s cardiocentesis procedure was no greater than 0.48 ± 0.01 lx (1.16 ± 0.04 μW/cm^2). The investigators have nearly 4 decades of experience in using this IACUC-approved cardiocentesis technique developed in the laboratory; there were no complications, such as moribundity or morbidity, due to anesthesia or cardiocentesis during the course of the investigation, and rats were immediately active after the procedure. Plasma samples were stored at −20 °C until assayed for melatonin, corticosterone, insulin, leptin, and TFA.

Arterial glucose, lactate, and acid–gas measurements. During the course of this study, arterial whole-blood samples were taken for measurements of pH, pO_2, pCO_2, glucose and lactate levels, and hematocrit by using a handheld analyzer (iSTAT1 Analyzer and CG4+ and CG8+ cartridges, Abbott Laboratories, East Windsor, NJ). Minimal levels of detection for pH, pO_2, pCO_2, glucose, and lactate were 0.01, 0.1 mm Hg, 0.1 mm Hg, 0.2 mg/dL, and 0.01 mmol/L, respectively.

Melatonin analysis. Arterial plasma melatonin levels were measured by using the melatonin rat ^{125}I radioimmunoassay kit (catalog no. BA 3500, Labor Diagnostika Nord, Nordham, Germany) and analyzed by using an automated gamma counter (Cobra 5005, Packard, Palo Alto, CA), as previously described.[16-19] The minimal detection level for the assay was 1 to 2 pg/mL.

Fatty acid extraction and analysis. Arterial plasma TFA, triglycerides, phospholipids, and cholesterol esters were extracted from 0.1-mL samples, as previously described.[4,5,16-19,61,62] Prior to extraction heptadecanoic acid (100 μg), which had been dissolved in chloroform (Fisher Scientific, Fair Lawn, NJ), was used as an internal standard. Methyl esters of fatty acids were analyzed by using a gas chromatograph (model no. 5890A, Hewlett Packard, Palo Alto, CA) fitted with a flame ionization detector (model no. 7673 A) autoinjector (model no. 7673 S), and integrator (model no. 3396A). All separations were done by using a 0.25-mm × 30-m capillary column (model no. 2380, Supelco, Bellefonte, PA) at 190 °C, with helium as the carrier gas (linear rate, 20 cm/s; split, 100:1). Injection port and detector were adjusted to 220 °C. All methyl esters were identified on the basis of their retention time compared with that of known standards. Minimal limit of detection for the assay was 0.05 μg/mL.

ELISA of corticosterone, insulin, and leptin. Arterial plasma samples were prepared in duplicate for measurement of corticosterone, insulin, and leptin levels by using chemiluminescent ELISA diagnostic kits for corticosterone (mouse–rat; protocol version 4-09/11; catalog no. 55-CORMS-E01, ALPCO, Salem, NH), insulin (rat, high range, protocol version V2.0; catalog no. 80-INSRTH-E01), and leptin (mouse–rat; protocol version 030112; catalog no. 22-LEPMS-E01). Samples were measured by using a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA) at 450 nM. Detection sensitivity for corticosterone, insulin, and leptin plasma analyses were 4.5 ng/mL, 0.124 ng/mL, and 10 pg/mL, respectively; lower limits of the assays were 15 ng/mL, 0.15 ng/mL, and 10 pg/mL, respectively; and coefficients of variation of all assays were less than 4.0%.

Statistical analysis. All data are presented as the mean ± 1 SD unless otherwise noted, using 6 animals per group, as reported in previous investigations[4-6,16-18,20] by statistical within-groups comparison; 6 rats in each group are included because duplicate determinations are required for confidence in the data. The rats in the red and clear cage groups were compared by using 2-way ANOVA for repeated measures followed by a Bonferroni multiple comparison test to evaluate statistical significance (Prism, GraphPad, La Jolla, CA). Differences between group means were considered statistically different at a P value of 0.05. The experiment was repeated once.

Results

Animal room illumination and caging spectral transmittance measurements. Daytime animal room illumination (mean ± 1 SD) at the center of the room and at 1 m above the floor (radiance detector facing upward toward light ballasts) had relatively small variance (n = 82 measurements) and was 420.54 ± 3.51 lx (172.42 ± 1.44 μW/cm^2). Measurements of photometric illuminance (lx) and radiometric irradiance (μW/cm^2) from outside and inside the front of each cage (Figure 1) made daily at position 1 through 4, as cages were shifted left-to-right at the same level (red-tinted cages, upper shelf; clear cages, lower shelf) on the caging racks and showed little to no intercage variability (n = 98 measurements per group; red-tinted cages: outside at front of cage, 174.98 ± 4.07 lx [71.74 ± 1.67 μW/cm^2]; inside at front of cage, 99.66 ± 2.24 lx [40.86 ± 0.92 μW/cm^2]; clear cages: outside at front of cage, 131.30 ± 3.51 lx [53.83 ± 1.44 μW/cm^2]; inside at front of cage, 99.02 ± 3.4 lx [40.58 ± 1.25 μW/cm^2]). Spectral power distributions of light measured through the wall of each cage type are illustrated in Figure 2. The data are typical of this type of fluorescent lamp, with signature peaks in the appropriate wavelengths.

Light testing measurements, including the interior radiometric and photometric values of the clear and red cages used in this study, are presented in Table 1. The measured CCT of the fluorescent lamps in the room was 3250 K; whereas the room CCT measurement and that for the clear cages did not differ, the red-tinted cage CCT was markedly lower than that for the room. The recorded irradiance, illuminance, and photon density values revealed key differences in light transmittance, depending on the color of the cage. We chose 4 peak irradiance values correlating to the mercury and phosphor emissions that are typical of this type of fluorescent light source[39] to compare peak amplitudes between cage types (Table 2). Compared with clear cages, red-tinted cages revealed substantial irradiance value differences near the blue–green portion (424 to 532 nm) of the visible spectrum, with smaller irradiance differences in the red portion (610 to 700 nm) of the visible spectrum, indicating that much less light at wavelengths below 550 nm is transmitted in

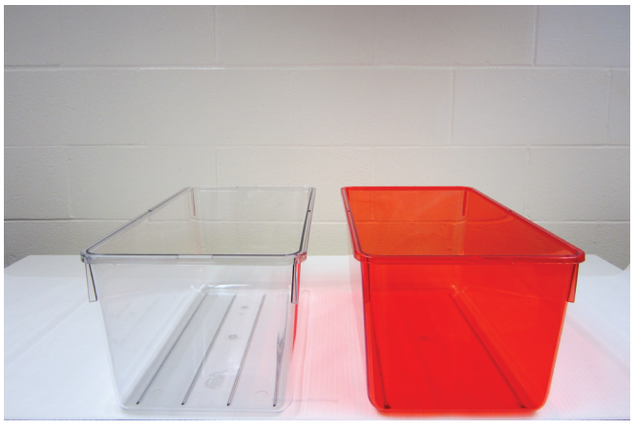

Figure 1. Photoimage showing the standard polycarbonate translucent clear (left, C) and red (right, R) rat cages. All animal cages were of equal dimensions (19 in. × 10.5 in. × 8 in.; wall thickness, 0.1 in.) and were autoclavable to 121 °C.

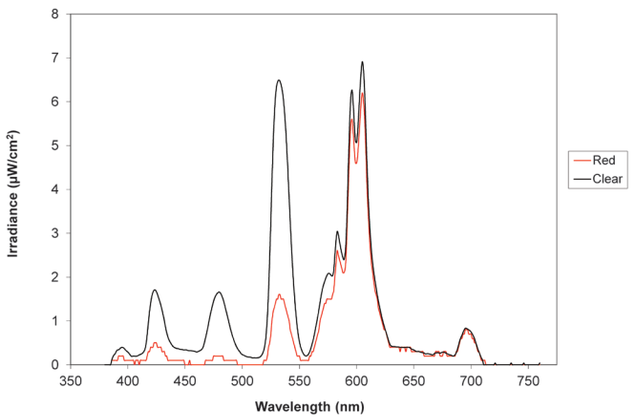

Figure 2. Measurements of spectral transmittance through standard polycarbonate translucent clear and red-tinted animal cages.

red-tinted cages than in the clear cage. This reduction in peak amplitudes in the lower region of the visible spectrum in the red-tinted cages provides the appearance perceived as red in the red-tinted cages. Table 3 illustrates these major differences in photon flux (across 50-nm wavelength bins) below 550 nm.

The Pearson correlation coefficient between cage types (data not shown) did not demonstrate a significant difference, on the basis of the linearity of the curves. Significant ($P < 0.05$) differences in amplitude between cage types can be seen clearly at several of the peaks (Figure 2), with red cages displaying smaller peak amplitudes in the short- and intermediate- wavelength portions of the visible spectrum.

Dietary, water intake, and body growth rates. Dietary and water intakes and body growth rates did not differ among rats maintained in the various caging groups during the course of this study. The daily dietary and water intakes were 9.5 ± 0.7 g and 12.7 ± 1.9 mL per 100 g body weight daily, respectively; daily body growth rates ($n = 36$ measurements per group) were 2.4 ± 0.2 g/d (rats in clear cages) and 2.4 ± 0.4 g/d (rats in red-tinted cages), respectively.

Plasma melatonin values. Diurnal rhythms in concentrations of plasma melatonin were similar among all rats and groups (Figure 3): low (less than 10 pg/mL) during daytime and significantly ($P < 0.001$) higher during the dark phase, with peak levels occurring between 2400 and 0400, and decreasing

Table 1. Radiometric and photometric values inside the translucent clear and red cages

	Clear cage	Red cage
Irradiance (μW/cm$^{2)}$	84	63
Photon flux (photons/cm^2/s)	1.20 x 10^{15}	0.72 x 10^{15}
Illuminance (lx)	238 lx	162 lx
CCT (K)	3250	2300

to a nadir between 1200 and 1600. Peak dark-phase melatonin levels for rats in clear cages were nearly 2-fold higher ($P < 0.05$) than those of rats in red-tinted cages. There were no differences in either the phase (for example, timing) or duration of the nocturnal melatonin signal between the 2 groups of rats. The integrated mean levels of melatonin over the 24-h period for rats in clear cages were nearly 2-fold higher than those of animals in red-tinted cages.

Arterial plasma TFA. We assessed the diurnal rhythms in the concentrations of arterial blood plasma TFA of female nude rats with free access to food and water (Figure 4). The plasma lipid levels followed that of the normal feeding pattern, as reported earlier.[16-18] Plasma TFA measured during the light phase (1600) were significantly ($P < 0.001$) less than those measured during the dark phase (0400) in both groups, but peak values (at 0400) did not differ between groups. In addition, calculated total TFA areas assessed over the 24-h day for curves shown in Figure 4 were not significantly different between rats in red-tinted (29.0 mg/mL) and compared with clear (30.3 mg/mL) cages.

Arterial blood glucose, lactate and acid–gas levels. Phase shifts in levels of arterial blood glucose, lactate), pO_2, and pCO_2 were determined by comparing the peak values (acrophases) from rats kept in red-tinted cages with those of rats in clear cages (Figure 5). A 'phase advance' was defined as a shift in a peak level to an earlier time (that is, from 1200 to 0800), whereas a 'phase delay' was defined as a shift to a later time (that is, from 0400 to 0800), as compared with values for rats housed in clear cages. Daily rhythms for arterial glucose and lactate concentrations (Figure 5 A and B) followed near-identical patterns in both groups and demonstrated 2 peaks, one occurring during the midlight phase (1200) and the other occurring during early dark phase (2000). Total calculated areas under the glucose curves over the 24-h day were lower ($P < 0.05$) in rats in red cages (1692 ± 149 mg/dL) than in those in clear cages (1829 ± 165 mg/dL). Blood glucose concentrations calculated over the 24-h day were 153.4 ± 2.4 mg/dL for rats in clear cages and 141.6 ± 1.5 mg/dL for those housed in red-tinted cages. Total calculated areas under the lactate curves over the 24-h day were lower ($P < 0.05$) in rats in red (13.87 ± 1.21 mmol/L) compared with clear (14.28 ± 1.25 mmol/L) cages. Blood lactate concentrations calculated over the 24-h day were 1.20 ± 0.01 mmol/L for the group in clear cages and 1.16 ± 0.01 mmol/L for that in red caging.

Daily rhythms in arterial pO_2 (Figure 5 C) were nearly identical between groups, with peak values occurring in the late light phase (1600) and lowest values in the middark phase (2400). Daily arterial pO_2 assessed over the 24-h day (Figure 5 C) for groups C and R shown in were significantly different from one another and are reported here as a mean ± SD for the 2 groups at 149.2 ± 2.2 and 139.7 ± 2.6 mm Hg, respectively ($n = 6$/group). Daily rhythms for arterial pCO_2 (Figure 5 D) were nearly identical for animals in groups C and R with a major peak value occurring during the light phase at 0800 followed by a minor peak at 2000. Total daily mean arterial pCO_2 values were significantly ($P < 0.05$) lower in group R than group C. Calculated average mean daily arterial pO_2 assessed over the

Table 2. Irradiance values (μW/cm^2) and comparisons of peak wavelength differences

Peaks		424 nm	480 nm (blue)	532 nm	605 nm (red)
Measured irradiances	Clear cage	1.71	1.66	6.49	6.91
	Red cage	0.47	0.19	1.57	6.67
Percentage difference		72.5%	88.6%	75.8%	9.8%

Table 3. Photon flux across 50-nm wavelength bins

	Photon flux (photons/cm^2/s)					
	400–450	451–500	501–550	551–600	601–650	651–700
Clear cage	7.28×10^{13}	9.27×10^{13}	3.16×10^{14}	3.20×10^{14}	2.84×10^{14}	6.20×10^{13}
Red cage	2.05×10^{13}	1.08×10^{13}	7.65×10^{13}	2.65×10^{14}	2.58×10^{14}	5.84×10^{13}

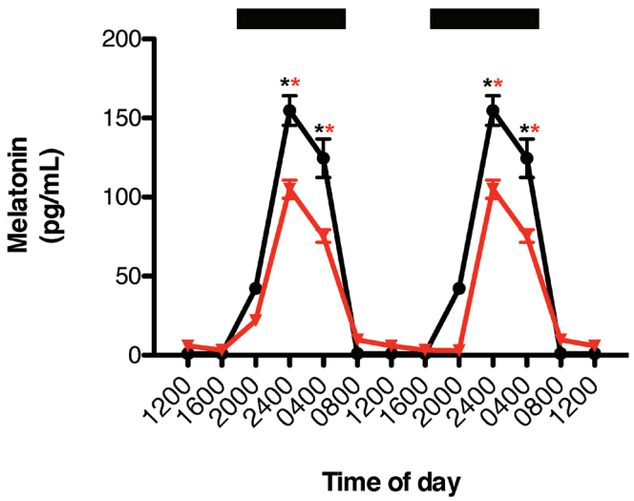

Figure 3. Diurnal plasma melatonin levels (pg/mL; mean ± 1 SD) of female nude rats (n = 6 per group) maintained for 6 wk in a controlled 12:12-h light:dark cycle (300 lx; 123 μW/cm^2; lights on, 0600) in either a standard polycarbonate translucent clear (black circles) or red-tinted (red triangles) rodent cage. Data for two 24-h periods are plotted. Rats were exposed to dark-phase lighting cycles from 1800 to 0600 (dark bars). Concentrations with asterisks are different ($P < 0.05$) from concentrations without asterisks of the same group: interaction of time and cage color, $P < 0.0001$; effect of time, $P < 0.0001$; effect of cage color, $P < 0.0001$.

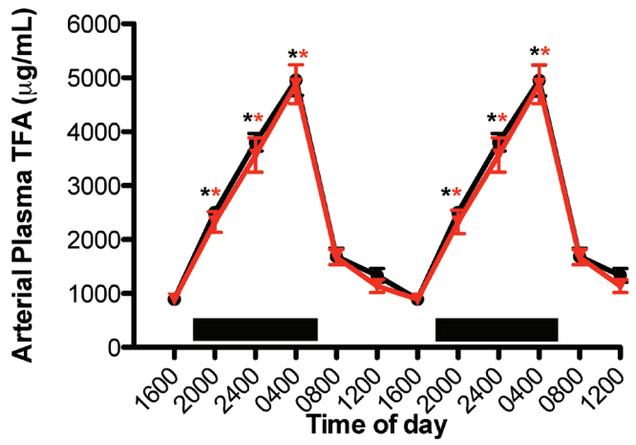

Figure 4. Diurnal changes in the blood plasma lipid concentrations in the arterial blood of adult female nude rats fed normal chow ad libitum and maintained in standard polycarbonate translucent clear (black circles) or red-tinted (red triangles) rodent cages. Rats were exposed to dark-phase lighting cycles from 1800 to 0600 (dark bars). TFA values (mean ± 1 SD; n = 6 per group) were the sums of myristic, palmitic, palmitoleic, stearic, oleic, linoleic, and arachidonic acids collected at the various time points. Data for two 24-h periods are plotted. Concentrations with asterisks are different ($P < 0.05$) from concentrations without asterisks of the same group: interaction of time and cage color, not significant; effect of time, $P < 0.0001$; effect of cage color, not significant.

24-h day for curves shown in Figure 5 D were significantly different from one another ($P < 0.05$) and are reported here as a mean ± SD for the 2 groups at 30.8 ± 0.5 and 28.8 ± 0.5 mm Hg.

Arterial blood pH, O_2 saturation, and hematocrit remained relatively constant for both groups over the 24-h day at 7.43 ± 0.071, 99.1 ± 0.01%, and 45.2 ± 0.05 (n = 72), respectively. These values are consistent with carotid arterial values in blood acid-gases determined in previous cardiocentesis investigations at this time of day.[16-19]

Arterial plasma corticosterone, insulin, and leptin concentrations. Plasma corticosterone levels (Figure 6 A) revealed clear differences between groups with regard to integrative concentrations. Values for arterial plasma corticosterone in both groups began to increase ($P < 0.05$) at the end of the light phase, with peak levels ($P < 0.05$) for animals in clear cages occurring at 1600 that eventually decreased to a nadir ($P < 0.05$) at the middark phase (2400). A second but lower-amplitude peak ($P < 0.05$) occurred in clear-caged rats near the end of the dark phase at 0400, decreasing to a nadir ($P < 0.05$) at 0800. Peak corticosterone levels for red-caged rats were phase-delayed 4 to 8 h compared with those of rats in clear cages, peaking ($P<0.05$) at 2400 and decreasing to a nadir during the light phase at 1200. Integrated plasma corticosterone concentrations calculated over the 24-h day were significantly ($P < 0.05$) different between rats in clear (350.5 ± 2.0 ng/mL [1016.5 ± 5.8 nmol/L]) compared with red-tinted (421.0 ± 2.0 ng/mL [1221.0 ± 5.8 nmol/L]) cages.

Similar to corticosterone concentrations, plasma concentrations of insulin (Figure 6 B) showed clear differences between groups with regard to daily rhythms and integrative levels. Values for arterial plasma insulin in animals in clear cages increased to highest levels 2 h after onset of the light phase (0800), with a secondary minor peak prior to onset of the dark phase (1600) and lowest levels occurring throughout the late light and early dark phases (1600 to 2400). In contrast, rats in red cages showed steadily increasing insulin levels from the onset of the dark phase. The duration of peak insulin levels was greater ($P < 0.05$) in red-caged than in clear-caged rats. In rats in clear cages, insulin levels rapidly declined over a 4-h interval from their peak at 0800 to their lowest levels at 1200, whereas red-caged rats experienced a gradual decline over 12 h from peak insulin concentration at 2400 to the lowest levels (1200).

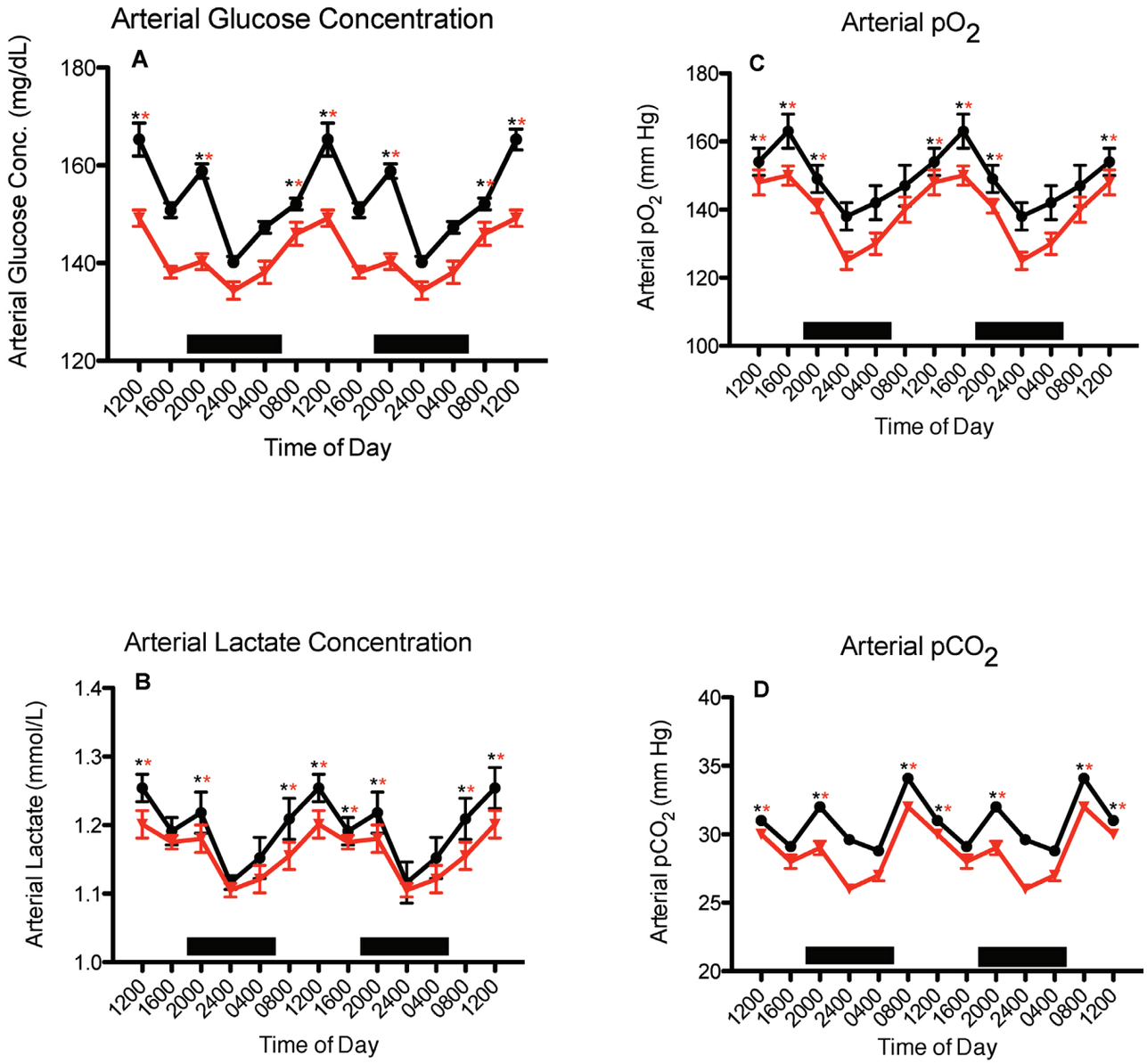

Figure 5. Diurnal changes in (A) glucose, (B) lactate, (C) pO_2, and (D) pCO_2 (mean ± 1 SD; $n = 6$ per group) in the arterial blood of female nude rats maintained in either a standard polycarbonate translucent clear (black circles) or red-tinted (red triangles) rodent cage. Rats were exposed to dark-phase lighting cycles from 1800 to 0600 (dark bars). Data for two 24-h periods are plotted. Levels with asterisks are different ($P < 0.05$) from levels without asterisks of the same group. Total calculated levels of glucose, lactic acid, PO_2, and PCO_2 were significantly different between rats housed in clear compared with red cages: interaction of time and cage color, $P < 0.0001$; effect of time, $P < 0.0001$; effect of cage color, $P < 0.0001$.

Integrated plasma insulin concentrations calculated over the 24-h day were significantly ($P < 0.05$) different between groups (clear cages, 2.60 ± 0.06 ng/mL; red cages, 1.65 ± 0. 05 ng/mL).

Plasma concentrations of leptin (Figure 6 C) also revealed clear differences between rats in clear compared with red-tinted cages. Rats in clear cages demonstrated a sharp peak in arterial plasma leptin levels at the middark phase (2400), but those in red cages had a much lower peak at this same time point. A nadir in blood leptin concentrations was reached at 0400 (clear cages) or 0800 (red cages). A second similar, albeit broader, peak in leptin levels occurred in both groups 16 h later, near the end of the light phase (1600 h); these secondary peaks did not differ between groups. Integrated plasma leptin concentrations calculated over the 24-h day were significantly different ($P < 0.05$) between groups (clear cages, 3.10 ± 0.05 ng/mL; red-tinted cages, 2.88 ± 0.06 ng/mL).

Discussion

Light influences mammalian circadian behavioral, physiologic, and metabolic processes in profound ways.[3,8,16-19,31,32] The master biologic clock is located in the SCN, is entrained by daily alteration of the light–dark cycle and the nocturnal melatonin rhythm, and influences these processes. Circadian rhythms in cellular physiology and metabolism become disrupted in the absence of signals from the SCN, in turn negatively influencing overall animal physiology and metabolism. Therefore, appropriate light and lighting cycles, as outlined in the *Guide*, are

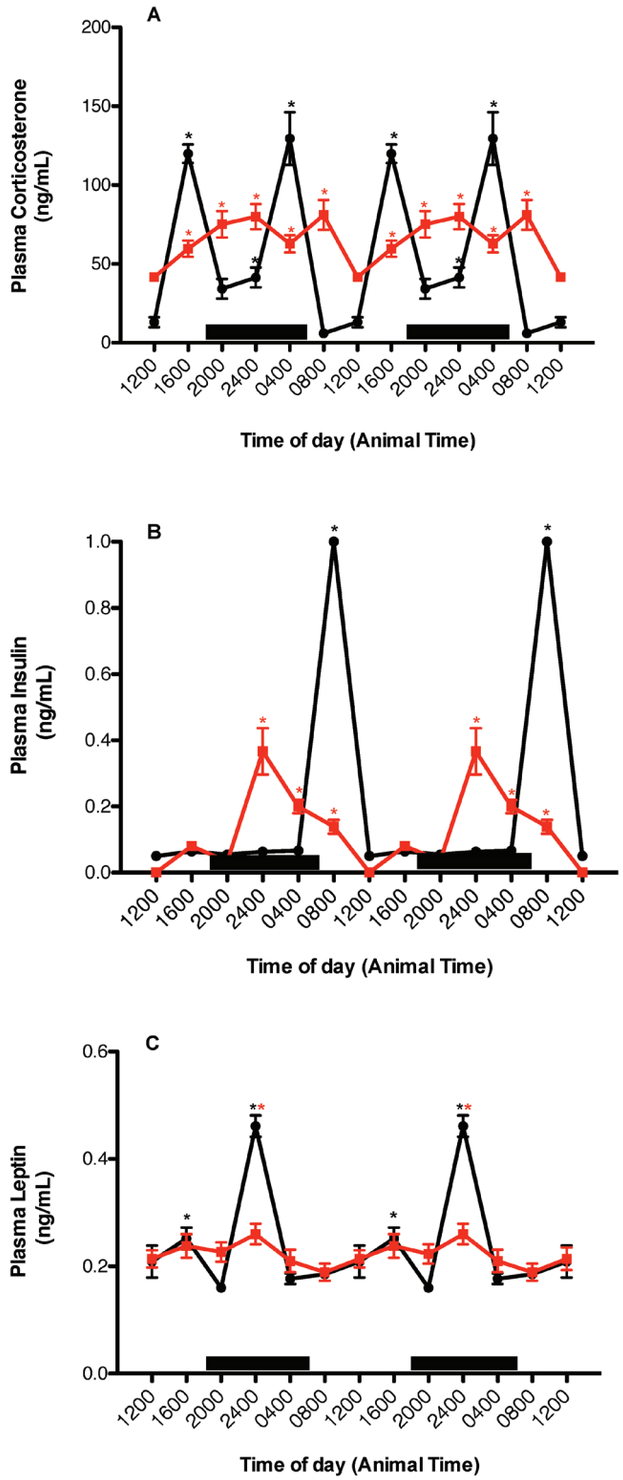

Figure 6. Diurnal changes in plasma (A) corticosterone, (B) insulin, and (C) leptin concentrations (mean ± 1 SD; $n = 6$ per group) in the arterial blood of rats maintained in either a standard polycarbonate translucent clear (black circles) or red-tinted (red triangles) rodent cages. Data for two 24-h periods are plotted. Rats were exposed to dark-phase lighting cycles from 1800 to 0600 h (dark bars). Concentrations with asterisks are different ($P < 0.05$) from concentrations without asterisks of the same group: interaction of time and cage color, $P < 0.0001$; effect of time, $P < 0.0001$; effect of cage color, $P < 0.0001$ (corticosterone), $P = 0.002$ (insulin), $P = 0.01$ (leptin).

essential for maintaining the health and wellbeing of laboratory animals used in scientific investigations, the results of which are often used for our understanding of human physiology and disease. Because red- and yellow-tinted lighting environments are often used in animal facilities to facilitate observation during both the light and dark phases, understanding of the influence these colors on the physiology and metabolism in laboratory animals is essential. Here, we examined the changes in various plasma measures of endocrine physiology and metabolism in pigmented female nude rats maintained in red-tinted laboratory cages.

The photic environments of the clear and red-tinted laboratory rodent cages we used in the current study reveal marked differences in the radiometric and photometric data obtained. These photic environments differed primarily in the spectral transmittance of light (color) passing into the cage. Irradiance, photon flux, illuminance, and CCT values for both the translucent clear and red-tinted polycarbonate cages were normalized relative to cage location on the holding racks. Thus, rats housed in either clear or red-tinted polycarbonate laboratory cages were maintained in environments in which both the intensity and duration of lighting remained constant during the light phase. We tested the hypothesis that daily rhythms of arterial blood melatonin, TFA, glucose, lactic acid, acid–gases, corticosterone, insulin, and leptin concentrations are altered in pigmented nude rats exposed to either light in cages that transmit less light at wavelengths below 550 nm as compared with those that receive more visible light (390 to 550 nm) transmitted through clear cages during the light phase. Except for the circadian rhythm in blood TFA levels, all physiologic and metabolic rhythms significantly changed in response to exposure to red light during the light phase of a 12:12-h light:dark cycle. Depending on the circulating factor measured, these alterations included changes in rhythm amplitude, phasing (for example, timing), duration, and combinations of these rhythm characteristics. These altered rhythms appeared to be independent from the SCN-generated rhythms in dietary intake of TFA, as discussed later.

Although the timing or duration of the nocturnal circadian plasma melatonin signal were not different between rats housed in the 2 different types of cages, there were very marked differences in the melatonin amplitudes. The amplitude of nocturnal plasma melatonin in the rats housed in clear cages was nearly 40% higher than that in rats housed in red-tinted cages. To our knowledge, this finding marks the first time that the exposure of a rodent species to a spectral transmittance of light minimalized in the 390- to 550-nm range during the light phase has been shown to induce a marked decrease in the melatonin amplitude during the subsequent dark phase. Our previous study[16] reported that melatonin levels for animals housed in blue-tinted polycarbonate cages (spectral transmittance of light enhanced in the 450 to 480 nm range) were more than 7-fold higher than those of rats housed in clear polycarbonate cages. The physiology by which either the wavelength or intensity of light (or their combination) during the light phase induces enhanced or, in this case, abrogated melatonin production during the subsequent dark phase of a 24-h day is unknown.

The phasing and durations of the daily oscillations in arterial glucose, lactate, pO_2, and pCO_2 in rats housed in red-tinted cages closely matched those in rats maintained in clear cages (Figure 5). However, both the individual and overall 24-h integrated levels of these analytes were significantly lower in rats in red compared with clear cages. These findings suggest that the basal metabolic rates of rats maintained in red-tinted cages were somewhat lower than those of rats housed in clear cages.

Elevated levels of corticosterone have long been associated with a variety of stressors, including anxiety, fear, pain, hemorrhage, infections, low blood glucose, and starvation.[22,30,42,57,64] Corticosterone, a potent glucocorticoid, acts on important metabolic tissues, including muscle, liver and adipose tissues, to alter metabolism and provide the animal with necessary bioenergetic fuels to cope with stress.[16] A circadian rhythm in circulating plasma corticosterone has been well documented.[16,17,22,30,43,64] Corticosterone peak amplitudes vary somewhat depending on the animal strain and sex, with female rats having somewhat higher daily concentrations and male rats demonstrating a more consistent circadian rhythm.[16,17] In our study, the rats housed in red compared with clear cages had large differences in the circadian rhythm of plasma corticosterone. Rats housed in translucent clear cages (Figure 6), maintained under a normal 12:12-h light:dark cycle showed 2 major peaks of circulating corticosterone levels, one near the end of the light phase followed by a second, slightly higher-amplitude, peak near the end of the dark phase. Although the integrated peak values over a 24-h period for rats maintained in red-tinted cages were somewhat higher (20%) and significantly different ($P < 0.05$) than those of the clear-cage group, there were no extreme fluctuations in peak amplitude, particularly during nighttime peak activity period. This pattern might suggest that the rats maintained in the red-cage environment have developed circadian corticosterone patterns reflective of a reduced allostatic load on the animals, which would be consistent with arterial blood glucose, lactate, pO_2, and pCO_2 metabolic findings presented previously (Figure 5).

In addition to corticosterone, the largest changes in circadian hormone rhythms were observed in plasma insulin and leptin in rats maintained in red-tinted cages. Shifts in insulin rhythm phasing accompanied by alterations in rhythm amplitude and duration were more prominent in rats housed in red-tinted as compared with clear cages. In comparison, the leptin rhythm was completely abolished in the red-tinted cage group. Plasma leptin levels normally are associated with feeding and insulin release, and the tight coupling of leptin to feeding may provide an additional meal-timing signal to the brain to accompany information on fat storage.[6,24,45,47] Consistent with findings reported previously,[16] the information presented here argue strongly that changes in the spectral transmittance of light that an animal experiences, particularly during daytime, are tightly coupled to both circadian leptin and insulin levels. Furthermore, the lower circadian glucose and insulin levels in rats maintained in red-tinted compared with clear cages suggest that the animals are more insulin-sensitive.[24] Additional studies now underway may provide important support in this regard.

Melatonin exerts regulatory effects on both glucose and lactic acid metabolism and on corticosterone, insulin, and leptin production in humans[6,42,45,47,] and rats.[22,24,43] One can speculate that the marked changes in the circadian amplitude of melatonin, particularly in rats exposed to light in the red-tinted cages, may have altered at least some of the daily rhythm changes observed in these analytes. Other mechanisms need to be considered as well, including melatonin-independent circadian hormonal or neural outputs from the SCN. Nevertheless, to our knowledge, this report is the first to show a direct association between differences in laboratory cage hue and accompanying spectral transmittance, abrogation of the normal nighttime melatonin signal, and disruptions in the circadian profiles of blood acid gases, glucose, lactate, corticosterone, insulin, and leptin in a mammalian species.

Consistency in the spectral quality of light (perceived as color), lighting intensity, and duration is essential in the maintenance of stable animal models in laboratory animal facilities around the world. Minor perturbations in these parameters and lighting protocols will affect virtually every biologic response associated with animal physiology and metabolism. Additional studies are needed to determine whether combined factors (for example, spectral transmittance and photon density) are required to evoke the biologic changes we found in the current investigation. Our study provides compelling evidence that, compared with rats maintained in translucent clear cages and exposed to the broad visual spectrum of light, rats maintained in red-tinted laboratory cages (which differed from the clear cages only in the spectral transmittance or quality of light that passed through the cage) develop chronobiologic disruptions in plasma measures of endocrine metabolism and physiology.

Taken together with the results of our previous study, the present findings indicate an important consideration for scientific investigators and animal care personnel in the development of future protocols and investigations as well as subsequent editions of the *Guide.*

Acknowledgments

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References

1. **Aggelopoulos NC, Meissl H.** 2000. Responses of neurons of the rat suprachiasmatic nucleus to retinal illumination under photopic and scotopic conditions. J Physiol **523:**211–222.
2. **Aschoff J, editor.** 1981. Handbook of behavioral neurobiology, biological rhythms, p 1–581. New York (NY): Plenum Press.
3. **Berson DM, Dunn FA, Takao M.** 2002. Phototransduction by retinal ganglion cells that set the circadian clock. Science **295:**1070–1073.
4. **Blask DE, Dauchy RT, Sauer LA, Holowachuk E, Ruhoff M, Kopff H.** 1999. Melatonin inhibition of cancer growth in vivo involves suppression of tumor fatty acid metabolism receptor-mediated signal transduction events. Cancer Res **59:**4693–4701.
5. **Blask DE, Brainard GC, Dauchy RT, Hanifin JP, Davidson LK, Krause JA, Sauer LA, Rivera-Bermudez MA, Dubocovich ML, Jasser SA, Lynch DT, Rollag MD, Zalatan F.** 2005. Melatonin-depleted blood from premenopausal women exposed to light at night stimulates growth of human breast cancer xenografts in nude rats. Cancer Res **65:**11174–11184.
6. **Boden G.** 2001. Obesity, free fatty acids, and insulin resistance. Curr Opin Endocrinol Diabetes Obes **18:**139–143.
7. **Brainard GC.** 1989. Illumination of laboratory animal quarters: participation of light irradiance and wavelength in the regulation of the neuroendocrine system, p 69–74 In: Guttman HN, Mench JA, Simmonds RC, editors. Science and animals: addressing contemporary issues. Bethesda (MD): Scientists Center for Animal Welfare.
8. **Brainard GC, Hanifin JP.** 2005. Photons, clocks, and consciousness. J Biol Rhythms **20:**314–325.
9. **Brainard GC, Hanifin JP, Rollag MD, Greeson JM, Byrne B, Glickman G, Gerner E, Sanford B.** 2001. Human melatonin regulation is not mediated by the 3-cone photopic visual system. J Clin Endocrinol Metab **86:**433–436.
10. **Brainard GC, Richardson BA, King TS, Matthews SA, Reiter RJ.** 1983. The suppression of pineal melatonin content and N-acetyl-transferase activity by different light irradiances in the

Syrian hamster: a dose–response relationship. Endocrinology **113:**293–296.

11. **Brainard GC, Richardson BA, King TS, Reiter RJ.** 1984. The influence of different light spectra on the suppression of pineal melatonin content in the Syrian hamster. Brain Res **294:**333–339.
12. **Brainard GC, Vaughan MK, Reiter RJ.** 1986. Effect of light irradiance and wavelength on the Syrian hamster reproductive system. Endocrinology **119:**648–654.
13. **Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ.** 2005. A review of the multiple actions of melatonin on the immune system. Endocrine **27:**189–200.
14. **Commission Internationale de l'Eclairage.** 2004. Proceedings of the CIE Symposium on Light and Health: Nonvisual Effects. Vienna (Austria): Commission Internationale de l'Eclairage.
15. **Dauchy RT, Blask DE, Sauer LA, Brainard GC, Krause JA.** 1999. Dim light during darkness stimulated tumor progression by enhancing tumor fatty acid uptake and metabolism. Cancer Lett **144:**131–136.
16. **Dauchy RT, Dauchy EM, Hanifin JP, Gauthreaux SL, Mao L, Beleancio VP, Ooms TG, Dupepe LM, Jablonski MR, Warfield B, Wren MA, Brainard GC, Hill SM, Blask DE.** 2013. Effects of spectral transmittance through standard laboratory cages on circadian metabolism and physiology in nude rats. J Am Assoc Lab Anim Sci **52:**146–156.
17. **Dauchy RT, Dauchy EM, Tirrell RP, Hill CR, Davidson LK, Greene MW, Tirrell PC, Wu J, Sauer LA, Blask DE.** 2010. Dark-phase light contamination disrupts circadian rhythms in plasma measures of physiology and metabolism. Comp Med **60:**348–356.
18. **Dauchy RT, Dupepe LM, Ooms TG, Dauchy EM, Hill CR, Mao L, Belancio VP, Slakey LM, Hill SM, Blask DE.** 2011. Eliminating animal facility light-at-night contamination and its effect on circadian regulation of rodent physiology, tumor growth, and metabolism: a challenge in the relocation of a cancer research laboratory. J Am Assoc Lab Anim Sci **50:**326–336.
19. **Dauchy RT, Sauer LA.** 1986. Preparation of 'tissue-isolated' rat tumors for perfusion: a new surgical technique that preserves continuous blood flow. Lab Anim Sci **36:**678–681.
20. **Dauchy RT, Sauer LA, Blask DE, Vaughan GM.** 1997. Light contamination during the dark phase in 'photoperiodically controlled' animal rooms: effect on tumor growth and metabolism in rats. Lab Anim Sci **47:**511–518.
21. **Davis S, Mirrick DK, Stevens RG.** 2001. Night shift work, light at night, and the risk of breast cancer. J Natl Cancer Inst **93:**1557–1562.
22. **De Boer SF, Van der Gugten J.** 1987. Daily variations in plasma noradrenaline, adrenaline, and corticosterone concentrations in rats. Physiol Behav **40:**323–328.
23. **Depres-Brummer P, Levi F, Metger G, Touitou Y.** 1995. Light-induced suppression of the rat circadian system. Am J Physiol **268:**R1111–R1116.
24. **Diaz B, Blazquez E.** 1986. Effect of pinealectomy on plasma glucose, insulin, and glucagon levels in the rat. Horm Metab Res **18:**225–229.
25. **Faith RE, Huerkamp MJ.** 2008. Environmental considerations for research animals, p 59–83. In: Hessler JR, Lehner NDM, editors. Planning and designing research animal facilities. Burlington (MA): Academic Press.
26. **Fall MW.** 1974. The use of red light for handling wild rats. Lab Anim Sci **24:**686–687.
27. **Freedman MS, Luca RJ, Soni B, von Schantz M, Munoz M, David-Gray Z, Foster RG.** 1999. Regulation of mammalian circadian behavior by nonrod, noncone, ocular photoreceptors. Science **284:**502–504.
28. **Gooley JJ, Chamberlain K, Smith KA, Sat Bir SK, Rajarathnam SMW, Van Reen E, Seitzer JM, Czeisler CA, Lockley SW.** 2011. Exposure to room light before bedtime suppresses melatonin onset and shortens melatonin duration in humans. J Clin Endocrinol Metab **96:**E463–E472.
29. **Grossweiner LI.** 1989. Photophysics, p 1–45. In: Smith KC, editor. The science of photobiology. New York (NY): Plenum Press.
30. **Gwosdow-Cohen A, Chen CL, Bosch EL.** 1982. Radioimmunoassay (RIA) of serum corticosterone in rats. Proc Soc Exp Biol Med **170:**29–34.
31. **Hanifin JP, Brainard GC.** 2007. Photoreception for circadian, neuroendocrine, and neurobehavioral regulation. **26:**87–94.
32. **Hanifin JP, Stewart KT, Smith P, Tanner R, Rollag M, Brainard GC.** 2006. High-intensity red light suppresses melatonin. Chronobiol Int **23:**251–268.
33. **Hansen J.** 2001. Increased breast cancer risk among women who work predominantly at night. Epidemiology **12:**74–77.
34. **Hattar S, Liao H-W, Takao M, Berson DM, Yau K-W.** 2002. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science **295:**1065–1070.
35. **Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW, Lem J, Biel M, Hofman F, Foster RG, Yau K-W.** 2003. Melanopsin and rod–cone photoreceptive systems account for all major accessory visual functions in mice photosensitivity. Nature **424:**76–81.
36. **Hofstetter JR, Hofstetter AR, Hughes AM, Mayeda AR.** 2005. Intermittent long-wavelength red light increases the period of daily locomotor activity in mice. J Circadian Rhythms **3:**8–16.
37. **Holley DC, Winget CW, Leon HA, editors.** 1988. Lighting requirements in microgravity—rodents and nonhuman primates. Moffett Field (CA): National Aeronautics and Space Administration, Ames Research Center.
38. **Illnerova H, Vanecek J, Hoffman K.** 1983. Regulation of the pineal melatonin concentration in the rat (*Rattus norvegicus*) and the Djungarian hamster (*Phodopus sungorus*). Comp Biochem Physiol A Comp Physiol **74:**155–159.
39. **Illuminating Engineering Society.** 2011 The lighting handbook, 10th ed. New York (NY): Illuminating Engineering Society.
40. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
41. **Kalsbeek A, Strubbe JH.** 1998. Circadian control of insulin secretion is independent of the temporal distribution of feeding. Physiol Behav **63:**553–558.
42. **Karlsson B, Knutsson A, Lindahl B.** 2001. Is there an association between shift work and having a metabolic syndrome? Results from a population-based study of 27,485 people. Occup Environ Med **58:**747–752.
43. **Kennaway DJ, Voultsios A, Varcoe TJ, Moyer RW.** 2002. Melatonin in mice: rhythms, response to light, adrenergic stimulation, and metabolism. Am J Physiol Regul Integr Comp Physiol **282:**R358–R365.
44. **Knutsson A, Akerstedt T, Jonsson BG, Orth-Gomer K.** 1986. Increased risk of ischaemic heart disease in shift workers. Lancet **2:**89–92.
45. **La Fleur SE, Kalsbeek A, Wortel J, van der Vliet J, Bujis RM.** 2001. Role for the pineal gland and melatonin in glucose homeostatsis: pinealectomy increases nighttime glucose concentrations. J Neuroendocrinol **13:**1025–1032.
46. **Li JC, Xu F.** 1997. Influence of light–dark shifting on the immune system, tumor growth, and life span of rats, mice, and fruit flies as well as on the counteraction of melatonin. Biol Signals **6:**77–89.
47. **Lima FB, Machado UF, Bartol I, Seraphim PM, Sumida DH, Moraes SMF, Hell NS, Okamoto MM, Saad MJ, Carvalho CR, Cipolla-Neto J.** 1998. Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. Am J Physiol **275:**E934–E941.
48. **Lucas RJ, Freedman MS, Munoz M, Garcia-Fernandez JM, Foster RG.** 1999. Regulation of the mammalian pineal by nonrod, noncone, ocular photoreceptors. Science **284:**505–507.
49. **Lynch HJ, Rivest RW, Ronsheim PM, Wurtman RJ.** 1981. Light intensity and the control of melatonin secretion in rats. Neuroendocrinology **33:**181–185.
50. **McCormack CE, Sontag CR.** 1980. Entrainment by red light of running activity and ovulation rhythms of rats. Am J Physiol **239:**R450–R453.
51. **Mead AP.** 1961. A response of the albino rat to red light. Anim behav **9:**225–226.

52. **McGuire RA, Rand WM, Wurtman RJ.** 1973. Entrainment of the body temperature rhythm in rats: effect of color and intensity of environmental light. Science **181:**956–957.
53. **McLennan IS, Taylor-Jeffs J.** 2004. The use of sodium lamps to brightly illuminate mouse houses during their dark phases. Lab Anim **38:**384–392.
54. **Moore RY.** 1983. Organization and function of a central nervous system oscillator: the suprchiasmatic hypothalamic nucleus. Fed Proc **42:**2783–2789.
55. **Podolin PL, Rollag MD, Brainard GC.** 1987. The suppression of nocturnal pineal melatonin in the Syrian hamster: dose-dependent curves at 500 nm and 360 nm. Endocrinology **121:**266–270.
56. **Poeggeler BH, Barlow-Walden LR, Reiter RJ, Saarela S, Menendez-Pelaez A, Yaga K, Manchester LC, Chen LD, Tan DX.** 1995. Red-light–induced suppression of melatonin synthesis is mediated by N-methyl-D-aspartate receptor activation in retinally normal and retinally degenerate rats. J Neurobiol **28:**1–8.
57. **Rahman SA, Kollara A, Brown TJ, Casper RF.** 2008. Selectively filtering short wavelengths attenuates the disruptive effects of nocturnal light on endocrine and molecular circadian phase markers in rats. Endocrinology **149:**6125–6135.
58. **Reiter RJ.** 1991. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr Rev **12:**151–180.
59. **Reiter RJ.** 1991. Pineal gland: interface between photoperiodic environment and the endocrine system. Trends Endocrinol Metab **2:**13–19.
60. **Reppert SM, Weaver DR.** 2002. Coordination of circadian timing in mammals. Nature **418:**935–941.
61. **Sauer LA, Dauchy RT.** 1988. Identification of linoleic and arachidonic acids as the factors in hyperlipemic blood that increases [^{3}H] thymidine incorporation in hepatoma 7288CTC perfused in situ. Cancer Res **48:**3106–3111.
62. **Sauer LA, Dauchy RT, Blask DE, Armstrong BJ, Scalici S.** 1999. 13-Hydroxyoctadienoic acid is the mitogenic signal for linoleic-acid–dependent growth in rat hepatoma 7288CTC in vivo. Cancer Res **59:**4688–4692.
63. **Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Fuchs CS, Colditz GA.** 2001. Rotating night shifts and risk of breast cancer in women participating in the Nurses' Health Study. J Natl Cancer Inst **93:**1563–1568.
64. **Seggie J, Sha BE, Uhler I, Brown GM.** 1974. Baseline 24-hour plasma corticosterone rhythm in normal, sham-operated, and septally lesioned rats. Neuroendocrinology **15:**51–61.
65. **Takahashi JS, DeCoursey PJ, Bauman L, Menaker M.** 1984. Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. Nature **308:**186–188.
66. **Van Cauter E.** 1998. Putative roles of melatonin in glucose regulation. Therapie **53:**467–472.
67. **Vestal BM, Hill JL.** 1972. Pattern vision of deermice (*Peromyscus*) under red light. J Mammal **53:**374–376.
68. **Zeitzer JM, Dijk D-J, Kronauer RE, Brown EN.** 1997. Photopic transduction implicated in human circadian entrainment. Neurosci Lett **232:**135–138.
69. **Zervanos SM, Davis DE.** 1968. Perception of red light in wood rats (*Neotoma floridana*). J Mammal **49:**759.