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Blue light from individual light masks directed at a single eye advances the breeding season in mares

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Summary

Reasons for performing study: Artificial lighting is commonly used to advance the breeding season in horses. Light masks have been developed that direct light at a single eye to inhibit the production of melatonin, the decoder of photoperiod for seasonally breeding animals.
Objectives: To investigate whether low-intensity blue light from light masks was effective at advancing the breeding season in mares.
Study design: Controlled experiment.
Methods: Data on reproductive activity was collected from 3 groups of mares maintained on Kentucky horse farms under various lighting conditions between 20 November 2011 and 10 February 2012: 59 nonpregnant, healthy Thoroughbred mares were used. On 1 December 2011, *Group 1* (n = 16) was housed indoors under barn lighting (250 Lux) until 23.00 h daily. *Group 2* (n = 25) wore light masks programmed to turn on from 16.30 h until 23.00 h daily and was maintained outdoors. *Group 3* (n = 19) was maintained outdoors under the natural photoperiod as control. At 2-week intervals, rectal ultrasound examinations were performed and blood was collected for progesterone analysis. Oestrous cyclicity was defined as the presence of follicles >20 mm diameter detected in conjunction with serum progesterone >1 ng/ml and confirmation of ovulation by transrectal ultrasound examination.
Results: On 10 February, the number of mares exhibiting oestrous cyclicity was 14/16 (87.5%) in *Group 1*; 20/25 (80%) in *Group 2*; and 4/19 (21%), in *Group 3*. Pairwise comparison of groups revealed no difference in the number of cycling mares between *Groups 1* and *2* (χ^2 test, P = 0.3348) whereas differences were observed between *Groups 1* and *3* (χ^2 test, P<0.0001) and *Groups 2* and *3* (χ^2 test, P<0.0003).
Conclusions: Low-intensity blue light to a single eye from a light mask is an effective alternative to maintenance of mares indoors under lights for advancing the breeding season. Mobile light therapy for horses could have economic benefits for the breeder by reducing the costs of maintaining mares indoors, and welfare benefits for horses by permitting outdoor maintenance.

Keywords: horse; melatonin; seasonality; progesterone; light mask; blue light

Introduction

Horses have a natural breeding season that extends from April to September in the northern hemisphere, a time coinciding with longer day length, grass growth and milder weather [1]. Melatonin is a pineal hormone produced during the hours of darkness, its pattern of secretion mimicking the light/dark patterns that occur during each 24 h diurnal cycle. It acts as the daily decoder of seasonal changes in day length and regulates the circa-annual reproductive cycles of seasonally breeding mammals [2,3].

The onset of the equine breeding season is triggered by increasing day length and concomitant reductions in melatonin secretion [4]. The mechanism of melatonin suppression has been well documented: light signals received by the retina and processed primarily by the photopigment melanopsin [5], located within the intrinsically photosensitive retinal ganglion cells, travel along the retino-hypothalmic tract to the hypothalamic suprachiasmatic nucleus [6]. The suprachiasmatic nucleus is connected to the pineal gland via the superior cervical ganglia and photic signals inhibit the release of the neurotransmitter norepinephrine, which therefore fails to stimulate the synthesis of melatonin [7,8].

During long days the inhibitory action of melatonin on the mare's reproductive axis is lifted and increased gonadotropin releasing hormone pulse frequency stimulates the anterior pituitary to release follicle stimulating hormone and luteinising hormone, which act in concert to promote the growth, development and ovulation of ovarian follicles [9–11].

The northern hemisphere specifies a universal birthday for Thoroughbred racehorses of 1 January. Breeders therefore desire foals born early in the year to produce mature yearlings and precocious 2-year-old racehorses. Studies show that annual earnings are significantly higher for horses born in January–February than for those born in April–June [12,13]. For breeders to satisfy industry timelines, it is necessary that they manipulate the mares' reproductively active period to meet the official start date of the breeding season on 15 February.

Beginning 1 December, it has become standard industry practice to extend day length for 8–10 weeks by exposing mares to artificial light until 23.00 h, allowing natural dawn to occur during the most sensitive phase of the 24 h cycle [4]. An artificial photoperiod of 16 h light: 8 h dark is facilitated using light from a 100 W light bulb in a 3.6 × 3.6 m stall [3,14], loosely described as 'enough light to read a newspaper'. Light therapy such as this can advance the breeding season by as much as 3 months [15].

Recent research indicates that light within the short-wavelength spectrum (465–485 nm) is most effective at inhibiting melatonin [16] as melanopsin is particularly sensitive to short wavelength, blue light [17,18]. A recent study in Thoroughbred mares has shown that melatonin can be successfully inhibited using low-level blue light (465 nm) from a light emitting diode (LED) source directed at a single eye [19]. We aim to determine if timed, low-intensity, blue light administered to a single eye from head worn light masks is as effective as indoor maintenance under white light at advancing the breeding season in mares.

Materials and methods

Animals

All experimental procedures were approved by the University College Dublin Animal Research Ethics Committee and the University of Kentucky Institutional Animal Care and Use Committee protocol number 2012-0928.

Healthy, Thoroughbred mares (n = 59), all aged between 5 and 24 years, were used for this experiment. All mares included in the study had a body condition score between 4 and 6 using the Henneke body condition scoring system [20] and there was no difference in average body condition score between groups (*Group 1*, 5.1 ± 0.7; *Group 2*, 5.1 ± 0.5; *Group 3*, 4.8

± 0.5, P>0.05). The experiment took place on 2 farms located in Fayette County, Lexington, Kentucky. *Group 1* (n = 16) was kept in a large pasture during the day and housed indoors in individual stalls at night. *Group 2* (n = 25) was maintained in 2 groups in large pastures during the day and night. *Group 3* (n = 19) was maintained on a separate farm, within an 8 km radius of the first, as a group in a large pasture during both day and night. There was no difference between the age ranges of mares within the different groups (*Group 1*, 12.6 ± 4.9; *Group 2*, 13.1 ± 4.4; *Group 3*, 11.4 ± 4, P>0.05). The experiment took place from 20 November 2011 to 10 February 2012 at longitude W -84.4° and latitude N 38.1°, which experienced temperatures that ranged from -10.6 to 20.6°C during this period. During the experiment *Group 1* grazed *ad libitum* during the day and had *ad libitum* access to hay at night. In addition, this group was supplemented with either 2.7 kg/day of a low-starch diet; Purina Wellsolve Low Starch[a], or of a sweet mix[b], or of mare nuts[b], depending on the individual needs of the mare as assessed by farm management. *Groups 2* and *3* grazed *ad libitum* during the day and night and received 1.4 kg/day of mare nuts[b]. During times of subfreezing temperature, frost or snow, *ad libitum* hay was provided in all pastures. Access to water was *ad libitum* for all groups for the duration of the study. Body condition scores did not vary markedly over the period of the study.

Light masks

The masks were made primarily of leather and designed to mimic the shape of racing blinkers, fitting snugly to the horses' heads. The mask had openings for the ears and eyes and covered the cheeks and head from the poll to half way down the face (Fig 1). Two buckle fastenings closed the mask under the horse's jaw and a Velcro section down the centre of the mask allowed for adjustable sizing. Attached to the buckles on the underside of the jaw was a small leather pouch, which contained the electronics box. A neoprene sleeve ran from the pouch up to the eye cup containing the light source, covering the electric wires connecting the LED to the battery pack. The eye cup was positioned on the outside of the eye opening for the horse's right eye. This permitted unimpaired vision of a handler on the traditional left side. The masks were handmade in Co. Kildare, Ireland by Berney Bros Saddlery.

Fig 1: A horse wearing the light mask that provided 50 lux blue light to the right eye. The light mask consists of a leather headpiece with a semi-rigid rubber cup containing a single blue-light emitting diode fitted on the inside of the rubber eye cup. The inner surface of the cup is covered with reflective aluminium foil to reflect light diffusely onto the eye. A Velcro fastening down the centre of the face permits adjustability.

The eye cup was modified from a full cup race hood[c]. It consisted of a black, semi-rigid rubber quarter sphere with a flattened outer lip on one edge, which facilitated attachment to the mask. The lip was sewn onto a semi-rigid half ring of leather, which was then sewn to the rim of the eye opening of the mask. The inner surface of the cup was covered with reflective aluminium foil tape. The LED was positioned inside the cup at the centre point, where the lip met the rim of the eye opening. The legs of the LED pierced the eye cup and were soldered to the electrical wires outside the eye cup within the neoprene sleeve. The LED was positioned to shine up onto the reflective foil surface, diffusing light onto the horse's cornea. This provided uniform, diffuse light and minimised distraction to the horse, permitting maintenance of normal behaviour. A 3 cm diameter hole was cut through the cup 1 cm below the LED to permit peripheral vision on the right side.

The LED used was a Kingbright 7.6 × 7.6 mm Super Flux LED Lamp L-7676CQBC-D Blue[d]. It has a luminous intensity of 1300 millicandelas when powered by a current of 70 mA. The peak wavelength was 468 nm, which is within the desired range for optimum suppression of melatonin in mammals. The spectral line half width was 25 nm, providing a broad margin for error in terms of the efficacy of the wavelength. The beam angle of the LED was 70° allowing for some degree of movement between the LED and the horse's eye. Each mask was individually calibrated using different levels of resistance to provide 50 Lux of diffuse light to the horse's eye. The Lux levels (illuminances) for each individual mask were adjusted and measured using a Lux meter (LX-1010 B Digital Lux Meter).

Each mask used 4 Duracell Pro-cell AA sized 1.5 V batteries (replaced at 2-week interval) and powered a circuit containing an Arduino[e] board, a switch (on/off), a clock and a set of resistors. The Arduino board was programmed to turn the LED on at 16.30 h each day and switch it off at 23.00 h every night. A resistor was connected in series with the LED to determine the current flow that produced illuminance of 50 Lux. In order to save power and prolong the battery lifetime, much of the Arduino functionality was put in a sleep mode. The circuit also included a real time clock module that kept time so that the Arduino could power the LED for specific intervals. The real time clock had its own small, independent battery so that it did not lose time when the switch for the main circuit was turned off.

Experimental protocol

Beginning on 1 December, *Group 1* received light therapy via artificial lighting indoors in individual stalls. The lights, which provided 250 Lux of light to each mare at eye level, were switched on at 16.30 h every day and remained on until 23.00 h each night. Also beginning on 1 December, *Group 2* received individual light therapy from head worn masks that provided 50 Lux of blue light to the right eye of each mare. The mask light turned on automatically at 16.30 h each day and turned off at 23.00 h every night. *Group 3* served as a control group and received no artificial light therapy, but was exposed to the natural environmental light/dark cycles for the duration of the experiment.

Beginning on 20 November and continuing at 2-week intervals throughout the experiment the mares underwent transrectal ultrasound examination and jugular venipuncture. Ovarian activity in terms of follicle number and size, presence or absence of *corpora lutea*, cervical tone and presence or absence of uterine oedema were recorded for each mare at every ultrasound examination and the blood samples were assayed to establish circulating progesterone concentrations. Oestrous cyclicity was defined as the presence of follicles >20 mm and the confirmation of an ovulation determined by the presence of a previously unrecorded *corpus luteum* or *corpus haemorrhagica* on an ovary in conjunction with progesterone levels >1 ng/ml. Transitional phase was defined as follicles >20 mm and associated physiological characteristics typical of oestrous activity but in the absence of progesterone levels indicative of ovulation

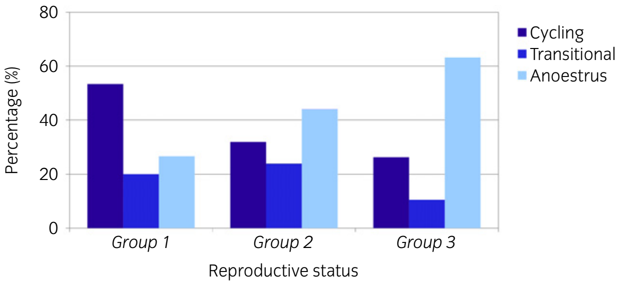

Fig 2: Reproductive status of all mares at the commencement of the study on 20 November 2011. *Group 1* (indoor barn lighting) had 9/16 (56.25%) cycling, 3/16 (18.75%) transitional and 4/16 (25%) anoestrus. *Group 2* (light masks) had 8/25 (32%) cycling, 6/25 (24%) transitional and 11/25 (44%) anoestrus. *Group 3* (no artificial light) had 5/19 (26.32%) cycling, 2/19 (10.53%) transitional, and 12/19 (63.16%) anoestrus.

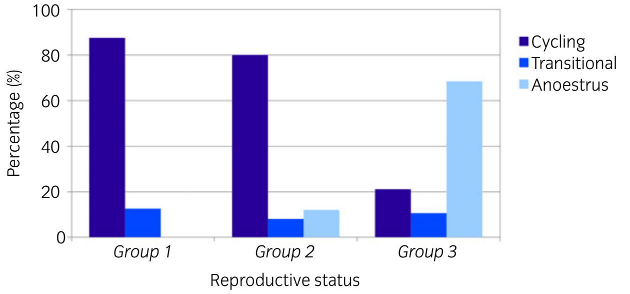

Fig 3: Reproductive status of all mares at the end of the study on 10 February 2012. *Group 1* (indoor barn lighting) had 14/16 (87.5%) cycling, 2/16 (12.5%) transitional and 0/16 (0%) anoestrus. *Group 2* (light masks) had 20/25 (80%) cycling, 2/25 (8%) transitional and 3/25 (12%) anoestrus. *Group 3* (no artificial light) had 4/19 (21.05%) cycling, 2/19 (10.53%) transitional and 13/19 (68.42%) anoestrus.

(<1 ng/ml). Anoestrus was defined as the absence of follicles >15 mm with no associated physiological characteristics typical of oestrous activity and progesterone levels <1 ng/ml.

Ophthalmic examinations were carried out at the start and end of the project. Hair shedding was assessed for *Groups 1* and *2* on 20 January. Using a latex glove each mare was rubbed once in the direction of hair growth along the back, flank and buttocks. The hair loss was graded based on coverage of the glove with hair on a scale of 1–4: 1 represents no hair loss; 2, mild hair loss; 3, moderate hair loss; and 4 represents heavy hair loss.

Progesterone assay

Whole blood was centrifuged and the serum removed and stored at -20°C until further processing. Serum progesterone concentrations were determined using a competitive binding enzyme-linked immunosorbent assay as previously described [21]. All reagents were purchased from Sigma-Aldrich[f]. Inter- and intra-assay coefficients of variation were 16.1% and 12.8%, respectively.

Data analysis

Mares from each group were classified as either cycling, transitional or anoestrus on 20 November and 10 February (the beginning and end of the study period). Pairwise comparisons were conducted using a Chi-squared and Fisher's exact test.

The number of mares within each group determined to have ovulated by 20 January and 10 February was calculated and pairwise comparisons were conducted using a Chi-squared and Fisher's exact test.

Mean serum progesterone concentrations from samples collected on 6 January, 20 January and 10 February were compared between mares classified as cycling or anoestrus across all groups using the Mann–Whitney *t* test for nonparametric data.

The hair loss scores from *Group 1* were compared with the hair loss scores from *Group 2* using an unpaired *t* test. Significance was defined as P<0.05 and data are presented as mean ± s.e.

All statistical analyses were conducted using GraphPad Prism Version 5.0 for Mac[g].

Results

The proportion of mares determined to be cycling, transitional or anoestrus was not different between *Group 1* and *Group 2* (P = 0.314), *Group 1* and *Group 3* (P = 0.11), or between *Group 2* and *Group 3* (P = 0.532) in mid November (Fig 2). At the end of the trial there was a difference in the proportion of mares exhibiting oestrous cyclicity between *Group 1* and *Group 3* (87.5% vs. 21%, respectively; P<0.0001) and also between *Group 2* and *Group 3* (80% vs. 21%, respectively; P<0.0003). There was no difference in oestrous cyclicity between *Group 1* and *Group 2* (87.5% vs. 80%, respectively; P = 0.335). Figure 3 illustrates the reproductive status of each group on 10 February.

The number of mares determined to have ovulated was not different between *Group 1* and *Group 2*, or between *Group 1* and *Group 3*, on 20 January. However, a difference was observed between *Group 2* and *Group 3* (P = 0.014) indicating that more mares wearing light masks had ovulated than in the nonlight exposed control group at this time. On 6 February, there was no difference in number of mares that had ovulated between *Group 1* and *Group 2* but significant differences existed between *Group 1* and *Group 3* (P<0.001) and between *Group 2* and *Group 3* (P<0.001, Fig 4).

Mean serum progesterone levels were significantly higher in mares classified as 'cycling' (n = 38) compared with 'noncycling' mares (n = 16) in samples collected after 1 January (P<0.0001).

There was no significant difference in mean hair loss scores between *Group 1* and *Group 2* (3.06 ± 0.17 vs. 2.95 ± 0.195, respectively; P = 0.665), with moderate hair loss observed in both. Ophthalmic examinations prior to and after the study revealed no effect of treatment on eye health.

Discussion

Artificial advancement of the equine breeding season has become standard management practice for Thoroughbred breeders. This study

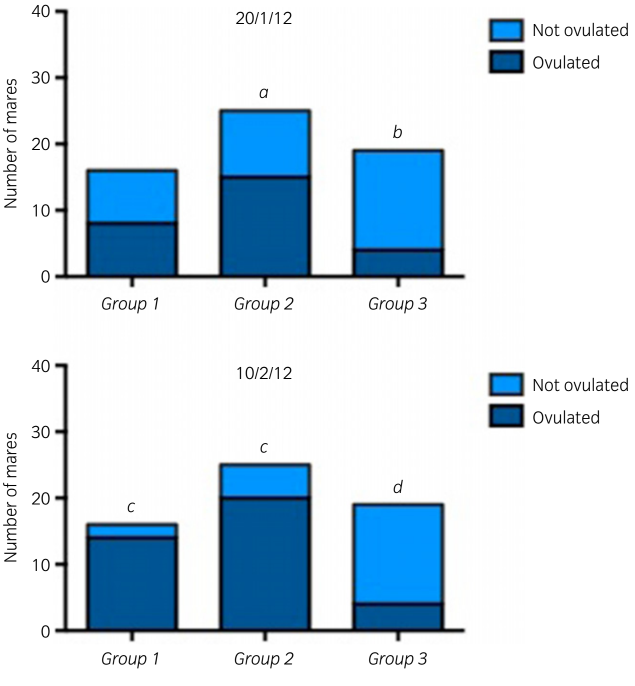

Fig 4: Comparison of number of mares determined to have ovulated from each group on 20 January (8/16, *Group 1*; 15/25, *Group 2*; 4/19, *Group 3*; top panel) and 10 February (14/16, *Group 1*; 20/25, *Group 2*; 4/19, *Group 3*; bottom panel). Differences between groups are denoted by italic lettering: *a*,*b* (P<0.05), *c*,*d* (P<0.001).

shows that the reproductively active period of a nonpregnant mare can be advanced using low-level blue light to one eye from head-worn light masks and that this method is as effective as maintaining mares indoors under artificial lighting.

While it was expected that a high proportion of mares in the barn-lit and light mask wearing groups would be cycling by the end of our study, what was surprising was the large proportion of mares from these groups that were still reproductively active at the beginning of the study in mid November. As reproductive examinations are not commonly carried out at this time of year, it is often assumed that mares enter winter anoestrus earlier in the year. However, we observed a transition to anoestrus that occurred between late December and early January in the mares that were still expressing cyclicity at the start of the study in our control group. Of interest is that a lower proportion of mares from *Group 3*, the control group, were still expressing oestrous cyclicity at this time.

It is believed that the maintenance history of each of the experimental groups contributed to their reproductive status at the beginning of the study. Due to the size of the study, it was not possible to randomly allocate mares into 3 groups from a homogeneous population. The control mares were maintained outdoors on a farm within an 8 km radius of the mares in the other experimental groups and had been living outdoors as a herd for several years prior to the study. Mares with constant exposure to the natural climatic challenges of the environment are known to exhibit a tighter breeding season and enter winter anoestrus, as is characteristic of feral mares [22]. While every effort was made to control for breed, body condition, age and nutritional intake, the management history of the animals in the experimental groups probably influenced their reproductive status in mid-November.

At the end of the study, 4/19 of the control mares were reproductively cycling despite having received no stimulatory light signal. This finding of a subset of mares that continued to cycle throughout the year is supported by previous studies [23,24] and is a phenomenon unique to the equine species.

Occasionally during the study incidents with the prototype masks, such as mud coating the reflective inner cup and masks breaking or falling off, meant that mares received either a lower intensity of light than intended or no light for a single night. This could have contributed to an inadequate light intensity failing to stimulate reproductive activity in the 3 mares from *Group 2* that remained anoestrus at the end of the trial. It is believed that an optimised mask design may increase the success rate achieved.

Our finding that low-level blue light from mobile light masks can successfully advance the breeding season in mares has implications for improving efficiency of equine breeding management. A recent survey of farm managers found that the cost of maintaining mares indoors under lights was approximately €120/mare/week (personal communication to B.A. Murphy). This figure was calculated based on combined feed, bedding, labour and energy costs. Assuming that artificial lighting programmes are initiated on 1 December and the earliest that a mare is covered is 15 February, the shortest duration a mare will be maintained under lights is until the first pregnancy check at Day 15 post covering. Thus the minimum duration of light therapy is 12 weeks (until 1 March). Our results suggest that the equine breeding season could now be advanced while the mares live outside in groups. This method is unlikely to reduce feed costs significantly, but bedding, labour and energy costs would be significantly reduced. In addition to the cost saving benefits, it has been reported that mares fed on fresh pasture ovulate earlier in the breeding season than mares fed on dry forage [25].

A previous investigation of the potential of delivering light to the mares eyes using white light-emitting blinkers as an alternative to stabling clearly showed the potential of this method [26]. The authors suggested that further technological advances were required before it became a viable option. The light mask described here represents the next step in the advancement of this technology towards a viable alternative for horse breeders, but with an important difference – the use of blue light delivered via LEDs to a single eye that has been shown to optimally inhibit melatonin levels necessary to provide the long-day photoperiod that stimulates the mare's reproductive axis [19].

Horses are naturally herd animals and the social instinct for bonding with individuals is an important component of their socioecology [27]. Domestication by man has led to horses being housed in individual stalls, fed high-energy concentrate feeds with heavy interference in natural breeding behaviours. Studies have shown that a lack of social interaction, a high-concentrate, low-forage diet and reduced locomotor activity can result in the development of stereotypic behaviour [28–31]. The development of management techniques that allow horses to be maintained outdoors, in social groups with access to grazing could reduce negative behaviours and risk of illness and therefore improve animal welfare.

This study reports that advancement of the breeding season using low-level light stimulation to one eye results in early shedding of the winter coat similar to what is seen in mares maintained under stable lighting. While a control group unexposed to artificial light was not examined for shedding in this study, it is commonly accepted that shedding of the heavier winter coat does not occur until much later in the season [32]. The relationship between photoperiod and seasonal changes in the equine pelage has previously been reported [33]. For many competitive equestrian disciplines such as showing and showjumping, the development of a smooth coat in summer is a sought after and a valuable characteristic of the natural equine breeding season. The ability to advance the onset of shedding using light therapy is a management technique already utilised by some sport horse trainers; however, the associated cost of housing could be considered prohibitive by many more. The ability to provide light therapy in a cost effective manner while the horses remain outdoors offers a novel method of achieving desired winter coat loss earlier in the year.

In conclusion, this study has shown that timed low-level blue light to one eye provided by a head worn mask is as effective as indoor barn lighting at advancing the equine breeding season, but with economic benefits for the breeder and welfare benefits for the horse.

Authors' declaration of interests

A priority preliminary patent application was filed in Ireland in May 2011 (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.

Ethical animal research

All experimental procedures were approved by the University College Dublin Animal Research Ethics Committee and the University of Kentucky Institutional Animal Care and Use Committee protocol number 2012-0928.

Sources of funding

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Authorship

B.A. Murphy contributed to study design, data collection, study execution, data analysis and interpretation and preparation of the manuscript. M.H. Troedsson contributed to study design, data analysis and interpretation and preparation of the manuscript. C.M. Walsh contributed to study design, data collection, study execution, data analysis and interpretation and preparation of the manuscript. E.M. Woodward contributed to study design, data collection, study execution, data analysis and interpretation and preparation of the manuscript. L.H. Fallon contributed to data collection, study execution, data analysis and interpretation and preparation of the manuscript. R.L. Prendergast contributed to data collection, study execution and preparation of the manuscript. J.P. Ryle contributed to data collection and study execution.

Manufacturers' addresses

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[c]Zilco, Christchurch, New Zealand.
[d]GaN, Taipei, Taiwan.
[e]Arduino, Almuñécar, Spain.
[f]Sigma-Aldrich, St. Louis, Missouri, USA.
[g]Graph Pad Software, San Diego, California, USA.

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