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The Effects of Coloured LED Light on Behaviour and Physiology in Healthy Horses

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Abstract: The use of colour light therapy to treat diseases and disorders in animals is increasingly common but only very little research has been conducted on the effects of illumination with coloured light. The present study examined to which extent coloured light causes behavioural and physiological responses in horses. Red, green, blue and yellow as well as white as a control colour were tested in 20 healthy stallions of the breed Freiberger. Coloured light was applied on five consecutive days in randomised order for 15 min each in a LED light illuminated box stall. Behaviour was observed during illumination, salivary cortisol levels were measured immediately thereafter. Elimination, activity, heart rate and temperature parameters were recorded before, during and after illumination. Results showed that "Drinking" occurred more often at yellow and body core temperature was higher at red and blue and lower at green and yellow, whereas body surface temperature was tendentially increased at all colours compared to the control white. For the majority of parameters, however, no effects were found and discovered effects often did not correspond to expectations deriving from principles of colour light therapy. A general effect of illumination, regardless of the colour, was further demonstrated for activity, heart rate and heart rate variability and absolute values of physiological and behavioural parameters indicated that the illumination with coloured light did not constitute a stressor but rather had a becalming effect on horses.

Keywords: Behaviour, body temperature, colour light, cortisol, horse, heart rate parameters.

1. INTRODUCTION

The use of colour light therapy in animals to treat diseases and disorders is increasingly common but only very little research has been conducted on the effects of coloured light on animals. Existing studies most often used animal models for human therapy, *e.g.* on wound healing [1, 2], osteoarthritis [3], motor function following embolic strokes [4], depression [5] or diabetes [6], however studies on the effectiveness of colour light therapy exist neither for horses nor other animals. Nevertheless, various forms of practical therapy applications are prevalently used in horses and other domestic animals as an alternative treatment method for disorders or diseases, *e.g.* dyspepsia, infections, abscesses, hematomata, arthritis, laminitis, muscle pain or nervousness [7]. After a diagnosis, the type of illumination, the choice of colour or colour combination and the duration of illumination are determined by a colour light therapist. Frequently applied types of application are selective irradiation of acupuncture points [8], partial irradiation on certain body areas [9] and total body exposure or room illumination for a holistic effect on the body. Recommendations on treatment durations vary from 10 to 20 minutes, sometimes repeated several times per day [9, 10]. Often specific therapy lamps (Bioptron®, Q.Light®) are used, whereas the usage of LED light provides an alternative which is also utilised in human medicine [11, 12].

The effects of colours and coloured light on the human body have traditionally been used for centuries. The practical application of colour light therapy in horses is based on an analogy to the human field or practical experience

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of animal colour light therapists. Red is described to be generally stimulating [7], leading to an increased heart rate, elevated blood circulation, and further to have a warming effect [9]. Blue is described to generally have a calming and relaxing effect by stimulating the parasympathetic nervous system [7, 10] and also to have a blood circulation reducing effect [7]. Green is described to induce general relaxation [7] which has a balancing effect on the cardiovascular system and further to have an anti-inflammatory effect [9]. Yellow is described to have a stimulating effect on nerves which can lead to increased activity or nervousness [10] and further is considered to be a "hot colour" [9]. Furthermore, coloured light is not only applied in alternative therapy but also in scientific medicine and research has been conducted on psychological and also therapeutic effects. Blue light (420 - 470 nm) for example is used for the treatment of jaundice in new-borns [13, 14] and Seasonal Affective Disorder in adults [15]. Iyilikci *et al.* [16] showed an antidepressant effect for blue light by stimulation of the melanopsin-containing ganglion cells of the retina, which was not ascertainable with red light. Also different effects of red and blue light were found by Weinzirl *et al.* [17] regarding changes in blood-oxygen levels in the human brain and muscles. Trimmer *et al.* [18] found evidence that a treatment with near-infrared light (810 nm) can restore axonal transport in model human dopaminergic neuronal cells to control levels, and thus to improve neuronal functions in patients with Parkinson's disease.

The impact of light on the body occurs either visually or physically and chemically by stimulation of photoreceptors in skin cells. Visual impact is causing an irritation of the sensory cells in the eye [19]. Therefore, colour vision might be a precondition for colour light therapy. Studies on horses' colour vision and their ability to discriminate certain wavelengths from grey have concluded a dichromatic colour vision. Pick *et al.* [20] obtained significant results regarding the discrimination of blue (462 nm) and red (700 nm), but not green (496 nm). Blackmore *et al.* [21], in turn, demonstrated the ability to see blue (470, 474, 482 nm), yellow (579, 582, 583 nm) and green (532, 533, 545 nm) and a tendency for red (609, 611, 615 nm). However, Smith and Goldman [22] observed horses with the ability to distinguish all four colours (blue 470 nm, red 617 nm, green 538 nm, yellow 581 nm) successfully, but reported some individuals with partial colour blindness. More important for the application of colour light therapy is the mechanism of physical and chemical impact by stimulation of photoreceptors in skin cells which generate neuronal and neuroendocrine signals [23, 24]. Hence, brightness and different wavelengths of light are supposed to specifically influence biochemical processes in body cells [7]. As a result, specialist literature on colour light therapy describes colour specific effects on body core temperature and heart rate in particular and on physical activity, relaxation or stress in general [7, 9, 10]. The temperature regulation centre is located in the hypothalamus and body core temperature is controlled endogenously [25]. Body surface temperature is subordinate to the regulation of body core temperature and participates additively in the production or release of heat [26]. The heart rate is related to circulation, respiration, temperature and metabolism; it is directly influenced by physical and psychological stressors [27] and a good indicator of activity-independent acute stress [28]. Furthermore, heart rate variability is an analytical measurement for fluctuations in the length of beat-to-beat-intervals [29]. Changes in heart rate and heart rate variability are caused by a dynamic but non-additive interplay among the sympathetic and parasympathetic nervous system in the cardiovascular control [29, 30]. Heart rate variability has been shown to be a sensitive parameter of the time characteristic of physical or psychological stress [30]. Another parameter in valuation of stress is the steroid hormone cortisol, whose secretion into blood, and further *via* passive diffusion into saliva, responds quickly to stressors [31 - 33]. Since blood sampling is invasive and thus a potential stressor itself, non-invasive salivary cortisol sampling is a means to measure stress responses [31].

The aim of this study was to obtain general knowledge on the effects of coloured light on horses and to evaluate effects described in specialist literature. For this purpose, the effects on various behavioural and physiological parameters were investigated in 20 healthy stallions of the breed Freiberger during short term exposure to coloured light at red, blue, green and yellow in comparison to white light as control. In connection with described physiological responses to coloured light, behavioural reactions, which allow general conclusions about possibly induced stress or relaxation by the illumination, were further taken into consideration.

2. ANIMALS, MATERIAL AND METHODS

2.1. Animals and Housing

The study was conducted with 20 healthy stallions of the breed Freiberger at the Swiss National Stud Farm[a]. The age of the animals ranged from 5 to 17 years (mean of 8.25 years), the coat colours matched the breed typically chestnut (n=6), liver chestnut (n=2), bay (n=10) and dark bay (n=2). Seventeen horses had winter coat, two were

[a] Agroscope, Swiss National Stud Farm, Avenches, Switzerland

wearing rugs and therefore had shorter coat and one horse was clipped and wearing a rug; rugs were taken off 1 h before individual testing. All horses were handled on a daily base, they were accustomed to being ridden, driven or walked in a horse walker and having access to paddocks. The stallions were housed individually in box stalls of 3.0 x 3.65 m, provided with crib, drinker, salt lick and straw bedding (Fig. **1**). Box stalls were built of solid wooden walls (up to a height of 1.30 m) and upper grill panels with gaps between bars of 5 cm (up to a height of 2.70 m), allowing visual and restricted physical contact. Housing was in accordance with the national animal welfare legislation [34]. Feeding with hay and concentrate occurred at 8:00 and 17:00, and concentrate was fed additionally at 12:00.
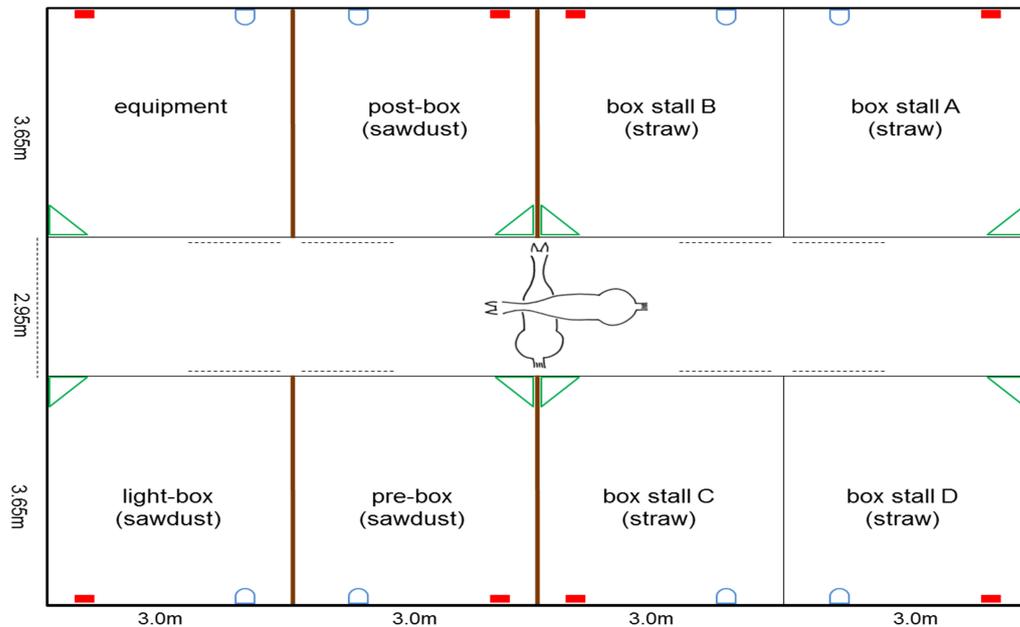
Fig. (1). Sketch of the experimental barn compartment at the Swiss National Stud Farm with four box stalls for housing of experimental animals and three experimental box stalls (pre-, light- post-box); each provided with crib, drinker and salt lick and littered with straw or sawdust.

The experiment took place in a separate barn compartment with eight box stalls, similar to all other barn compartments at the Swiss National Stud Farm (Fig. **1**). The horses were brought to the experimental barn compartment one day before their first testing and stayed there for the entire test week; four box stalls were used for housing the animals, three for the experiment and one for equipment (Fig. **1**). During the five experimental days, housing and feeding was performed as usual but the horses were not ridden or driven. Instead, they walked for 1 h in a horse walker in the morning and had 1.5 h individual access to a paddock (150 m^2) every day. Except for feeding, mucking out and littering the box stalls, all handling and data recording was conducted by one person.

2.2. Experimental Test Procedure

The horses were tested during five weeks between November and January; in each test week four horses attended the experiment. Each horse was tested individually on five consecutive days (Monday to Friday) with the colours red, green, blue and yellow as well as white as control colour. A given animal was exposed to only one colour per day and test procedures for each horse always started at the same time of the day (9:00, 10:30, 13:00 or 14:30). Allocation of colours to the different horses and test days was randomised considering the following rules:

a. For all possible combinations of weekdays (Mon - Fri) and times of the day (9:00, 10:30, 13:00 or 14:30), every colour was realised once within the five test weeks.
b. All possible sequences of any two colours must be realised within the five test weeks and the number their occurrences was as equal as possible.
c. As many different colours as possible were tested on a given day.

The daily test procedure took about 75 min and was subdivided into three experimental phases (pre-, light- and post-phase) of 20 min each. Between experimental phases the horse was tied in the alley for 5 min (Table **1**). Pre-, light- and

post-phase each took place in different experimental box stalls (pre-, light- and post-box) which were provided with crib, drinker and salt lick and littered with sawdust (Fig. **1**). To prevent physical interactions with neighbouring horses during testing, the side walls of experimental box stalls were shut with wooden panels up to a height of 2.70 m.

Table 1. Daily experimental test procedure for each horse with respective location (pre-, light- and post-box, alley) and recorded behavioural or physiological parameters at given phases or measurement times.

	Pre-phase		Light-phase		Post-phase	
Location	Pre-box	Tied in alley	Light-box	Tied in alley	Post-box	Tied in alley
Time [min]	0 - 20	20 - 25	25 - 45	45 - 50	50 - 70	70 - 75
Behaviour			x			
Elimination	x		x		x	
Activity (ALT-Pedometer)	x		x		x	
Heart beat parameters (Polar®)	x		x		x	
Body core temperature (Microlife Vet-Temp)		x		x		x
Body surface temperature (FLIR Systems)		x		x		x
Salivary Cortisol (Salivette®)				x		

The experimental barn compartment was illuminated with ordinary fluorescent strip lights; lamps were turned on all day (7:00 - 12:00 / 13:00 - 17:00) and displayed the standard lighting in pre-, light and post-box. The exposition to the coloured light was conducted for 15 min after 5 min acclimatisation. The light-box was equipped with two high power LED lamps on the ceiling (at a height of 3.65 m) to illuminate the entire rear wall and about 0.5 m of the floor (Fig. **2**). The LED lamps[b] each contained 48 RGB-LEDs (16 red, 16 green, 16 blue) to generate the electromagnetic spectrum from 470 - 620 nm by additive colour mixture. To illuminate the light-box with red (625 nm), green (534 nm) and blue (470 nm), the corresponding LEDs were used with their maximum output (100%). Yellow (571 nm) was mixed with red (output 41.2%) and green (output 58.8%), whereas white was mixed with red (output 29.4%), green (output 35.3%) and blue (output 35.3%). The wavelengths of test colours were consistent with horses' ability of colour vision. For all colours, the intensity of illumination was controlled to about 240 lux. To focus the illumination on the rear wall, the side walls were painted in black and the rear wall was coated with reflecting white dispersion paint.

Fig. (2). Light-box with ceiling-mounted LED lights at white light and horse during illumination with red, green, blue and yellow light (from left to right).

2.3. Data Recording

For data analysis, only 14 min (minute 6 - 19) of each experimental phase (pre-, light-, and post-) were used accounting for adaption of the horse to the experimental box stalls.

2.3.1. Behaviour

Activity was recorded continuously by means of an ALT-Pedometer[c] and stored as the sum of single step events per minute. The pedometer was attached to the horses' left hind leg above the fetlock. Data was transferred to a

[b] LED lamp HEDERA, Zumtobel Licht AG, Zurich, Switzerland

[c] ALT-Pedometer, Ingenieurbüro Holz, Falkenhagen, Germany

computer using associated ALT-Pedometer-Software[d] and the mean steps per minute were calculated. Due to a problem with the logger capacity, data of 10 horses only was available for the analysis.

During the exposition to the coloured light, the horses were filmed, allowing real time observation out of sight of the animals *via* a monitor. Behavioural parameters and position (Table **2**) within the light-box were recorded continuously using Interact[®e]. The frequency of defecation and urination was documented additionally in pre- and post-phase by counting droppings and urine stains.

Table 2. Ethogram of observed behavioural parameters and position in the light-phase.

Behaviour	Recording unit	Description
Arousal indicative behaviour		
Head banging	frequency	Vigorous vertical head movement
Head shaking	frequency	Rapid torsional neck movement, 1 - 10 direction changes, maximum interruption of 2 s between bouts
Pawing	frequency	Raising a front leg and scraping it backwards over the floor, 1 - 10 leg movements, switching legs possible, maximum interruption of 2 s between bouts
Snorting	frequency	Strong exhaling through the nostrils with audible sound
Tail swishing	frequency	Strong movement of the tail root, 1 - 5 changes of direction, maximum interruption of 2 s between bouts
Comfort behaviour		
Body shaking	frequency	Horizontal movement of the body, 1 - 10 changes of direction, maximum interruption of 2 s between bouts
Rolling	frequency	Lying down, rubbing the body with wavy or rolling movement on the ground and getting up
Scratching	frequency	Scratch itself with mouth, head, leg or to an object, 1 - 10 changes of direction of single movement, maximum interruption of 2 s between bouts
Elimination		
Defecating	frequency	Defecation
Urinating	frequency	Urination
Exploration		
Exploration of litter	frequency, duration (s)	Mouth max. 1/2 head length above ground
Locomotion		
Change of sector	frequency	Horse changes between the two sectors of light-box (half of the box near or away from illuminated rear wall), horse is located in the sector in which more than half of its body stands
Turning in circles	frequency	Turning around its body axis to minimum 180 and maximum 360 degrees
Oral activities (non-stereotypic)		
Chewing	frequency, duration (s)	Masticatory movement with an empty mouth, maximum interruption of 2 s between bouts
Drinking	frequency	Mouth in drinker and swallowing movements, maximum interruption of 2 s between bouts
Licking salt lick	frequency	Licking the salt lick, maximum interruption of 2 s between bouts
Tongue activity	frequency	Licking of an object (other than salt lick) or the area around the mouth, maximum 2 s interruption
Yawning	frequency	Opening of the mouth by straight or lateral movement of the mandible
Resting behaviour		
Hind leg resting	duration (s)	One hind leg strained, the other bent, switching legs possible, maximum interruption of 2 s between bouts
Lying	frequency	Lying in a ventral or lateral position
Vocalisation		
Whinnying	frequency	Species-typical vocalisation
Position		
Distance to light	duration (s)	Horse in the half of the box near illuminated rear wall
Orientation to light	duration (s)	Body axis towards or parallel to illuminated rear wall

2.3.2. Physiological Parameters

Heart rate parameters were measured continuously with the Polar® heart rate monitoring system[f], using an elastic surcingle with two electrodes attached behind the fore legs aligned to position of saddle girth. Ultrasound gel was used to optimise the contact between electrodes and skin. The recorded data was readout with associated Polar® software[g]. Data was corrected for artefacts (filtration rate: moderate, minimal protection zone: 6) with the maximum acceptable value for identified errors set at 10%. With this precondition, 51 incorrect measurements of in total 300 data points were

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not included in the data analysis, excluding one horse completely. The mean heart rate and heart rate variability, measured as RMSSD (root mean square of successive beat-to-beat differences in milliseconds), were determined for each phase.

Body core and body surface temperature were measured after each experimental phase. Body core temperature was measured rectally using a thermometer[h]; for each measurement time, three values were taken and averaged. Body surface temperature was measured using an infrared thermal camera[i]. After each phase, photos of the right and left side of the body were taken from a distance of about 6 m. The pictures were analysed with associated IRwin OLE software[j]. Each photo was corrected for ambience temperature and atmospheric humidity at the time of photographing, using time-matched stable climate data recorded by a weather station[k] placed in the barn. In each photo three areas on the horse were marked (1. trapezoid along the jugular groove to the base of the neck, up to the withers and back to the throat, 2. rectangle stretched between stifle and back, limited by the heart rate monitor girth, 3. rectangle stretched between stifle and dock) and the average temperature was determined for each area. Finally, the mean average surface temperature of the horse was calculated including all three areas of the left and right body side. The three horses that were wearing rugs outside the experimental procedure were not included in the analysis of body surface temperature.

Salivary cortisol levels were determined following the light-phase. Samples were taken by holding a cotton swab[l] with a clamp and placing it in the mouth for about 15 seconds. The wadding was centrifuged (15 min, 3000 rpm) after experiments finished on a given day and afterwards deep-frozen (-85°C) until analysis. Samples were analysed by the Laboratory of Veterinary-Physiology (Vetsuisse Faculty, University of Berne, Switzerland) by use of a Salimetrics® analysis kit[m]. Analysis was performed according to the manufacturer [35] and results were expressed in nmol/ml.

2.4. Statistical Analysis

Statistical analysis was conducted in R (The R Project for Statistical Computing, Version 2.15.0 [36]) using generalised linear mixed-effects models; lme method (package 'nlme' [37]) and glmer method (package 'lme4' [38]). For parameters recorded only in the light-phase, explanatory variables included "colour" (factor with five levels: white, red, green, blue, yellow) as fixed effect and "animal nested within test week" as random effect. For parameters recorded in all phases of the test procedure (pre-, light-, post-phase), explanatory variables included "colour" (factor with five levels: white, red, green, blue, yellow), "phase" (factor with three levels: pre-, light-, post-phase) and their interaction "colour*phase" as fixed effects and "day nested within animal within test week" as random effect. Models with only main effects were compared to models additionally including the interaction of the main effects by means of Akaike information criterion (AIC values), in order to determine the best fitting model for each parameter. The models with the lower AIC values [39] were chosen as final models and presented as results; models with only the main effects were found to be the best fitting for all parameters.

The lme method was used with type I sum of squares to calculate the *p*-values for explanatory variables. Statistical assumptions of all models were checked using graphical analysis of residuals (normal distribution, homoscedasticity). To meet the statistical assumptions, some outliers were excluded (in maximum 7 data points per response variable) and some of the response variables were transformed (log-transformation for activity, heart rate parameters and body surface temperature, for behavioural parameters, see Table **3**). If transformation was not successful to achieve normal distribution of residuals, the response variables were dichotomised and the glmer method was used (Table **3**). For calculation of *p*-values with glmer method, maximum models were compared to minimum models (parameters observed only in light phase: models with fixed effect "colour" *vs.* models with no fixed effect; parameters observed in all three phases: models with fixed effects "colour" and "phase" *vs.* models with fixed effect "colour" or fixed effect "phase"). For some behavioural parameters, statistical analysis was not possible due to very low frequencies (Table **3**).

While model selection was performed according to AIC values, *p*-values of main effects were presented in order to support understanding [40]. In addition, model predictions with estimated means and 95% confidence intervals were

calculated for every experimental situation ("colour" or combination of "colour" and "phase") on basis of the respective model and used for interpretation of the effects in case of statistically significant results.

2.5. Ethical Approval

Ethical approval for the implementation of the study was obtained from the Vaud Cantonal Veterinary Office, Switzerland (Approval No. 2265, 2009).

3. RESULTS

3.1. Behaviour

In comparison to white, "drinking" occurred more often at yellow (χ^2_4 = 14.62, p = 0.0056; (Fig. **3**). Colour did not affect any of the other behavioural parameters, the position (see Table **3**), or activity ($F_{4,36}$ = 0.92, p = 0.5; (Fig. **4**). However, activity was decreased in the light-phase compared to the pre- and post-phase ($F_{2,92}$ = 5.39, p = 0.0061; (Fig. **4**), regardless of colour. Defecation was observed more often in the pre-phase compared to the subsequent phases, regardless of colour (χ^2_4 = 21.18, p = 0.017; (Fig. **5**)).
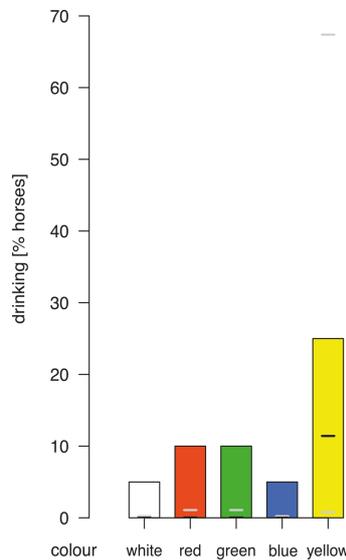
Fig. (3). Percentage of horses showing "drinking" in relation to colour, including model predictions with estimated means (black lines) and 95% confidence intervals (grey lines).

Table 3. Behavioural parameters and position observed in the light-box during illumination with different colours. Raw data given as means and ranges (min - max) of recording unit (in brackets for parameters analysed as dichotomised variables) and percentage of horses (n=20) showing the respective behaviours. Statistical results with selection of model (including eventual transformation for lme method or distributional family for glmer method), statistical unit, test statistics and p-values (< 0.05 in bold).

	White	Red	Green	Blue	Yellow			
Behaviour and position [recording unit]			Ø; min - max % horses			Model [statistical unit]	test statistics	p-value
Head banging [frequency]	0.25; 0 - 4 10%	0.1; 0 - 1 10%	0 0%	0 0%	0.35; 0 - 7 5%	no statistics		
Head shaking [frequency]	1.4; 0 - 5 55%	(1.95; 0 - 5) 70%	(1.8; 0 - 9) 65%	(1.25; 0 - 6) 50%	(2.25; 0 - 20) 60%	glmer (binomial) [incidence]	χ^2_4= 2.52	p = 0.64
Pawing [frequency]	(1.7; 0 - 9) 55%	(3.75; 0 - 25) 45%	(1.85; 0 - 22) 35%	(0.9; 0 - 8) 30%	(1.3; 0 - 10) 40%	glmer (binomial) [incidence]	χ^2_4 = 3.64	p = 0.46
Snorting [frequency]	(1.05; 0 - 5) 55%	(0.95; 0 - 3) 55%	(1.6; 0 - 9) 60%	(0.9; 0 - 4) 65%	(1.55; 0 - 6) 45%	glmer (binomial) [incidence]	χ^2_4 = 1.95	p = 0.74

(Table 5) contd.....

Behaviour and position [recording unit]	White	Red	Green	Blue	Yellow	Model [statistical unit]	test statistics	p-value
			Ø; min - max % horses					
Tail swishing [frequency]	0 0%	0 0%	0.05; 0 - 1 5%	0.05; 0 - 1 5%	0 0%	no statistics		
Body shaking [frequency]	0 0%	0.05; 0 - 1 5%	0.05; 0 - 1 5%	0.05; 0 - 1 5%	0.2; 0 - 3 10%	no statistics		
Rolling [frequency]	0 0%	0.05; 0 - 1 5%	0 0%	0 0%	0.05; 0 - 1 5%	no statistics		
Scratching [frequency]	(2.25; 0 - 14) 70%	(1.6; 0 - 6) 55%	(0.95; 0 - 7) 45%	(1.8; 0 - 7) 60%	(2.05; 0 - 10) 55%	glmer (binomial) [incidence]	$\chi^2_4 = 2.84$	$p = 0.58$
Defecating [frequency]	(0.02; 0 - 1) 10%	(0.04; 0 - 1) 20%	(0.03; 0 - 1) 15%	(0.05; 0 - 2) 15%	(0.03; 0 - 1) 15%	glmer (binomial) [incidence]	$\chi^2_4 = 5.36$	$p = 0.25$
Urinating [frequency]	0 0%	0 0%	0 0%	0 0%	0 0%	no statistics		
Exploration of litter [frequency]	3.8; 0 - 16 90%	3.85; 0 - 16 75%	3.25; 0 - 11 80%	3.55; 0 - 15 75%	4.5; 0 - 17 80%	lme (log) [frequency]	$F_{4,76} = 0.26$	$p = 0.9$
Exploration of litter [duration (s)]	58.3; 0 - 212.9 90%	56.6; 0 - 233.5 75%	59.4; 0 - 301.3 80%	50.5; 0 - 187.1 75%	53.2; 0 - 336.0 80%	lme(log) [duration (s)]	$F_{4,76} = 0.19$	$p = 0.95$
Change of sector [frequency]	8.1; 0 - 36 85%	8.7; 0 - 26 80%	6.15; 0 - 18 80%	6.6; 0 - 17 80%	8.9; 0 - 32 75%	lme (sqrt) [frequency]	$F_{4,76} = 0.26$	$p = 0.9$
Turning in circles [frequency]	(0.3; 0 - 2) 20%	(0.7; 0 - 5) 30%	(1; 0 - 8) 25%	(0.5; 0 - 4) 30%	(0.65; 0 - 4) 40%	glmer (binomial) [incidence]	$\chi^2_4 = 3.30$	$p = 0.51$
Chewing [frequency]	4.3; 0 - 12 90%	5.3; 0 - 15 90%	6.35; 0 - 22 90%	6.85; 2 - 20 100%	6.9; 0 - 20 90%	lme [frequency]	$F_{4,74} = 1.12$	$p = 0.36$
Chewing [duration (s)]	28.3; 0 - 94.2 90%	36.8; 0 - 185.4 90%	46.5; 0 - 163.0 90%	46.7; 10.2 - 136.9 100%	39.5; 0 - 101.6 90%	lme [duration (s)]	$F_{4,73} = 1.5$	$p = 0.21$
Drinking [frequency]	(0.05; 0 - 1) 5%	(0.1; 0 - 1) 10%	(0.1; 0 - 1) 10%	(0.05; 0 - 1) 5%	(0.25; 0 - 1) 25%	glmer (binomial) [incidence]	$\chi^2_4 = 14.62$	$p = 0.0056$
Licking salt lick [frequency]	(0.3; 0 - 3) 20%	(0.15; 0 - 2) 10%	(0.45; 0 - 3) 20%	(0.35; 0 - 4) 10%	(0.4; 0 - 3) 20%	glmer (binomial) [incidence]	$\chi^2_4 = 2.36$	$p = 0.67$
Tongue activity [frequency]	(0.55; 0 - 5) 20%	(0.15; 0 - 1) 15%	(0.45; 0 - 2) 30%	(0.45; 0 - 6) 20%	(0.8; 0 - 4 40%)	glmer (binomial) [incidence]	$\chi^2_4 = 4.63$	$p = 0.33$
Yawning [frequency]	1.2; 0 - 7 40%	1.85; 0 - 12 45%	2; 0 - 10 55%	1.45; 0 - 4 55%	1.7; 0 - 7 40%	glmer (binomial) [incidence]	$\chi^2_4 = 2.60$	$p = 0.63$
Hind leg resting [duration (s)]	65.88; 0 - 100 95%	63.29; 1.8 - 100 100%	69.4; 13.9 - 100 100%	66.17; 18.5 - 99.8 100%	64.01; 0 - 100 90%	lme (logit) [% of time]	$F_{4,73} = 0.55$	$p = 0.7$
Lying [frequency]	0.05; 0 - 1 5%	0 0%	0 0%	0 0%	0 0%	no statistics		
Whinnying [frequency]	0 0%	0.05: 0 - 1 5%	0 0%	0 0%	0 0%	no statistics		
Distance to light [duration (s)]	35.39; 0 - 100 85%	26.82; 0 - 94.4 75%	35.44; 0 - 100 85%	37.25; 0 - 100 80%	44.33; 0 - 100 75%	lme (logit) [% of time]	$F_{4,76} = 0.53$	$p = 0.72$
Orientation to light [duration (s)]	23.99; 0 - 100 95%	27.05; 0 - 100 75%	27.58; 0 - 100 85%	25.96; 0 - 98.4 80%	13.17; 0 - 100 75%	lme (logit) [% of time]	$F_{4,76} = 0.48$	$p = 0.75$
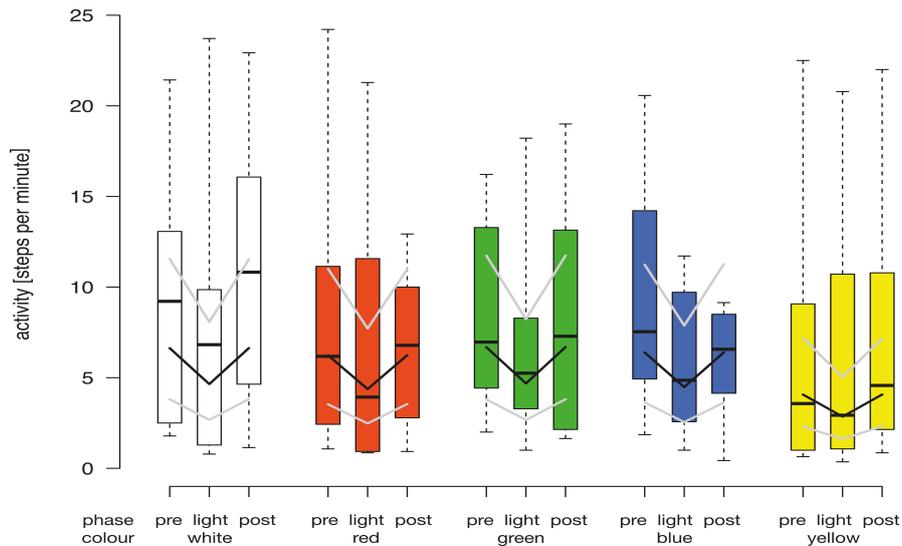
Fig. (4). Activity [steps per minute] in relation to colour and experimental phase (pre-, light-, post-phase). Raw data are given as boxplots with medians, interquartile and absolute ranges, in addition to model predictions with estimated means (black lines) and 95% confidence intervals (grey lines).
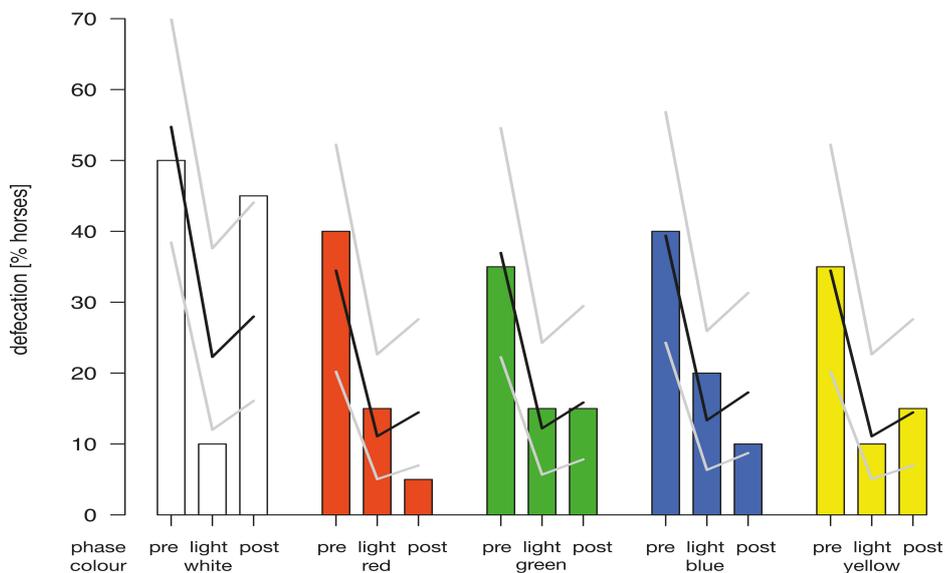
Fig. (5). Percentage of horses showing "defecation" in relation to colour and experimental phase (pre-, light-, post-phase), including model predictions with estimated means (black lines) and 95% confidence intervals (grey lines).

3.2. Physiological Parameters

No significant effect of colour was found on heart rate ($F_{4,68} = 0.36$, $p = 0.8$; (Fig. **6**)) or heart rate variability (RMSSD; $F_{4,69} = 0.5$, $p = 0.7$; (Fig. **6**)). However, compared to the pre-phase, heart rate was decreased in the light-phase and returned to pre-phase level in the post-phase ($F_{2,147} = 8.54$, $p = 0.0003$; (Fig. **6**)). The heart rate variability remained at pre-phase level in the light-phase but increased in the post-phase ($F_{2,148} = 7.54$, $p = 0.0008$; (Fig. **6**)). In comparison to white, body core temperature was increased at red and blue and decreased at green and yellow ($F_{4,76} = 2.5$ $p = 0.048$; (Fig. **7**)). A general decrease in body core temperature was recorded over the course of test procedure, regardless of colour ($F_{2,193} = 8.5$, $p = 0.0003$; (Fig. **7**)). The body surface temperature, however, tended to be increased at red, green, blue and yellow in comparison to white ($F_{4,63} = 2.3$, $p = 0.069$; (Fig. **7**)) but generally decreased at the end of the light-phase, before increasing slightly after the post-phase ($F_{2,163} = 12.4$, $p < 0.0001$; (Fig. **7**)). Colour had no effect on cortisol levels ($F_{4,75} = 0.67$, $p = 0.6$); mean salivary cortisol concentration after the light-phase was 1.14 ± 0.6 nmol/L.
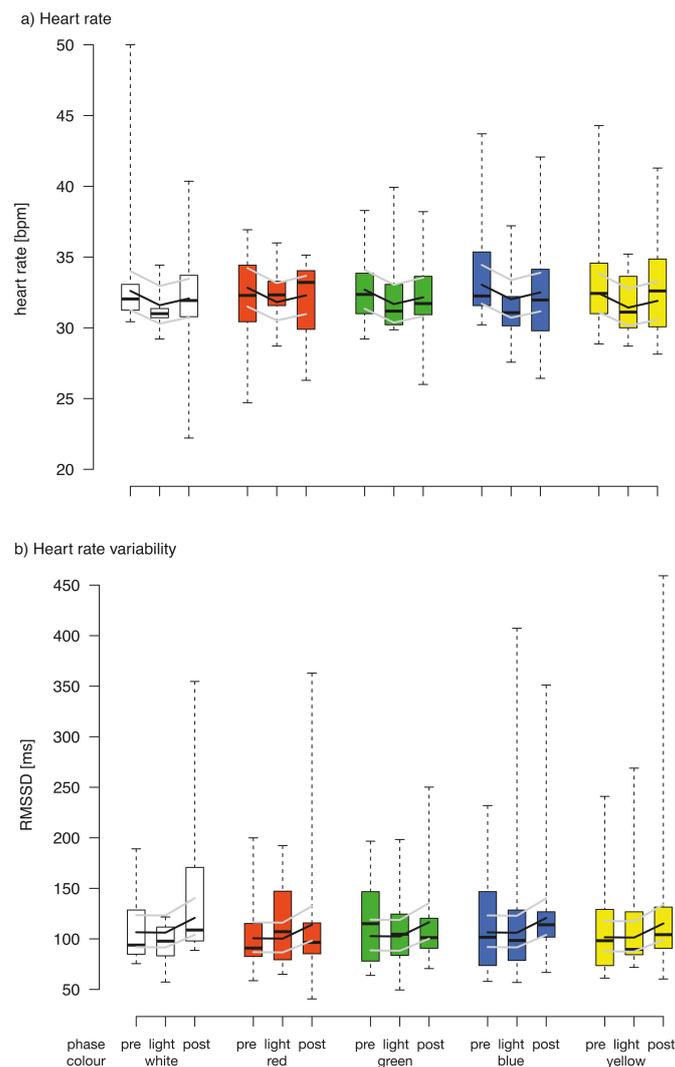
Fig. (6). a) Heart rate [beats per min] and **b**) Heart rate variability as RMSSD [ms] in relation to colour and experimental phase (pre-, light-, post-phase). Raw data are given as boxplots with medians, interquartile and absolute ranges, in addition to model predictions with estimated means (black lines) and 95% confidence intervals (grey lines).

4. DISCUSSION

The selection of behavioural and physiological variables was sought to assess the variety of possible responses to coloured light under standardised conditions. An effect of coloured light was found on "drinking" as well as body core and body surface temperature. However, the obtained results were often not consistent with the literature on colour light therapy and, furthermore, no effect was found on many parameters, even though clearly described in literature. Red is described to be generally stimulating in horses [7], leading to an increaed heart rate, elevated blood circulation and inducing a warming effect [9]. Based thereon, effects regarding enhanced activity, cortisol levels, heart rate, and temperature parameters were expected. However, the results showed only an increase in body core and surface temperature, which was consistent with the postulated warming effect. Blue is described to generally have a calming and relaxing effect on horses [7]. Expectations regarding reduced activity and heart rate were not met. Furthermore, blue is used for treatment of diarrhoea, colic and costiveness due to stimulation of the parasympathetic nervous system [7, 10] wherefore effects on "defecating" and heart rate variability were expected; however, no effects were found in this study. Blue is also designated to have a blood circulation reducing effect which is used for treatment of fever [7]; results obtained in this study somehow refuted this by showing an increase in body core and surface temperature. Green is associated with the induction of general relaxation in horses [7] which has a balancing effect on the cardiovascular system and is commonly referred to as being a colour with an anti-inflammatory effect [9]. Expected effects were a reduction in activity and heart rate, whereas results did not correspond. Furthermore, the illumination with green caused
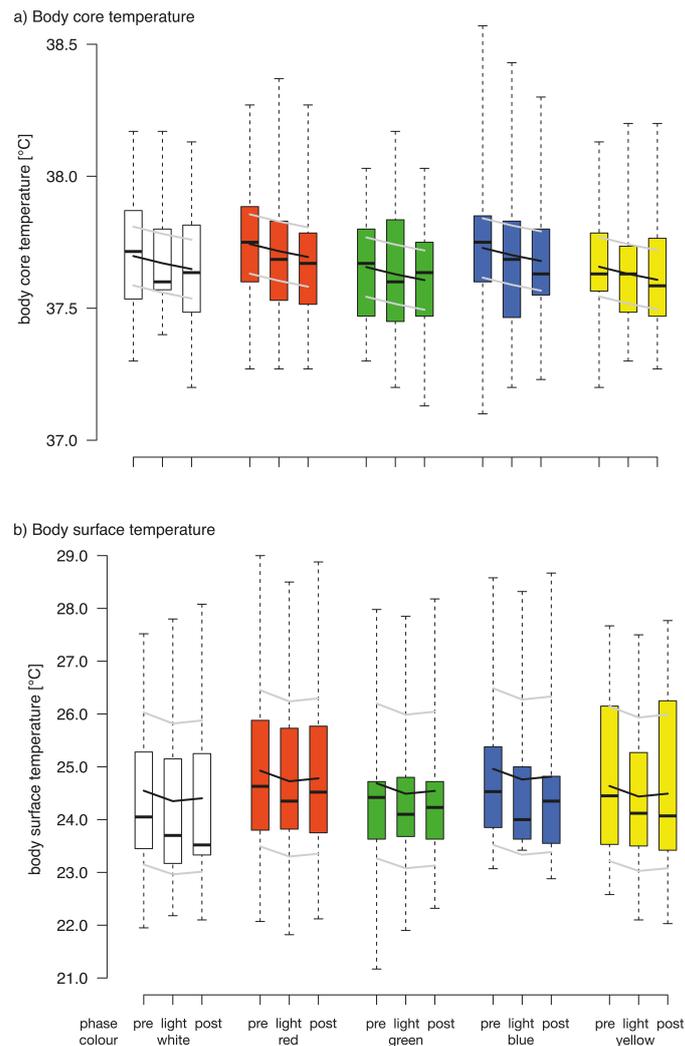
Fig. (7). a) Body core temperature [°C] and **b**) Body surface temperature [°C] in relation to colour and experimental phase (pre-, light-, post-phase) measured subsequent to the respective phase. Raw data are given as boxplots with medians, interquartile and absolute ranges, in addition to model predictions with estimated means (black lines) and 95% confidence intervals (grey lines).

a decrease in body core temperature and an increase in body surface temperature, although literature did not describe any explicit effects. Assuming that colours should have had a similar effect on both parameters, the obtained results showed inverse effects which cannot be explained. For yellow, Muths [10] described a stimulating effect on nerves which can lead to increased activity or nervousness, but no effect on activity was found in this study. "Drinking" occurred more often at yellow, whereas no explicit statements about water intake were found in literature. Furthermore, body core and surface temperature decreased, although yellow is considered to be a stimulating and "hot colour" [9], for which reason an increase of body core temperature was expected.

The expected effects were based on results of research in humans or experiences of horse colour light therapists. Yet, obtained results were often inconsistent with expectations. Since the coloured light, type and duration of illumination used were consistent with practical therapy application, a variety of factors might have caused differing results. This study was conducted with healthy horses, whereas animals with specific disorders or diseases are treated in colour light therapy. Healthy horses may be affected to a lesser extent or not at all. Different coat thickness or coat colour could have influenced the mechanism of stimulation of photoreceptors in skin cells [23, 24]. However, practical colour light therapy is applied independently of season and no references on changing effectiveness by coat thickness or coat colour were found. In general, no statements can be made on the emphasis of impact on the eyes compared to impact on skin cells, as the illumination of the light-box probably acted through both mechanisms. Nevertheless, the experimental test procedure might has affected some parameters (*e.g.* activity, heart rate or heart rate variability), but the extent of interference cannot be assessed. Although handling before and between experimental phases was kept to a

necessary minimum, it cannot be excluded that the horses were being disturbed and possible effects of coloured light were covered. The same applied for the alternation of experimental box stalls. However, experimental box stalls were next to each other and very similar to habitual box stalls. Furthermore, the test procedure was carried out in a similar way for every colour and the randomised order of colours should have accounted for possible effect of habituation with progressive test days. Another possible explanation for inconsistencies between expected effects and obtained results might be based on basically differing effects of colours on humans and horses. Humans learn physiologically determined effects of colours at an early stage of life [41]; red embodies such as "caution" or "heat", blue usually represents "coldness". Animals, on the other hand, need to respond very quickly to colour stimuli in nature and these reactions are often congenital [41]. In view of this, certain colour stimuli in natural environment may have different meanings for horses than for humans, wherefore principles deriving from light therapy in humans cannot be applied directly onto horses. As a further aspect, the effects of colours in light therapy could be mediated by the person applying the light. It has been shown repeatedly, that humans are able to transfer expectations to horses; Hama *et al.* [42] showed that humans with a negative attitude towards a horse caused an increase in heart rate only by stroking the horses' neck and Bridgeman and Pretty [43] found a correlation of the riders' and horses' heart rate during dressage exercise. Chamove *et al.* [44] also described that a horse owners attitude regarding an obstacle course correlated with the actual reactions of their horse. Thus a person, *i.e.* a colour light therapist and/or horse owner, must be considered as a direct confounding factor on the horse during application of colour light therapy. In the present study, however, light was applied without the presence of a person during illumination, wherefore this influence should have been eliminated and the reactions of the horses should correspond exclusively to the respective effects of the colours on the horses.

An effect of the experimental phase, regardless of colour, was apprehended for all parameters recorded in multiple phases. "Defecating" appeared more frequently in the pre-phase than in subsequent phases. It is less likely that this result was caused by a direct reaction to the initial experimental preparation as a sign of alertness, as Rietmann *et al.* [45] or Fureix *et al.* [46] recorded low defecation rates during mental handling stress tests. However, defecation was probably caused by elimination marking behaviour in stallions [47, 48]. Values of body core temperature were within the physiological range [49] but a general decrease was recorded across experimental phases. An effect of the stable climate (min/max: 4.5/14.6 °C) can be ruled out, as Cross *et al.* [50] substantiated that ambient temperatures within a range of 5 - 30 °C have no effect on body core temperature in horses. An influence of the circadian rhythm of the body core temperature can further be excluded; according to Green *et al.* [51] the minimum occurs in the morning and the maximum is reached in the evening. The experimental animals were tested between 09:00 and 16:00, therefore, body core temperature should have increased with subsequent phases. A possible explanation might be the correlation of activity and body core temperature due to physiological regulatory circuits [52]. Accordingly, the generally low activity in the experimental box stalls could have led to a decrease of body core temperature. The same interpretation can be made for the decrease of body surface temperature in the course of treatment. Including only horses with winter coat in the statistical analysis, the horses' body surface was not only well insulated but also well adapted to the ambient climate. For this reason, the experimental treatment itself should not have led to a change in heat production. Submitting that colours should have had a similar effect on body core and surface temperature, inverse effects at green and yellow cannot be explained. Activity during treatment was generally low and the horses were less active in the light-phase compared to both the pre- and post-phase. Locomotion is described in association with arousal [53]; consequently, the horses appeared to be not aroused in experimental box stalls and results suggested that illumination with coloured light had an activity-lowering effect. This assumption is supported by the results of "hind leg resting", which was averagely shown for over 60% of the time during illumination; a behaviour which is known to indicate a relaxed position during resting in standing position [54]. Similar to activity, heart rate was decreased in the light-phase compared to the pre-phase and post-phase. However, the heart rate of the experimental animals was mainly in range of heart rate at rest [46, 55] in all three phases. The horses' heart rate variability also ranged mainly within basal values of parasympathetic activity [56, 57] in all three phases. Compared to the pre- and light-phase, however, the RMSSD was increased in the post-phase, indicating a stronger parasympathetic influence [29, 58]. Altogether, the decrease in heart rate without a change in heart rate variability during illumination may reflect a decreased sympathetic influence without activation of the parasympathetic branch. However, a more detailed analysis of heart rate variability may be necessary to enlighten the interactive effect of the sympatho-vagal activity during and after the illumination with coloured LED light in healthy horses.

In conclusion, there was no indication that either the treatment itself or the illumination with coloured light caused stress in the animals; a conclusion which is confirmed by levels of salivary cortisol ranging within basal concentration [59 - 61]. Noting that light plays an important role in the control of biological and physiological mechanisms [62], it can

be assumed that the illumination, regardless of colour, had a generally becalming effect on the horses.

CONCLUSION

The present study showed an effect of coloured light on one behavioural parameter as well as the body core and surface temperature. For the majority of parameters, however, no effects were found and discovered effects often did not correspond with the expectations deriving from the principles of colour light therapy. A general effect of the illumination, regardless of the colour, was further demonstrated for activity and heart rate parameters, indicating a decreased sympathetic influence during and an increased parasympathetic influence after the illumination. Values of physiological parameters were generally within basal ranges. Considering the low activity and the fact that only a small minority of observed behaviours were affected, the illumination with coloured light seemed to be not stressful for the horses, but rather had a generally becalming effect. The application of coloured light, however, needs verification by further research, wherefore horses with described disorders and diseases should be examined before, during and after colour light therapy.

LIST OF ABBREVIATIONS

LED	=	Light-Emitting Diode
RMSSD	=	Root Mean Square of Successive Beat-to-Beat Differences

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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