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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.

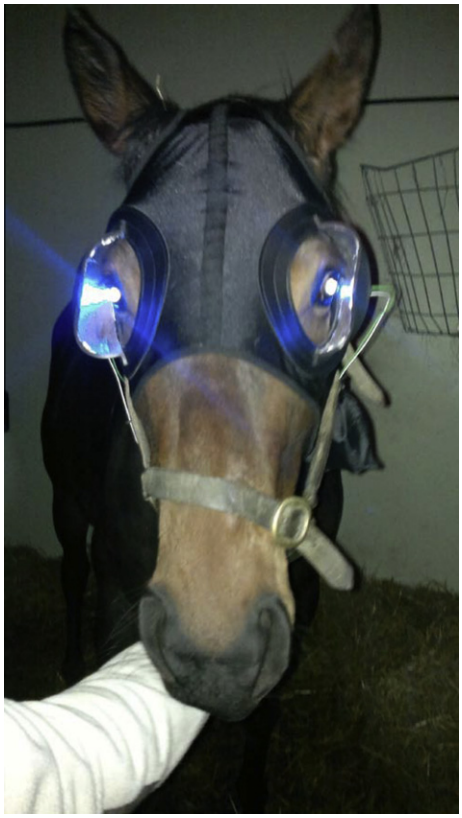

Fig. 1. Photograph of a horse wearing the light mask that provided light to both eyes. The light mask consisted of a modified full cup racing blinkers (Zilco) with a single blue LED (Kingbright 7.6 mm × 7.6 mm Super Flux LED Lamp L-7676CQBC-D Blue, GaN) fitted on the inside of each eye cup. The inner 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

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Table 1
Sampling and lighting schedule.

Zeitgeber time	Time	Night 1	Night 2	Single eye[a]	Both eyes[a]
11	18:00	Light	Light	Horse 1 + 2	Horse 3 + 4
12	19:00				
13	20:00		Dark	Horse 1 + 2	Horse 3 + 4
14	21:00	Dark	Lux 10	Horse 1 + 2	Horse 3 + 4
15	22:00		Dark	Horse 1 + 2	Horse 3 + 4
16	23:00		Lux 50	Horse 1 + 2	Horse 3 + 4
17	00:00	Lux 3	Dark	Horse 1 + 2	Horse 3 + 4
18	01:00		Lux 100	Horse 1 + 2	Horse 3 + 4
19	02:00		Dark	Horse 3 + 4	Horse 1 + 2
20	03:00		Lux 100	Horse 3 + 4	Horse 1 + 2
21	04:00		Dark	Horse 3 + 4	Horse 1 + 2
22	05:00		Lux 50	Horse 3 + 4	Horse 1 + 2
23	06:00		Dark	Horse 3 + 4	Horse 1 + 2
0	07:00		Lux 10	Horse 3 + 4	Horse 1 + 2
1	08:00		Light	Horse 3 + 4	Horse 1 + 2

[a] Single eye and both eyes columns refer to the crossover design implemented on night 2.

by the manufacturer, the efficiency of the extraction method was >90%, while the assay had an estimated functional sensitivity of 0.9 pg/mL (coefficient of variance – 10%) and an estimated analytical sensitivity of 0.3 pg/mL. This assay has been used previously to examine MT levels in equine serum (Murphy et al., 2011).

Data analysis

A two-way repeated measure ANOVA (treatment × lux level) was used to analyse data collected on night 2, where treatment refers to light exposure to a single eye or to both eyes. The light sample was comprised of data points from the samples collected at ZT 11 and the dark sample from data points collected from ZT 13. Bonferroni's post hoc tests were used when an overall $P < 0.05$ was found. A one-way ANOVA was used to assess significant differences across all lux levels, including data from night 1. In this case, the light sample was comprised of the data points from samples collected at ZT 11 on both nights 1 and 2 and the dark sample represents combined data from ZT 14 on night 1 and ZT 13 on night 2. Bonferroni's post hoc-tests were again used to assess significant differences between specific lux levels. Data was analysed using GraphPad Prism Version 4.0 for Windows (GraphPad Software). In all cases, significance was assessed as $P < 0.05$. The data distribution is presented as box and whisker plots in Figs. 2 and 3.

Results

Melatonin analysis

Two-way repeated measures ANOVA revealed no significant treatment × light intensity interaction ($P = 0.703$) for melatonin levels in horses receiving either light to one eye or light to two eyes (Fig. 2). However, an effect of light intensity was observed

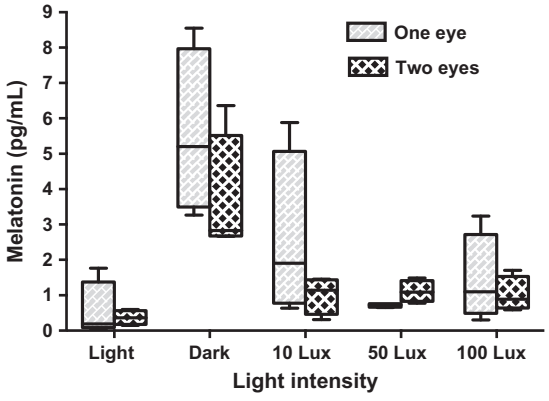

Fig. 2. Box and whisker plots of serum melatonin levels following 1 h exposure to varying intensities of light to one or two eyes. Medians are indicated by the midline; first and third quartiles by the open box; and minima and maxima by the lower and upper whiskers. ANOVA revealed no significant differences in melatonin inhibition between treatments at light intensities – light (approximately 200 lux), dark (< 0.1 lux), 10, 50 and 100 lux respectively.

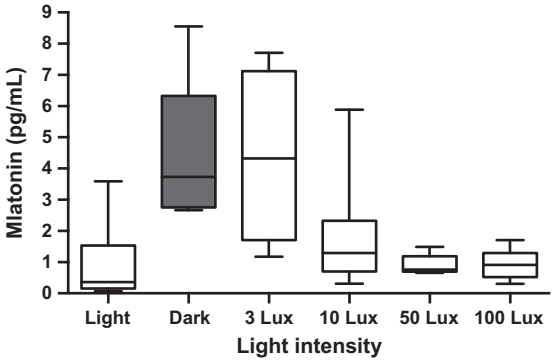

Fig. 3. Box and whisker plots of serum melatonin levels in the horse at various light intensities – light (approximately 200 lux), dark (<0.1 lux), 3 lux, 10 lux, 50 lux and 100 lux. Medians are indicated by the midline; first and third quartiles by the open box; and minima and maxima by the lower and upper whiskers. ANOVA results comparing dark levels are indicated by * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$), respectively.

($P < 0.0001$) with lowest melatonin values observed for light, 50 and 100 lux. The one-way ANOVA revealed an overall significant difference across lux levels ($P < 0.05$). Bonferroni's multiple comparison test revealed a significant difference between dark and 10 lux ($P < 0.05$), 50 lux ($P < 0.001$), 100 lux ($P < 0.01$) as well as light ($P < 0.001$). There was no difference between melatonin levels at 3 lux and dark. The data distribution is presented as box and whisker plots in Figs. 2 and 3. During the hour of darkness between each administration of an experimental light intensity, serum melatonin levels were observed to rise to pre-treatment dark levels. This finding is presented in box and whisker plots of the data in Fig. 4.

Discussion

Inhibition of melatonin using low wavelength blue light has not previously been tested in the equine species. We used LEDs set in a head mask to investigate the inhibitory effect of blue light on melatonin secretion in the horse. The results indicate that blue light inhibits the production of melatonin and that there is no difference in this inhibitory effect when light is administered to one or both eyes. We demonstrated that 10 lux or higher blue light is sufficient for suppression of melatonin to the levels observed under artificial barn lighting.

Light input to the circadian system is primarily mediated by the intrinsically photosensitive retinal ganglion cells (Berson et al., 2002), which contain the photopigment melanopsin (Provencio et al., 2000). These melanopsin rich cells relay neural signals to the brain communicating photoperiodic information to the circadian clock in the SCN (Berson et al., 2002). Investigations into the wavelength of light that best stimulates melanopsin for optimum melatonin inhibition demonstrate that light within the short wavelengths range of 465–480 nm is most effective in humans, monkeys and mice (Brainard et al., 2008; Dacey et al., 2005; Hattar et al., 2003). We chose a wavelength within this range to test the efficacy of blue light for inhibiting melatonin for the first time in horses.

To date, the inhibition of melatonin secretion by administering light to a single eye has not been tested in any species. The results from this study show clearly that this can be achieved and that there is no significant difference between the levels of inhibition achieved by shining light in a single eye vs. both eyes. This has important practical implications for horses where mobile light therapy involving two eyes would not be practical in an outdoor environment. Previous research in rodents has indicated that

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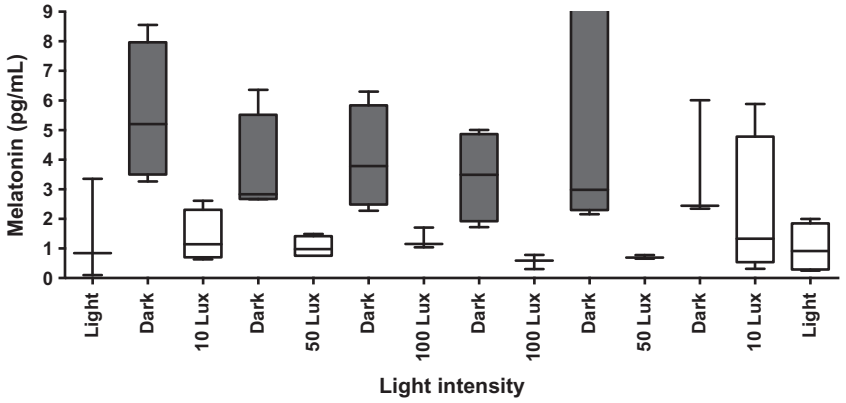

Fig. 4. Box and whisker plots of serum melatonin levels collected each hour on night 2. Samples were collected during the final 5 min of exposure to an hour of light (approximately 200 lux), dark (<0.1 lux), or an experimental light intensity. The initial sample was taken at ZT 11, in the hour before dusk or lights-off and the final light sample was collected at ZT 1 following dawn or lights-on. The data presented are the combined results from all four horses. Medians are indicated by the midline; first and third quartiles by the open box; and minima and maxima by the lower and upper whiskers.

unilateral enucleation resulting in a 50% decrease in retinal photoreceptors and their associated RHT projections does not reduce the ability of the SCN to respond to light (Stephan et al., 1982). Furthermore, a study measuring cFOS levels (used to indicate the neural response to light stimulation) in unilaterally enucleated rats demonstrated that a 50% reduction in photic signals from the eyes to the brain did not result in a corresponding 50% reduction in the levels of circulating cFOS. As the authors suggest, this implies that there is no significant decrease in the neural response to light signals received by a single eye (Beaulé et al., 2001). These results were later replicated and the conclusions were confirmed by a subsequent study (Muscat and Morin, 2005).

The ability to inhibit melatonin using light from an LED source could lead to new management techniques. The potential applications of a light-emitting device worn by horses are numerous. Manipulation of the equine breeding season, traditionally conducted under artificial light indoors, could be achieved while the horses remain outside, thereby reducing management costs and improving health and welfare. Furthermore, it has been previously demonstrated that the exposure of pregnant mares to extended light from 1st December significantly reduced gestation lengths and showed a trend towards the production of higher birth weight foals (Hodge et al., 1982). It is assumed that the reluctance to maintain pregnant mares indoors under artificially lengthened days is due to the excessive cost of the procedure.

Light therapy is also commonly used as a treatment for circadian rhythm disorders such as seasonal affective disorder (SAD), sleep disorders, depression and jetlag in humans (Pail et al., 2011). Global transportation of horses is a common occurrence and there is evidence to suggest that horses may well be impacted by jetlag (Murphy et al., 2007, 2011). Research into the use of light therapy in humans following transmeridian travel has shown that it accelerates circadian re-entrainment to a new time zone, reducing performance deficits (Boulos et al., 2002). Blue wavelength light is an alternative to white light therapy and has enhanced benefits including more effective inhibition of melatonin (West et al., 2011), improved alertness, cognitive ability and wellbeing (Chellappa et al., 2011). Blue light therapy, as described in this study, has potential applications in horses subject to transmeridian travel.

Conclusions

The current study demonstrates that low level blue light inhibits melatonin production in the horse and could provide opportunities for improving and developing multiple equine management procedures that rely on the suppression of melatonin.

Conflict of interest statement

A priority preliminary patent application has been filed in Ireland (Patent # S2011/0245) by University College Dublin entitled 'An apparatus and method for inhibiting melatonin synthesis in a horse' which describes an invention similar to the light mask used in the current study.

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References

Arendt, J., 1995. Melatonin and the Mammalian Pineal Gland, First Ed. Chapman and Hall, London, UK, p. 1.

Beaulé, C., Arvanitogiannis, A., Amir, S., 2001. Entrainment to a discrete light pulse at dawn is associated with acute suppression of FOS in the shell region of the suprachiasmatic nucleus. In: Proceedings of the Annual meeting of the Society for Neuroscience, San Diego, 2001 (Abstract 182.6).

Benloucif, S., Guico, M., Reid, K., Wolfe, L., L'hermite-Baleriaux, M., Zee, P., 2005. Stability of melatonin and temperature as circadian phase markers and their relation to sleep times in humans. Journal of Biological Rhythms 20, 178–188.

Berson, D.M., Dunn, F.A., Takao, M., 2002. Phototransduction by retinal ganglion cells that set the circadian clock. Science 295, 1070–1073.

Boulos, Z., Macchi, M.M., Stürchler, M.P., Stewart, K.T., Brainard, G.C., Suhner, A., Wallace, G., Steffen, R., 2002. Light visor treatment for jet lag after westward travel across six time zones. Aviation, Space, and Environmental Medicine 73, 953–963.

Brainard, G.C., Hanifin, J.P., Greeson, J.M., Byrne, B., Glickman, G., Gerner, E., Rollag, M.D., 2001. Action spectrum for melatonin regulation in humans: Evidence for a novel circadian photoreceptor. The Journal of Neuroscience 21, 6405–6412.

Brainard, G.C., Sliney, D., Hanifin, J.P., Glickman, G., Byrne, B., Greeson, J.M., Jasser, S., Gerner, E., Rollag, M.D., 2008. Sensitivity of the human circadian system to short-wavelength (420-nm) light. Journal of Biological Rhythms 23, 379–386.

Burkhardt, J., 1947. Transition from anoestrus in the mare and the effects of artificial lighting. The Journal of Agricultural Science 37, 64–68.

Chellappa, S.L., Steiner, R., Blattner, P., Oelhafen, P., Götz, T., Cajochen, C., 2011. Non-visual effects of light on melatonin, alertness and cognitive performance. Can blue-enriched light keep us alert? PLoS ONE 6, e16429.

Chemineau, P., Guillaume, D., Migaud, M., Thiéry, J., Pellicer Rubio, M., Malpaux, B., 2008. Seasonality of reproduction in mammals: Intimate regulatory mechanisms and practical implications. Reproduction in Domestic Animals 43, 40–47.

Cleaver, B., Grubaugh, W., Davis, S., Sheerin, P., Franklin, K., Sharp, D., 1991. Effect of constant light exposure on circulating gonadotrophin levels and hypothalamic

gonadotrophin-releasing hormone (GnRH) content in the ovariectomized pony mare. Journal of Reproduction and Fertility Suppl 44, 259–266.

Dacey, D.M., Liao, H.W., Peterson, B.B., Robinson, F.R., Smith, V.C., Pokorny, J., Yau, K.W., Gamlin, P.D., 2005. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. Nature 433, 749–754.

Gooley, J.J., Lu, J., Fischer, D., Saper, C.B., 2003. A broad role for melanopsin in nonvisual photoreception. The Journal of Neuroscience 23, 7093–7106.

Hanifin, J.P., Brainard, G.C., 2007. Photoreception for circadian, neuroendocrine, and neurobehavioral regulation. Journal of Physiological Anthropology 26, 87–94.

Hattar, S., Lucas, R.J., Mrosovsky, N., Thompson, S., Douglas, R., Hankins, M.W., Lem, J., Biel, M., Hofmann, F., Foster, R.G., 2003. Melanopsin and rod cone photoreceptive systems account for all major accessory visual functions in mice. Nature 424, 76–81.

Hodge, S.L., Kreider, J., Potter, G., Harms, P., Fleeger, J., 1982. Influence of photoperiod on the pregnant and postpartum mare. American Journal of Veterinary Research 43, 1752–1755.

Karsch, F., Bittman, E., Foster, D., Goodman, R., Legan, S., Robinson, J., 1984. Neuroendocrine basis of seasonal reproduction. Recent Progress in Hormone Research 40, 185–232.

Langlois, B., Blouin, C., 1998. Effect of a horse's month of birth on its future sport performance II. Effect on annual earnings and annual earnings per start. EDP Sciences, 67–74.

Moore, R.Y., Lenn, N.J., 1972. A retinohypothalamic projection in the rat. The Journal of Comparative Neurology 146, 1–14.

Murphy, B.A., Elliott, J.A., Sessions, D.R., Vick, M.M., Kennedy, E.L., Fitzgerald, B.P., 2007. Rapid phase adjustment of melatonin and core body temperature rhythms following a 6-h advance of the light/dark cycle in the horse. Journal of Circadian Rhythms 5, 5.

Murphy, B.A., Martin, A.M., Furney, P., Elliott, J.A., 2011. Absence of a serum melatonin rhythm under acutely extended darkness in the horse. Journal of Circadian Rhythms 9, 3.

Muscat, L., Morin, L.P., 2005. Binocular contributions to the responsiveness and integrative capacity of the circadian rhythm system to light. Journal of Biological Rhythms 20, 513–525.

Pail, G., Huf, W., Pjrek, E., Winkler, D., Willeit, M., Praschak-Rieder, N., Kasper, S., 2011. Bright-light therapy in the treatment of mood disorders. Neuropsychobiology 64, 152–162.

Palmer, E., Guillaume, D., 1992. Photoperiodism in the equine species – What is a long night? Animal Reproduction Science 28, 21–30.

Provencio, I., Rodriguez, I.R., Jiang, G., Hayes, W.P., Moreira, E.F., Rollag, M.D., 2000. A novel human opsin in the inner retina. The Journal of Neuroscience 20, 600–605.

Reppert, S.M., Weaver, D.R., 2002. Coordination of circadian timing in mammals. Nature 418, 935–941.

Sharp, D., 1980. Environmental influences on reproduction in horses. The Veterinary Clinics of North America. Large Animal Practice 2, 207–223.

Stephan, F.K., Donaldson, J.A., Gellert, J., 1982. Retinohypothalamic tract symmetry and phase shifts of circadian rhythms in rats and hamsters. Physiology and Behavior 29, 1153–1159.

Thapan, K., Arendt, J., Skene, D.J., 2001. An action spectrum for melatonin suppression: Evidence for a novel non-rod, non-cone photoreceptor system in humans. The Journal of Physiology 535, 261–267.

West, K.E., Jablonski, M.R., Warfield, B., Cecil, K.S., James, M., Ayers, M.A., Maida, J., Bowen, C., Sliney, D.H., Rollag, M.D., 2011. Blue light from light-emitting diodes elicits a dose-dependent suppression of melatonin in humans. Journal of Applied Physiology 110, 619–626.

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