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Blue light from light-emitting diodes directed at a single eye elicits a dose-dependent suppression of melatonin in horses

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ABSTRACT

The production of melatonin during night-time hours decodes day length for seasonally breeding animals. The use of artificial light to advance the breeding season in mares is common practice within the equine industry. Four healthy Thoroughbred mares were used to evaluate the minimum intensity of light required to inhibit serum melatonin. Mares were fitted with indwelling jugular catheters and using a crossover design blood samples were collected following 1 h exposure to light (barn lighting approximately 200 lux), dark (<0.1 lux), and 3, 10, 50, and 100 lux intensities. The light source was a light-emitting diode (LED; 468 nm) directed at either a single eye or both eyes. All treatments, except the sample collected after 1 h exposure to light, occurred during the dark phase of the 24 h cycle. Serum melatonin levels were determined by radioimmunoassay.

Two-way repeated measures ANOVA revealed that there was no difference between the level of melatonin inhibition achieved when light was administered to one or two eyes ($P = 0.7028$). One-way ANOVA of melatonin levels at light intensities of 10, 50 and 100 lux were significantly different to dark ($P < 0.05$) and not different to light ($P > 0.05$) intensities. There was no difference between melatonin levels at 3 lux ($P > 0.05$) and dark intensities. The threshold level of low wavelength light required to inhibit melatonin production in the horse lies between 3 and 10 lux. Melatonin inhibition can be achieved by exposing a single eye to low wavelength blue light. This is a novel finding with important implications for management of artificial lighting regimens in horses.

Introduction

The nightly rise of melatonin secretion by the pineal gland is considered to be one of the most stable outputs from the circadian clock and represents one of the best characterised mammalian adaptations to life on a rotating planet (Arendt, 1995; Benloucif et al., 2005). Optical radiation signals received by the retina are translated into neural signals that travel via the retino-hypothalamic tract (RHT) to the suprachiasmatic nucleus (SCN). The SCN is the site of the master mammalian circadian clock and serves to synchronise internal physiology with the external environment (Reppert and Weaver, 2002). The SCN projects via the superior cervical ganglia to the pineal gland to stimulate production of melatonin in the absence of light (Moore and Lenn, 1972).

Melatonin secretion plays a major part in the regulation of the circa-annual reproductive cycles of seasonally breeding mammals (Karsch et al., 1984; Sharp, 1980; Burkhardt, 1947) and represents the daily decoder of seasonal changes in day length. Horses are naturally long-day breeders and their reproductive system relies on the recognition of a shortened duration of melatonin secretion as the longer days of spring approach. Changes in the duration of melatonin secretion constitute a signal to the neural structures controlling the secretion of gonadotropins from the pituitary gland. A short duration of melatonin is stimulatory in the horse and reduces the inhibition of gonadotropin releasing hormone (GnRH) pulse frequency, thus acting as a cue to activate the annual reproductive phase (Cleaver et al., 1991).

Manipulation of this physiological mechanism by artificially exposing mares to lengthened hours of light for 8–10 weeks beginning as early as 15th November has been shown to successfully accelerate the onset of the equine reproductive season in the Northern hemisphere (Palmer and Guillaume, 1992). The universal birthday for many horse breeds is 1st January. This industry-wide crucial date creates a demand for foals born early in the year in order to produce mature yearlings and precocious 2-year old racehorses. Research has shown that annual earnings are significantly higher for Thoroughbred horses born in January and February than for those born from April to June (Langlois and Blouin, 1998; Chemineau et al., 2008), encouraging breeders to advance the mare's breeding season artificially. A photoperiod regimen of 16 h light:8 h dark (Palmer and Guillaume, 1992) provided by a 100 W light bulb in a 12 foot (3.65 m) by 12 foot stall is the standard industry protocol used to achieve this (Burkhardt, 1947). However, no research has

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been conducted to date to determine the minimum level of light required to inhibit melatonin secretion in the horse.

As photic pathways involved in circadian, neuroendocrine and neurobehavioural responses in the retina are independent of those pathways that convert light signals to neural signals in the visual system, the level of light required to inhibit melatonin is not related to vision, but instead to the level required to stimulate the SCN (Gooley et al., 2003). Melanopsin has been identified as a photopigment responsible for mediating these non-visual responses (Provencio et al., 2000). It is found in a novel set of photoreceptors called intrinsically photosensitive retinal ganglion cells (ipRGCs; Berson et al., 2002; Hanifin and Brainard, 2007) and works in conjunction with photopigments in rods and cones. The action spectra for these photoreceptors show peak sensitivities in the short-wavelength region of the visible spectrum and studies conducted in mice and humans indicate a peak sensitivity range between 459 nm and 484 nm in the blue-light spectrum (Brainard et al., 2001; Thapan et al., 2001).

The use of blue light-emitting diodes (LEDs) as a source of light for melatonin suppression is a relatively new concept. Light in the blue light spectrum (465–485 nm) has been found to facilitate more accurate and efficient levels of melatonin inhibition in humans by providing the optimum wavelength of light for stimulation of the SCN (West et al., 2011).

The primary aim of this study was to investigate the inhibitory effect of short-wavelength light on melatonin secretion in the horse using blue LEDs at various light intensity (lux) levels. A secondary aim was to determine whether light administration to a single eye is sufficient to stimulate this response.

Materials and methods

Animals

All experimental procedures were approved by the University College Dublin Animal Research Ethics Committee prior to commencement of the study.

Four healthy, 5-year old Thoroughbred mares (*Equus caballus*) were used for this experiment. For the duration of the study (3 days), the mares were housed in a custom-built, light-proof, fully ventilated barn under a light schedule that mimicked the natural external photoperiod (light/dark cycle). The experiment was carried out in mid-March, a time corresponding with the vernal equinox and a 12 h light:12 h dark (LD12:12) natural photoperiod, at longitude W6.8, latitude N53.2 in Co. Kildare, Ireland. During daylight hours, the light was provided by fluorescent bulbs in the barn and light intensity at eye level was recorded as approximately 200 lux using a handheld lux meter (LX-1010 B Digital Lux Meter). Access to hay and water was ad libitum and small amounts of concentrated mixed grain feed were provided at intervals during the experiment. The temperature in the barn remained relatively constant throughout the study (approximately 10 °C ± 0.5 °C).

Light masks

Four sets of full cup racing blinkers (Zilco) were purchased. Two sets of the blinkers were fitted with a single blue LED (Kingbright 7.6 mm × 7.6 mm Super Flux LED Lamp L-7676CQBC-D Blue, GaN) on the inside of the left eye cup and the right eye cup was covered over with a light-proof material. The other two masks were fitted with blue LEDs in both cups (Fig. 1). The internal surface of each blinker cup was lined with a layer of reflective aluminium foil. Each LED was positioned to shine light onto the inside of the blinker cup such that light reflected off the reflective surface back onto the cornea. In this way the light was diffuse, uniform and not distracting to the horse. The LED lights were powered by a PP3 9 volt battery running through a circuit containing a switch (on/off), a 75 Ω resistor and a 20 kΩ potentiometer (variable resistor). The potentiometer allowed adjustment of the LED light intensity to each of the four experimental lux levels (3, 10, 50, and 100). The lux levels were measured and adjusted throughout the study with the aid of a luxmeter. The LEDs had a peak wavelength of 468 nm. The levels were selected as they were lower than normal barn lighting, at which melatonin had previously been shown to be inhibited (Murphy et al., 2011).

Experimental protocol

On the afternoon before the experiment, the left jugular furrow of each mare was clipped and surgically prepared for the placement of indwelling jugular catheters (MILA International). Each catheter was secured in place using suture (3 metric Monosof nylon, Gosport) and bandage and flushed with 1% heparin in 0.9% saline solution. During darkness hours, blood samples were collected using dim red light from handheld torches.

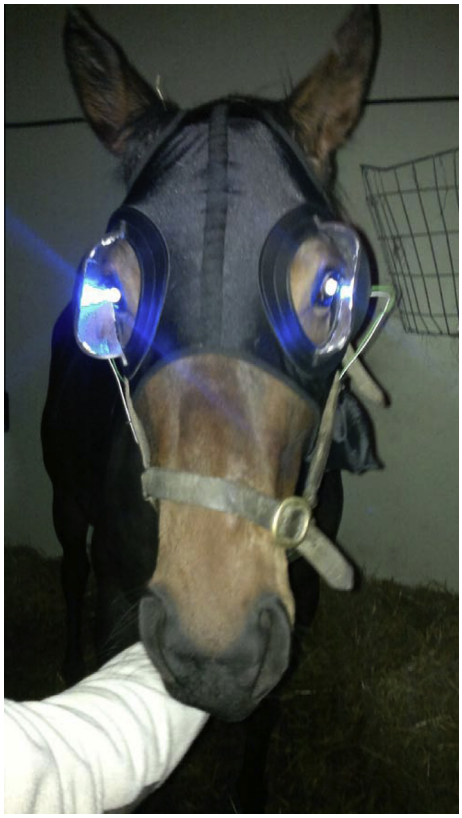

Fig. 1. Photograph of a horse wearing the light mask that provided light to both eyes. The light mask consisted of a modified full cup racing blinkers (Zilco) with a single blue LED (Kingbright 7.6 mm × 7.6 mm Super Flux LED Lamp L-7676CQBC-D Blue, GaN) fitted on the inside of each eye cup. The inner surface of the cup was covered with reflective aluminium foil to reflect light diffusely onto the eye. For the single eye light mask, only a single LED was fitted in the right eye cup and the right eye cup was covered over with a light-proof material.

Zeitgeber (ZT) time refers to the time of day relative to the LD cycle with ZT 12 indicating the time of lights-off. This experiment was conducted over two consecutive nights. On night 1, a light sample was taken at ZT 11, a dark sample was taken at ZT 14, and a further sample taken following an hour-long exposure to the lowest lux level (3 lux) at ZT 16. On this night, horses 1 and 2 wore the masks with 3 lux provided to a single eye and horses 3 and 4 had 3 lux directed to both eyes. This allowed for observation of the horses' reactions to the masks before exposure to the higher light intensities. On night 2, a crossover design was used, such that all four horses were exposed to each of the remaining 3 lux levels (10, 50 and 100 lux) in one eye and in both eyes. On night 2, horses 1 and 2 wore the light masks with light provided to a single eye at ZT 14, 16 and 18; horses 3 and 4 wore the light masks that provided light to both eyes at these times. The light masks were then swapped such that horses 1 and 2 received light to both eyes at ZT 20, 22 and 0, while horses 3 and 4 wore the single eye light masks at these times.

Each hour of exposure to light was followed by an hour of darkness, which permitted the investigation of the melatonin response subsequent to LED light exposure. As each time interval was an hour in length, the samples were taken in the last 5 min of each interval. The schedule of sampling and lighting conditions is outlined in Table 1. Blood was left at room temperature for 2 h and then stored at 4 °C for 24 h. The following day, samples were centrifuged for 15 min at 1600 *g* and 4 °C and the serum decanted. Serum samples were stored at −20 °C until assayed.

Melatonin radioimmunoassay (RIA)

Melatonin was measured using a Bühlmann melatonin RIA kit (RK-MEL2, ALPCO Diagnostics). Serum aliquots (500 μL) were column-extracted using a vacuum manifold (Visiprep-DL Solid Phase Extraction Vacuum Manifold) according to the directions of the manufacturer and reconstituted in 500 μL of incubation buffer solution provided with the kit. Aliquots of the reconstituted extracted samples (200 μL) were assayed in duplicate in a single assay. The intra-assay coefficients of variance for quality controls were 2.5% and 17.0%, respectively. As documented