The Effect of Altering Routine Husbandry Factors on Sleep Duration and Memory Consolidation in the Horse

Greening, Linda[1*]; Downing, Josh[2]; Amiouny, Daniela[2]; Lekang, Line[2] & McBride, Sebastian[2]

[1]Hartpury University, Gloucester, GL19 3BE, UK;

[2]IBERS, Penglais Campus, Aberystwyth, SY23 3DA, UK

*linda.greening@hartpury.ac.uk (+44 01452 702349)

Abstract

Sleep is a critically important behaviour for all mammals due to its fundamental role within homeostatic/circadian systems and memory consolidation. As a large and vigilant prey species that is highly sensitive to stimuli at night, the horse sleeps less than other mammalian species. For this reason, the domestic environment has the potential to greatly affect the duration and quality of equine sleep. This study aimed to determine the effect of environmental factors on equine sleep stages, and whether this would influence cognitive performance during a spatial memory task. Ten riding school horses (mixed breed/ height/ sex; average age 14.9 +2.4 years) were randomly assigned to two groups (n=5) within a five-week crossover repeated measures design experiment. Each group experienced a combination of one of two light conditions (lights on = Treatment; lights off = Control), and one of two bedding depth treatments (15cm bed = control; 5cm bed = treatment) for six days. Duration of sleep stage behaviours (standing Non-Rapid Eye Movement [NREM]), sternal NREM, sternal Rapid Eye Movement [REM] and lateral REM) were measured continuously using CCTV infrared cameras. For the spatial memory task, latency, number of correct responses, and differences between these parameters during training and testing days were measured. A repeated measures general linear model assessed the effects of treatment conditions on duration of sleep stage, and changes in sleep stage over time (bedding and light set as within-subject factors).

Wilcoxon Signed-Rank and paired t-tests determined differences in memory task parameters between treatments. Comparing Treatment Bedding with Control Bedding conditions, horses spent on average significantly less time in lateral REM (0.34 ± 0.12 versus 0.46± 0.13hrs; p= 0.032) and sternal NREM (0.64 ± 0.10 versus 0.80 ± 0.12hrs; p= 0.007), and significantly more time in standing NREM (3.69 ± 0.76 versus 3.17 ± 0.77; p= 0.024). Only sternal REM was significantly affected during the Treatment Light condition compared to control conditions (0.53 ± 0.07 versus 0.67 ± 0.11; p= 0.031). Interactions between day and treatment were apparent for specific sleep stage behaviours indicative of acclimatisation. No significant effects (p>0.05) of Treatment Light or Bedding conditions were detected for performance during the spatial memory test. Overall, horses exposed to sub-optimal conditions tended to display significantly less time in recumbent sleep stages (NREM and REM) and increased time in a standing NREM stage. The impact of reduced sleep on equine cognition requires further study.

Keywords: Equine, Nocturnal, Behaviour, Sleep, Bedding, Light.

1.0 Introduction

Sleep is one of the most critically important behaviours to all domestic animals due to its fundamental role within homeostatic and circadian systems (Toth and Bhargava, 2013). Both of the primary sleep stages (Non-Rapid Eye Movement [NREM] and Rapid Eye Movement [REM]) regulate a range of physiological processes including neuroendocrine modulation, restorative functions, and memory consolidation (Beccuti & Pannain, 2013; Mavanji et al., 2012; Toth and Bhargava, 2013). Thus, reduced sleep and states of sleep deprivation cause changes in a range of cognitive, emotional and physiological states such as cognitive impairment including reduced spatial memory (Guan et al.,

2004), increased levels of anxiety and aggression, and depletion of glycogen stores along with changes in appetite (McEwen, 2006).

The ability of animals to sleep is affected by their environment, for example, different light and temperature conditions affect the duration and type of sleep (via changes in melatonin levels) in a range of animal species (Redlin, 2001; Gooley et al., 2011; Siegel, 2005). For prey species, the perceived risk of predation is also influential on the amount of sleep that the animal experiences (Lima et al., 2005), which is greatly affected by the size of the animal and its ability to access secure sleep locations within its environment. Larger species, for example, tend to be more exposed within their environment and therefore display higher levels of vigilance throughout the night (Alison and Cincetti, 1976). This can also lead to a greater potential disruption of sleep in these species when exposed to novel stimuli during sleep periods (Campbell and Tobler, 1984).

The horse is an example of a large prey species that engages in less sleep compared to other mammalian species for the previous reasons identified (Siegel, 2005). On average the horse achieves 3.82hrs for total sleep time (2.88 hrs NREM and 0.63 hrs REM) within a 24hr period (Greening and McBride, in preparation). However, unlike other large herbivores, the horse sleeps for relatively small amounts of time in the recumbent position tending to achieve the majority of sleep whilst standing (average 21% vs. 79%, respectively) (Dallaire, 1986). This can be reduced further if the horse is not habituated to the environment (Ruckebusch *et al* 1970). Whilst the horse is able to achieve NREM sleep in both standing and recumbent positions, REM sleep can only be effectively achieved during recumbency due to the muscle atonia that occurs within this sleep stage (Ruckebusch *et al* 1970). Thus, reluctance of the horse to enter the recumbent position within the stable can have a welfare and performance effect due to a reduction in REM sleep.

Other factors within the domestic environment can potentially affect horses' ability to adopt a recumbent position for the purpose of sleep. For example, the average duration of laterally recumbent behaviour is reportedly higher for straw bedding (44.0 minutes) compared to shavings

and/or straw pellets (21.6 minutes) (Pedersen et al., 2004; Greening et al., 2013; Werhahn et al., 2010) with bedding depth also being important in this respect (Pedersen et al., 2004 Werhahn et al., 2010; Modena and Greening, 2018). Although no research has been directly carried out on the effect of lighting conditions on equine sleep stages, light (between 3 and 10 lux) is known to affect melatonin production in the horse (Walsh et al., 2013). Thus, artificial lighting within the stable environment will undoubtedly have an effect on the animal's ability to enter into stages of sleep. Traditional practice is that artificial lights are turned off within the stable environment overnight, however, in some instances this may not always be the case. In addition, late-night checks on horses involving lights being turned on could affect melatonin cycles and subsequently, sleep patterns.

The aim of this study was to determine the effects of altering the environment (bedding depth and light) on the duration of different sleep stages (NREM and REM) in the stabled horse. In addition, to determine the functional and welfare consequences of potential sleep deprivation, the study also assessed the effects of bedding depth and light on performance within a spatial memory task.

2.0 Materials and Methods

2.1 Animals

Ten school horses (6 geldings, 4 mares; mixed breeds; average age 14.9 $\pm$2.4 years; average height 163.5 $\pm$7.4cm, none displaying stereotypic behaviours) were observed in their usual 3.6m x 3.6m stable, experiencing the same feeding schedule (three forage rations presented morning, midday and evening) and similar amounts of exercise, all stabled on the same yard at Aberystwyth University. During the study, subjects were routinely stabled for 24 hours Monday to Friday then turned out to pasture for eight hours on both Saturday and Sunday according to their normal routine. As standard practice in all stables, approximately two thirds of the stable floor surface was covered by rubber matting and a straw bed leaving one third as bare concrete that was cleaned as

97 required throughout the day. Horses were accustomed to lights off at 20:00 (post final check), and a
98 straw bedding substrate of 15cm depth. Stables were organised around the periphery of the barn so
99 that study subjects were able to see stables and horses within the barn. Stable half doors were
100 open, with metal bars enclosing the top half of three walls of the stable but not the back wall,
101 facilitating some sensory communication with neighbours. Observations took place between
102 November and December 2019 (mean regional air temperature 5.75°c/ 42.85 days of sunshine/
103 sunset between 16:00 and 16:20, Metoffice.gov.uk) with individual placement of rugs on the horses.

104 The study was given ethical approval from the Aberystwyth University Animal Welfare and Ethical
105 Review Board (AWERB).

106

107 *2.2 Experimental design*

108 Horses were randomly assigned to two groups balanced for sex and age (Group A [n=5] or Group B
109 [n=5]) and exposed to two treatment conditions (Light and Bedding) within a two factor, crossover
110 repeated-measures experimental design (Table 1). Each treatment lasted for six days plus a one day
111 wash-out period. Groups were staggered in the treatment sequence by seven days (Group B started
112 seven days earlier and Group A finished seven days later) due to the logistics of applying treatment
113 conditions within the same stable environment (Table 1). The disadvantage of this short temporal
114 difference was considered to be outweighed by having all animals within the same experimental
115 environment with similar work and husbandry routines. The Control Light condition involved the
116 normal turning off (at 20:00) of fluorescent tube lighting (2 lux) overnight, whereas the Treatment
117 Light condition maintained the fluorescent tube lighting on (180 lux) during this period. The
118 fluorescent tube lighting was 'warm white' with primary spectral peaks of 490nm, 550nm and
119 625nm. The Control Bedding condition was bedding at normal height of 15cm and Treatment
120 Bedding was maintained at the lower height of 5cm. A yard stick marked at 5cm and 15cm was

121 used to measure bedding depth from the same position every morning that enabled the addition or
122 removal of bed as required.
123
124 Table 1. Details of treatments during the crossover experimental design for groups A and B.
125
126 *2.3 Sleep measurement*
127 High-quality security infrared cameras (Reolink H.264 Digital Video Recorder and ANNKE model
128 N28WEB) were used to record behaviour of all study subjects for 24 hours across seven consecutive
129 days during the five week study period, according to a predetermined ethogram (Table 2). Cameras
130 were mounted above the stable that enabled a field of vision spanning the entire floor of the stable
131 and prevented study subjects from interfering with the cameras. Duration of behaviour was
132 recorded (hours) using continuous focal behavioural sampling over a 24 hour period for 6 days per
133 experimental week for each horse, that was reviewed by three observers. Several training sessions
134 were held to ensure accurate agreement on the four sleep stage behaviours and this was followed
135 with an inter-observer reliability measurement using sample behaviour footage (R^2 =1).
136
137 Table 2. Definition of equine behavioural sleep stages (adapted from Hartman & Greening, 2019)
138
139 2.4 Spatial memory task
140 Both groups were tested on the spatial memory task during two of the combined treatments periods
141 1) Control Light/Control Bedding and 2) Treatment Light/Treatment Bedding. The task required
142 subjects to use distal spatial cues (signage, door apertures and view gallery) to locate a salient object
143 (food within a bucket) by disregarding local cues (bucket colour), based on a standard Morris maze
144 methodology used in rodents (Hamilton et al., 2007). The task was conducted in an indoor school
145 (60m x 30m with a combi-ride waxed surface) containing a number of distal cues that study subjects
146 were all familiar with (Figure 1).

The task comprised of a training session and a testing session, conducted on day four and day five of each combined treatment period (Dallaire 1986). During the training session, horses were required to learn the location of a 26 litre bucket containing food (10g of cereal-based concentrate [EQUERRY Minty Treats; N Yorkshire, UK], placed approximately 3 meters from a corner of the arena. The colour of the bucket (local cues) was either yellow or black (e.g. figure A). The colour of the bucket containing food changed with each presentation, the location of which was predetermined using a random number generator. For half the horses, the local cues comprised of one yellow bucket and three black buckets whilst the other half of the group were given one black bucket and three yellow buckets to reduce the potential influence of salience of local cues.

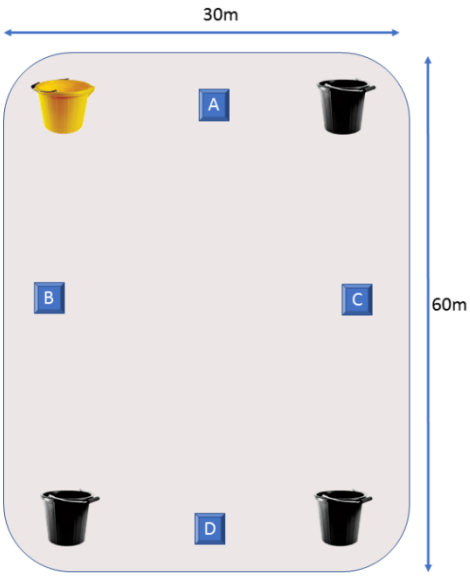


Figure 1. Arena set up for the spatial memory text. A, B, C, D denotes starting position for horse release. The position of the coloured buckets was pseudorandomly ordered to appear in different corners for each trial, whilst the food reward was maintained within one corner location of the arena.

During the training session, each horse was first led around the inside of the arena to inspect and eat from all the buckets, which contained food (pre-training phase). The horse was then led around the outside of the arena for 5 minutes whilst food was placed in the designated bucket. The first trial

started when the horse was released (lead rope unclipped from head collar) at a pre-designated random position and left to autonomously navigate the arena for 120 seconds. The lead rope was reattached to the head collar at the end of the 120 second period or once the horse had located the food. The horse was then led outside the arena to enable preparation for the next trial. The training phase continued until the horse reached a) learning criterion (four consecutive correct responses) or b) a maximum number of trials set at 18. A correct response was described as eating from the bucket containing food within 120 seconds. If horses did not respond for five consecutive trials, the training session was terminated and the horse returned to its stable. The testing session occurred on the day immediately following the training session and followed the same protocol, minus the pre-training phase. Trials were conducted until the horse reached a) the learning criterion or b) the maximum number of trials set at 6. Correct and incorrect responses and latency to locate food was recorded (seconds) for each trial giving the following measures of task performance: mean training latency (Md^1), mean testing latency (Md^2), mean difference of training-testing latency (Md^1 - Md^2), mean correct number of training responses (%d^1), mean correct number of testing responses (%d^2), and training-testing difference in correct responses (%d^1- %d^2). The latency for failed trials was recorded as 120 seconds. Data from horses that disengaged from the task (5 consecutive non-responses) were not included in the final data set. Even though the order of treatments was counterbalanced across the two groups of animals, in order to reduce the effects of learning due to repeat testing over the two treatment periods (and thus maximise treatment effects), a memory wash out session was conducted between the two treatment periods. Here, the salience of the previous location was diminished by leading all horses to baited buckets in all four locations (120 seconds per location) (Hamilton et al., 2007).

2.5 Data analysis

The sleep data sets (Standing NREM, Sternal NREM, Sternal REM and Lateral REM), met the assumptions for parametric statistical analysis and were analysed using repeated measures general

linear model with bedding (control and treatment) and lighting (control and treatment) set as within-subject factors. To assess the effects of light and bedding conditions on the overall duration of sleep stage, the duration data over the 6 days of treatment were averaged and the data tested using repeated measures general linear model with bedding and light set as within-subject factors. To assess the effects of light and bedding conditions on the total amount of sleep, the duration data over the 6 days of treatment for each sleep stage were also averaged and the data tested using repeated measures general linear model with bedding and light set as within-subject factors. To assess changes in sleep stage over time during the treatment period (from days 1 to 6), data were also assessed using repeated measures general linear model but with days (1, 2, 3, 4, 5 and 6), bedding and light set as within-subject factors. Pairwise comparisons at each time point were made using a Bonferroni correction for multiple comparisons (uncorrected p value x no. of pairwise comparisons [6]). Violations of sphericity were tested using Mauchly's test of sphericity and appropriate corrections applied (Greenhouse-Geisser, Huynh-Feldt) where required.

For the analysis of the spatial memory task, each variable and derived variable was analysed to statistically evaluate differences in performance between the first and the second memory test. The mean testing latency data for the control treatment significantly deviated from a normal distribution ($D(9)=0.306$, $p=0.015$) and the Wilcoxon signed-rank test was therefore used to analyse this variable of the memory data set. The remaining data sets (mean training latency, mean difference latency, correct percentage of training responses, correct percentage of testing responses, difference in percentage of correct responses) met the assumptions for parametric statistical analysis and were analysed using a paired t test. Interobserver variation was tested using Kendalls' coefficient of concordance and gave a value of (Kendall's Wa= 1; chi squared =12; df= 3; p =0.007). All statistical analyses were carried out using SPSS 25 and statistical significance was set at $p \leq 0.05$.

Results

Sleep

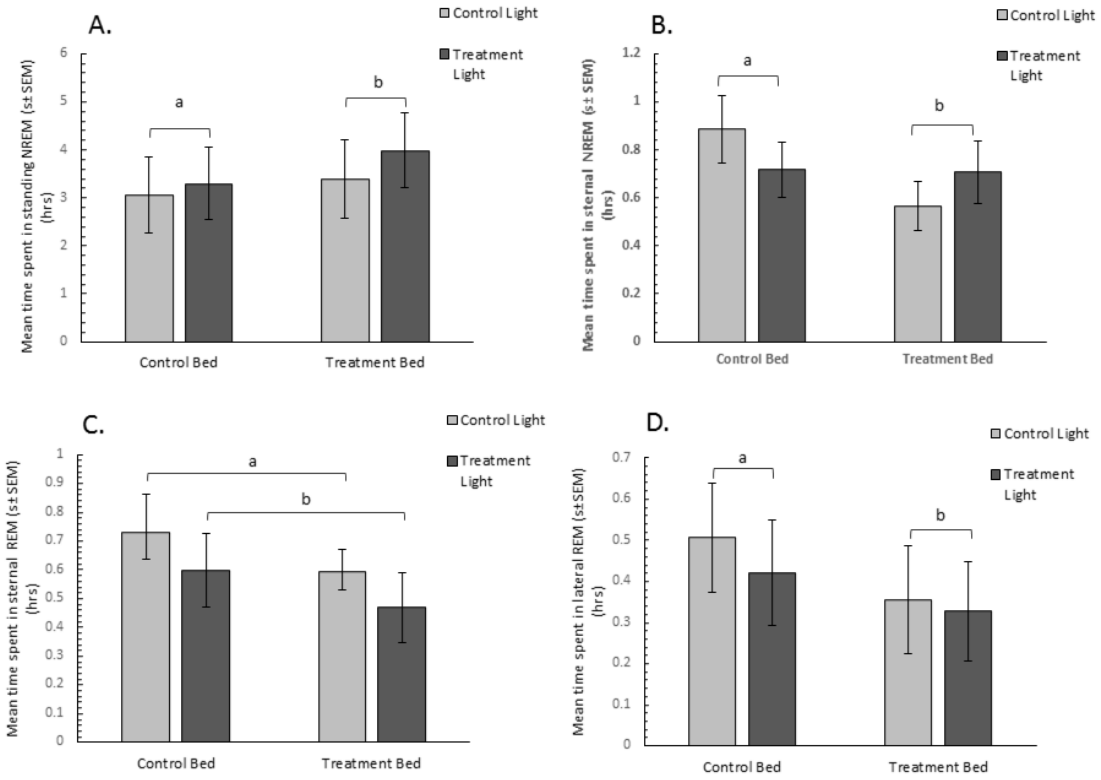
Mean Duration

During the Light (lux 2) and Bedding (15cm) control condition, horses slept for an average of 5.18 ±0.88 hrs with an average of 3.94 ±0.85 hrs (76.1%) and 1.4 ±0.13 hrs (23.9 %) spent in NREM and REM sleep stages respectively. There was no significant effect ($F(1,9)=0.01$, p=0.76) noted for Control Bedding (5.19 ±0.78 hrs) versus Treatment Bedding (5.10 ±0.82 hrs) or the Control Light ($F(1,9)=0.45$, p=0.52) (5.26 ±0.77 versus the Treatment condition (5.04 ±0.84 hrs) on the total amount of sleep.

There was no significant effect ($F(1,9)=3.54$, p=0.09) of Treatment Bedding (4.43 ±0.79 hrs) versus Control Bedding (3.98 ±0.81 hrs) or Treatment Light (4.35 ±0.76) versus Control Light (3.95±0.84 hrs) ($F(1,9)=2.27$, p=0.17) on the total amount of NREM sleep. There was no significant effect ($F(1,9)=4.31$, p=0.07) of the Treatment Bedding (0.87 ±0.11) versus Control Bedding (1.13 ±0.12 hrs), nor for the Treatment Light (0.91 ±0.11) versus Control Light (1.09 ±0.11hrs) ($F(1,9)=4.24$, p=0.07) on the total amount REM of sleep. However, horses spent significantly more time in standing NREM sleep for the Treatment Bedding (5cm) compared with the Control Bedding (15cm) (3.69 ± 0.76 versus 3.17 ± 0.77; $F(1,9)=7.303$, $p= 0.024$;) (Figure 2A.). Horses also spent significantly less time in sternal NREM sleep (0.64 ± 0.10 versus 0.80 ± 0.12hrs; $F(1,9)=12.238$, $p= 0.007$) (Figure 2B), and significantly less time in lateral REM (0.34 ± 0.12 versus 0.46 ± 0.13hrs; $F(1,9)=6.450$, $p= 0.032$) during the Treatment Bedding week compared with the Control Bedding (Figure 2D). Horses spent significantly less time in sternal REM during the Treatment Light compared to the Control Light (0.53 ± 0.07 versus 0.67 ± 0.11; $F(1,9)=6.511$, $p= 0.031$) (Figure 2C). No statistical difference was found in the duration of sternal REM between Control and Treatment Bedding ($F(1,9)=2.317$, $p= 0.162$), nor in the amount of time spent in lateral REM ($F(1,9)=0.758$, $p= 0.407$), sternal NREM ($F(1,9)=0.037$, $p= 0.853$) and standing NREM ($F(1,9)=2.981$, $p= 0.118$) between the two light conditions . There were no significant interaction effects between the Bedding and Lighting treatments for any of the sleep

242 stages: Standing NREM (F(1,9)=0.410, p= 0.538); Sternal NREM (F(1,9)=4.203, p= 0.071); Sternal REM

243 (F(1,9)=0.012, p= 0.917); Lateral REM, (F(1,9)=0.946, p= 0.356).



244

245 Figure 2. Mean (±SEM) (n=10) time spent in the 4 different sleep behaviours (A. standing NREM, B.

246 sternal NREM, C. lateral REM and D. sternal REM over 6 days of each treatment (Control Bedding

247 [15cm], Treatment Bedding [5cm], Control Light (lux 2), Treatment Light (lux 180). Significant

248 differences between treatments are indicated by different subscripts (p ≤0.05).

249

250 <u>Changes over time</u>

251 The durations of sleep stage per day for each treatment are presented in Figure 3. There was no

252 significant interaction effect of treatment (light and bedding) with day for Standing NREM

253 (F(1,9)=0.872, p=0.507; F(1,9)= 1.092, p=0.378) or sternal REM, (F(1,9)=0.752, p=0.527; F(1,9)=

254 0.135, p=0.983). For Sternal NREM, there was a significant interaction of the Treatment Light

255 (F(1,9)=4.208, p=0.011) with day but not for the Treatment Bedding (F(1,9)=0.661, p=0.534). Post

256 hoc tests with Bonferroni correction revealed a significant difference between Treatment Light

conditions for Day 2 (p=0.025) but no significant difference between Treatment Light conditions for other days. For Lateral REM, there was no significant interaction for the Treatment Light (F(1,9)=3.049, p=0.056) with day nor for Treatment Bedding (F(1,9)=1.613, p=0.221).

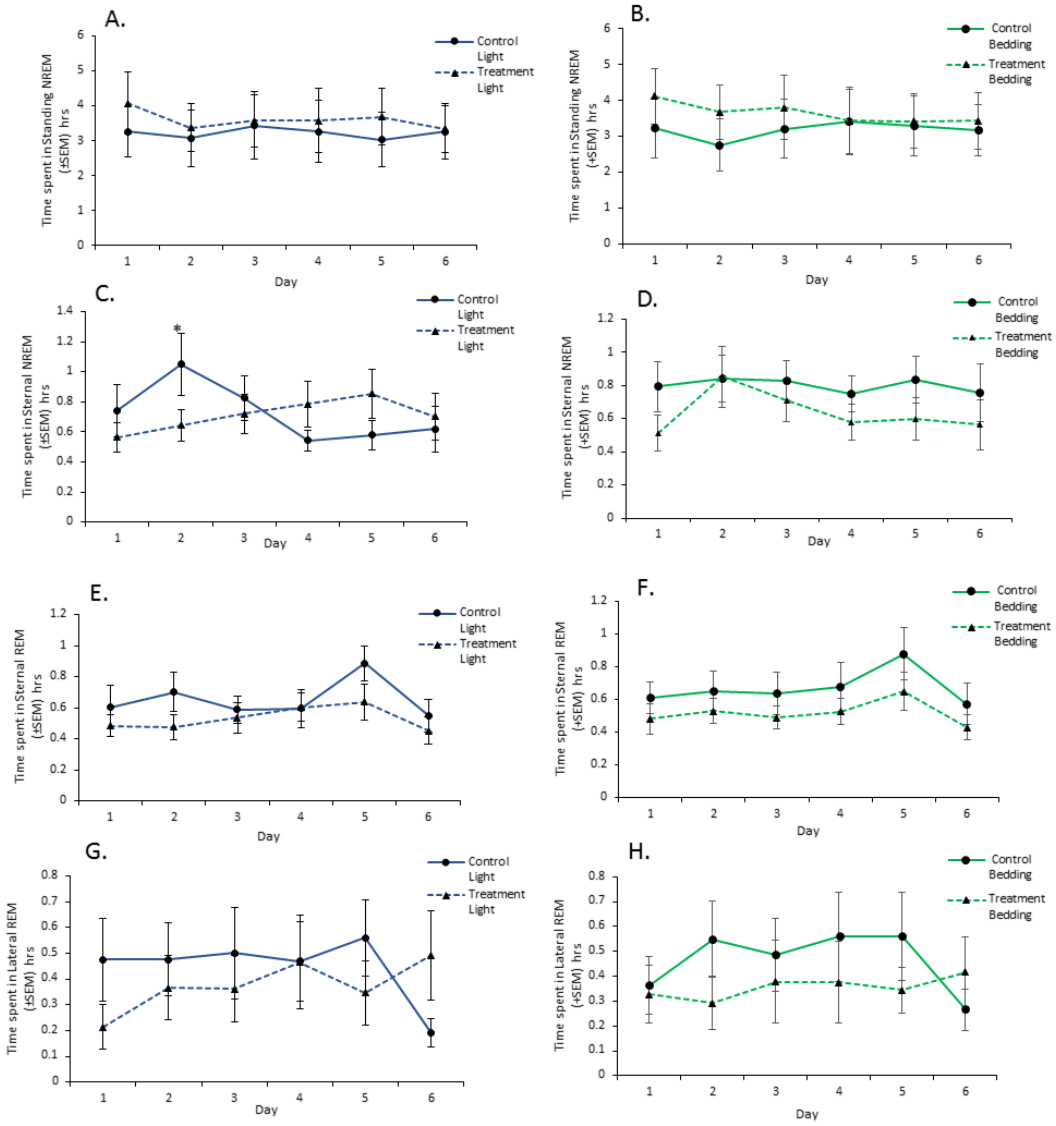


Figure 3. Mean (±SEM) (n=10) time spent in the 4 different sleep stages: standing NREM (A,B), sternal NREM (C,D), lateral REM and (E,F), sternal REM (G,H) over 6 days of each Treatment (Control Light [lux 2], Treatment Light [lux180]), Control Bedding [15cm], Treatment Bedding [5cm]). * Significant differences between treatments at each time point (Day)($p \leq 0.05$).

Memory

A full set of memory test results was obtained for 9 out of the 10 horses. One horse completely disengaged from the task during the second set of memory testing by standing still at the starting point inside the arena. There was no statistical difference between the combined control condition (15cm bedding/lux 2) and the combined treatment condition (5cm bedding, lux 180) for training latency ($t(8) = 0.855$, $p = 0.417$), testing latency ($Z = -0.415$, $p = 0.678$) (figure 4A.) and mean difference latency ($t(8) = 2.064$, $p = 0.073$). There was no statistical difference between the combined control and treatment conditions for the percentage correct training responses ($t(8) = -0.684$, $p = 0.513$), percentage correct testing responses ($t(8) = 0.632$, $p = 0.545$), or difference in percentage of correct responses ($t(8) = -1.791$, $p = 0.111$) (Figure 4B).

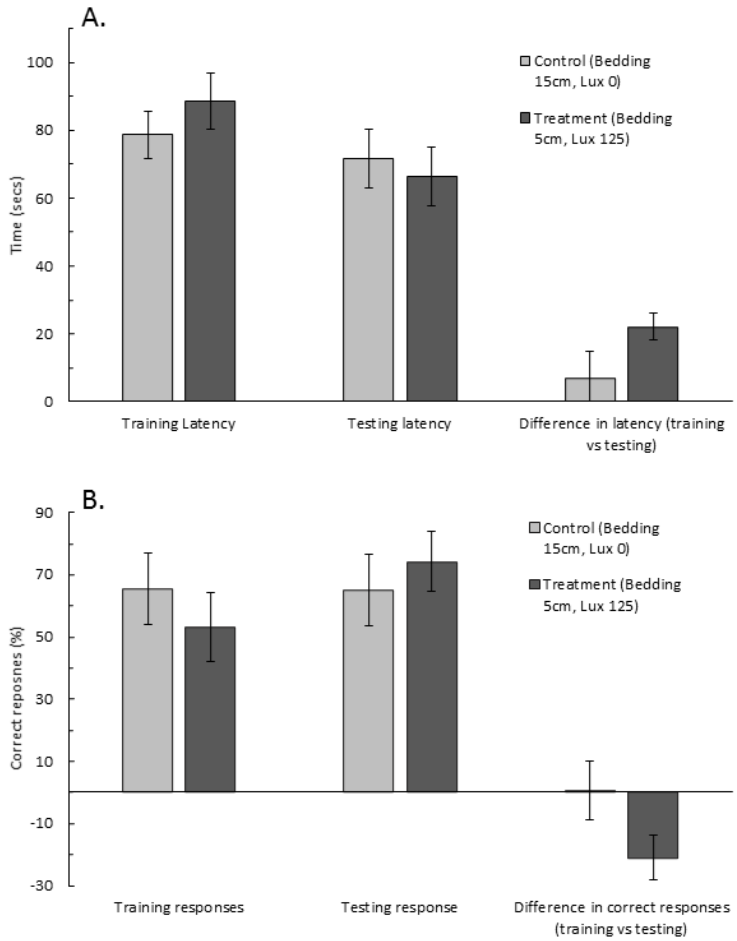


Figure 4. A. Means (±SEM) for training latency (s), testing latency (s) and difference latency (s) for the combined Control (15cm bedding, lux 2) and Treatment (5cm bedding, lux 180) conditions for all

horses (n=9). B. Means (± SEM) for correct percentage of training responses (%), correct percentage of testing responses (%) and difference in percentage of correct responses (training vs testing, %) for the combined Control (15cm bedding, lux 2) and Treatment (5cm bedding, lux 180) conditions for all horses (n=9).

4.0 Discussion

4.1 Baseline sleep values and variability

Horses slept on average for a total of 5.2±0.88 hours/day during the Control conditions week (15cm bedding, lights off), which is higher than has been previously reported for total sleep time (TST) for horses (Zepelin, 2000; Wohr et al., 2016). Whilst the use of behavioural observations to quantify equine sleep stages comes with a risk of over or under estimating time spent in each sleep stage, the equine sleep positions observed in this study aligned strongly with the descriptions and characteristics described in the EEG study by Williams et al. (2008). In the present study, REM sleep during the control period represented on average 23.9% of TST (1.23hrs/day), which falls within the range of 15% to 27.9% of TST for REM sleep previously reported for stabled horses (Kalus, 2014; Williams et al.2008; Dallaire and Ruckebusch, 1974a). NREM sleep during the control period (76.1% of TST; 3.94hrs/day) was also similar in percentage of TST to previous EEG data (Kalus, 2014). The present study observed horses for 24 hours compared to previous studies of stabled horses that tend to report data from nocturnal observations only. Whilst most sleep occurs at night, stabled horses do rest during the day (Ruckebusch, 1972), and in the present study several horses engaged in sternal NREM, sternal REM and even lateral REM during the daytime period. Only 70% of horses achieved at least one bout of REM sleep every day/night indicating inter-horse variability in sleep profiles. Previous work has suggested that sleep variability in horses may be due to differences in age, diet, daily exercise or their ability to adopt a recumbent position (Crowell-Davis, 1994; Duncan, 1980; Dallaire and Ruckebusch, 1974b; Ruckebusch et al., 1970). In this study, the variation in age

range of the horses observed in the present study was low, there were no reported injuries and all horses accustomed to the same management routine and daily exercise. Sleep (quantity and quality) in other species has also been reported to be genetically determined (Sehgal & Mignot, 2011). The majority of horses used in this study were classed as 'sports horse' types containing varying proportions of the Thoroughbred breed which may also have had an effect on time spent in the different sleep stages Considering the effect of breed on the occurrence of sleep may, therefore, be important for future equine sleep studies.

4.2 NREM sleep

Sternal NREM was significantly reduced with the treatment bedding (5cm) whilst standing NREM significantly increased. Standing behaviour has previously been used to detect general discomfort within sub-optimal environments for both cattle (Relic et al., 2012) and horses (Chung et al., 2018). In the present study, rubber matting was used in every stable, which may have improved overall comfort within the stable as indicated by the overall duration of TST which fell within the normal levels previously recorded for the horse. The increase in standing NREM appeared to compensate for the loss of sternal NREM and this was more apparent at the start of the treatment period (Figure 3b) suggesting some acclimatisation to the reduced bedding substrate.

Overall, no significant effects of light were recorded for total sternal and standing NREM sleep duration, which would suggest that light contamination did not significantly influence the occurrence of NREM sleep stages. There was a significant interaction of Light Treatment with day for sternal NREM, with a significant difference between treatments at day 2 followed by a crossover trend between experimental conditions over the 6 days (Figure 3c). This potentially suggests some initial effect of the Light Treatment on duration of NREM sleep but also potential adaptation as the treatment progressed. The artificial light/dark cycle in the Control Light condition may therefore act as a 'Zeitgeber', where artificial light appears to initially interrupt the natural patterns/ rhythm of sleep experienced under treatment conditions when left on. Previous work in other species has also

shown that dim light (~ 5 lux) can have a significant effect on NREM and REM sleep patterns (Stenvers et al., 2016; Batra et al., 2020). Thus, the lights off condition (2 lux) in this study may still have contained sufficient light intensity to have a physiological effect on sleep, thereby reducing the overall comparative effect with the lights on treatment. Overall, a thinner bed reduced recumbent NREM sleep stages, whilst light appeared to have a short-term effect on the occurrence of sternal NREM specifically.

4.3 REM sleep

The Treatment Bedding resulted in a significant reduction in the average duration of lateral REM, and a trend towards reduced duration in sternal REM and total REM (lateral and sternal combined). The reduction in sternal REM was not as pronounced as lateral REM which may be explained by the latter compensating the former. There were no significant interactions of day during the Control and Treatment Bedding conditions with both REM stages consistently lower during the 5cm Bedding Treatment for the majority of the six-day period. There was, however a peak in lateral REM on day six under the Treatment Bedding condition indicating that a longer observation period might be advantageous for future studies. The data overall confirm that recumbent positions increase when deeper bedding is used, indicative of increased comfort as has previously suggested in other studies (Werhahn et al., 2010) irrespective of bedding type (Greening and Modena, 2019). Additional attributes of bedding can also influence recumbency including texture, softness, smell, cleanliness and insulation properties (Pedersen et al., 2004; Kwiatkowska-Stenzel et al., 2016). In the present study, dirty and wet bedding was removed every morning and evening, however, the reduction in bedding depth may have exacerbated wetness and made the Treatment Bedding more unappealing in this respect. Throughout the study, some of the horses ate their bedding, as seen previously (Greening et al., 2014), which may have affected bedding depth towards the end of the 24 hours.

354 The Treatment Light condition significantly affected the average total duration of time spent in
355 sternal REM, with a similar but non-significant pattern for lateral REM and REM across the two states
356 (lateral and sternal recumbency). The reduced effect of light on lateral REM suggests that this may
357 be more an obligatory sleep stage for the horse and mirrors the results of a study comparing the
358 influence of light and dark environments on recumbent behaviour of dogs housed in kennels (Houpt
359 et al., 2019). Whilst there was no significant effect of day on the occurrence of either REM sleep
360 stage, the trend towards interaction between treatment light and days 1 and 6 for lateral REM
361 indicates some potential acclimatisation to light contamination. This again suggests that an
362 observation period longer than 6 days might be advantageous in future studies.

363

364 *4.3 The influence of the environment on memory test completion*

365 Although no significant effects of the treatment condition (lights on, 5cm bedding) were apparent
366 for any of the parameters measured during the spatial memory task, there was a slight trend
367 towards increased mean difference latency (training vs testing) for treatment versus the control
368 condition. The latter was predominantly due to horses under treatment conditions taking longer to
369 complete the task during the training phase as opposed to the testing phase. Memory function
370 following some disruption to sleep stages as previously described therefore did not appear to be
371 impaired as initially hypothesized, which may be due to a number of reasons. Firstly, the significant
372 reduction in overall sleep and/or specific sleep stages may not have been sufficient to affect memory
373 consolidation due for example to the compensatory increase in standing NREM sleep during
374 treatment conditions. Sleep deprivation studies that have reported effects on cognition often
375 physically prevent with study subjects to prevent them from sleeping (for example, Zhang et al.,
376 2013; Rahman et al., 2013), but this was not the purpose of this study. Secondly, the significant
377 reduction in sleep stage duration reported in this study may not reflect the level of sleep quality that
378 the animal is experiencing, the latter of which may not have been affected by the treatment

379 condition. Cycling into REM sleep immediately following NREM is reported to help stabilize
380 transformed memories (Rasch and Born, 2013). Thus, measuring sleep quality, particularly the
381 relationship between REM and NREM cycles, may be a more useful approach to understand the
382 interplay between sleep and memory consolidation. Recording this and additional measures of sleep
383 quality such as sleep fragmentation, may therefore be critical for future sleep studies. Thirdly, sleep
384 reduction is also known to have an impact on the neuroendocrine system which may have indirectly
385 affected cognitive performance. For example, previous research indicates that ghrelin, a hormone
386 promoting hunger, increases with sleep restriction, whereas leptin, a hormone contributing to
387 satiety perception decreases (Morselli et al., 2010). Thus, horses with reduced sleep may have been
388 more motivated to engage in the food-reward task. Increased levels of motivation have previously
389 been found to compensate for deficits in cognitive performance in humans (Alhola and Polo-Kantola
390 2007) and other species (Ward, Winiger et al. 2015) and this factor may need to be taken into
391 consideration for future work. Finally, horses under control conditions appeared to perform only
392 marginally better in the testing compared to the training phase of the spatial memory test. This may
393 also suggest that overnight memory consolidation was not required given the cognitive demands of
394 the task.
395
396 *4.3 Conclusion*
397 The duration of equine sleep stages was found to be affected by both the depth of bedding and
398 lighting conditions. During sub-optimal conditions horses tended to display significantly reduced
399 time in recumbent states for both NREM and REM sleep, which was compensated by increased time
400 in the standing NREM stage. The impact of reduced sleep on the animal was not fully determined in
401 this study. Although the data suggested that there might be some effect on spatial memory, a more
402 cognitively demanding task may better differentiate the performance-related consequences of
403 different levels of sleep duration and quality. This study specifically highlights the influence of

bedding depth on sleep behaviour, and how minimal alterations can be made in the stable environment to better accommodate sleep behaviour with potentially positive outcomes for equine welfare.

Acknowledgements: We would like to thank Hannah Appleton, Jennifer Lawrence and Caryl Thomas at LLuest Riding Centre for aiding and supporting the day-to-day running of the research trial. We would also like to thank Dr Matthew Parker at the University of Portsmouth for his guidance on adapting the spatial memory task for use in horses.

References

Alhola, P. and P. Polo-Kantola (2007) Sleep deprivation: Impact on cognitive performance. *Neuropsychiatric disease and treatment.* 3(5): 553-567.

Allison, T. and Cicchetti, D.V. (1976) Sleep in mammals: ecological and constitutional correlates. *Science.* 194(4266): 732-734.

Batra, T., Malik, I., Bhardwaj, S.K., Kumar, V., (2020) Sleep in unnatural times: illuminated night negatively affects sleep and associated hypothalamic gene expressions in diurnal zebra finches. *Proceedings of the Royal Society B-Biological Sciences*, 287.

Beccuti, G., & Pannain, S. (2011) Sleep and obesity. *Current opinion in clinical nutrition and metabolic care*. 14(4): 402–412.

Boyd LE, Carbonaro DA and Houpt, K, A. (1988) The 24-Hour Time Budget of Przewalski Horses. *Appd. An. Behav. Sci.* 21: 5-17.

Campbell, S.S. and Tobler, I. (1984) Animal sleep: a review of sleep duration across phylogeny. *Neuro. & Biobehavioral Rev*. 8(3): 269-300.

Chung, E.L.T., Khairuddin, N.H., Azizan, T.R.P.T. and Adamu, L. (2018) Sleeping patterns of horses in selected local horse stables in Malaysia. *J. Vet. Behav*. 26: 1-4.

Dallaire, A. (1986) Rest behavior. *Vet Clin North Am Equine Pract.* 2(3): 591-607.

428 Dallaire, A. and Ruckebusch, Y. (1974) Sleep and wakefulness in the housed pony under different
429 dietary conditions. *Canadian J. Comp. Med*. 38(1):6 5.
430 Dallaire, A. and Ruckebusch, Y. (1974) Sleep patterns in the pony with observations on partial
431 perceptual deprivation. *Phys. & Behav*. 12(5): 789-796.
432 Gooley, J. J., Chamberlain, K., Smith, K. A., Khalsa, S. B., Rajaratnam, S. M., Van Reen, E., Zeitzer, J.
433 M., Czeisler, C. A., & Lockley, S. W. (2011) Exposure to room light before bedtime suppresses
434 melatonin onset and shortens melatonin duration in humans. *J. Clin. Endo. & Met.* 96(3): E463–
435 E472.
436 Greening, L. and McBride, S., A Profile of equine sleep; implications for equine welfare. In
437 preparation.
438 Greening, L., Shenton, V; Wilcockson, K; Swanson, J. (2013) Investigating duration of nocturnal
439 ingestive and sleep behaviours of horses bedded on straw versus shavings. *J. Vet. Behav.* 8 (2): 82-
440 86.
441 Guan, Z., Peng, X. and Fang, J. (2004) Sleep deprivation impairs spatial memory and decreases
442 extracellular signal-regulated kinase phosphorylation in the hippocampus. *Brain research*. 1018(1):
443 38-47.
444 Hamilton, D.A., Akers, K.G., Weisend, M.P. and Sutherland, R.J. (2007) How do room and apparatus
445 cues control navigation in the Morris water task? Evidence for distinct contributions to a movement
446 vector. *J. Exp. Psych: An. Behav. Proc*. 33(2): 100.
447 Hartman, N. & Greening, L. (2019) A preliminary study investigating the influence of auditory
448 stimulation on the occurrence of nocturnal equine sleep related behaviour in stabled horses. *J. Eq.*
449 *Vet. Sci*. 82: https://doi.org/10.1016/j.jevs.2019.07.003
450 Houpt, K.A., Erb, H.N. and Coria-Avila, G.A. (2019) The Sleep of Shelter Dogs Was Not Disrupted by
451 Overnight Light Rather than Darkness in a Crossover Trial. *Animals*. 9(10): 794.
452 Jonckheer-Sheehy, V.S. and Houpt, K.A. (2015) Management methods to improve the welfare of
453 horses used in research. *Lab animal.* 44(9): 350-358.

Kalus, M. (2014) Sleep behaviour and physiology of sleep in horse based polysomnography investigations. *Inaugural dissertation printed with permission of the Veterinary Faculty of the Ludwig Maximillian University of Munich*.

Kwiatkowska-Stenzel, A., Sowińska, J. and Witkowska, D. (2016) The effect of different bedding materials used in stable on horses behavior. *J. Eq. Vet. Sci*. 42: 57-66.

Lima, S.L., Rattenborg, N.C., Lesku, J.A. and Amlaner, C.J. (2005) Sleeping under the risk of predation. *An. Behav*. 70(4): 723-736.

Mavanji, V., Billington, C.J., Kotz, C.M. and Teske, J.A. (2012) Sleep and obesity: a focus on animal models. *Neuroscience & Biobehavioral Reviews*. 36(3): 1015-1029.

McEwen, B.S. (2006) Sleep deprivation as a neurobiologic and physiologic stressor: allostasis and allostatic load. *Metabolism.* 55: S20-S23.

Met Office (2020) https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-and-regional-series

Mills, D.S., Eckley, S. and Cooper, J.J. (2000) Thoroughbred bedding preferences, associated behaviour differences and their implications for equine welfare. *An. Sci*. 70(1): 95-106.

Modena, F. and Greening, L. (2019) The influence of shavings bed thickness on nocturnal recumbent behaviour in horses. *Poster presentation and published in the proceedings of the 9th Alltech-Hartpury Student Conference, May 2019.*

Morselli L, Leproult R, Balbo M, Spiegel K. (2010) Role of sleep duration in the regulation of glucose metabolism and appetite. *Best Pract Res Clin Endocrinol Metab*. 24(5), pp687-702.

Murphy, J., Hall, C. and Arkins, S., (2009) What horses and humans see: a comparative review. *Int. J. Zoo.* 2009: 1-14

Murphy, B.A., Walsh, C.M., Woodward, E.M., Prendergast, R.L., Ryle, J.P., Fallon, L.H. and Troedsson, M.H.T. (2014) Blue light from individual light masks directed at a single eye advances the breeding season in mares. *Eq. Vet. J*. 46(5): 601-605.

479 Pedersen GR, Søndergaard E, Ladewig J. (2004) The influence of bedding on the time horses spend
480 recumbent. *J Equine Vet Sci*, 24: 153-158
481 Raap, T*.,* Pinxten, R. and Eens, M. (2015) Light pollution disrupts sleep in free-living animals. *Sci. Rep.*
482 5: 13557.
483 Rahman, A., Languille, S., Lamberty, Y., Babiloni, C., Perret, M., Bordet, R., Blin, O.J., Jacob, T.,
484 Auffret, A., Schenker, E. and Richardson, J. (2013) Sleep deprivation impairs spatial retrieval but not
485 spatial learning in the non-human primate grey mouse lemur. *PLoS One*, *8*(5): p.e64493.
486 Rasch, B. and Born, J. (2013) About sleep's role in memory. *Physiological reviews*. 93: 681-766
487 Redlin, U. (2001). Neural basis and biological function of masking by light in mammals: suppression
488 of melatonin and locomotor activity. *Chronobiology international*. 18(5): 737-758.
489 Relić, R., Hristov, S., Joksimović-Todorović, M., Davidović, V. and Bojkovski, J. (2012) Behavior of
490 cattle as an Indicator of their health and welfare. *Bulletin of University of Agricultural Sciences and*
491 *Veterinary Medicine Cluj-Napoca. Veterinary Medicine*. 69(1-2).
492 Ruckebusch, Y., Barbey, P., and Guillemot, P. (1970). Les états de sommeil chez le cheval (*Equus*
493 *Caballus). Cr Seanc. Soc Biol.* 164: 658-665.
494 Ruckebusch, Y. (1975) The Hypnogram as an index of adaptation of farm animals to changes in their
495 environment. *Appd. An. Ethol.* 2: 3-18.
496 Sehgal, A., and Mignot, E. (2011) Genetics of sleep and sleep disorders. *Cell.* 146(2): 194–207
497 Siegel, J.M. (2005). Clues to the functions of mammalian sleep. *Nature.* 437(7063): 1264-1271.
498 Stenvers, D.J., van Dorp, R., Foppen, E., Mendoza, J., Opperhuizen, A.L., Fliers, E., Bisschop, P.H.,
499 Meijer, J.H., Kalsbeek, A., Deboer, T. (2016) Dim light at night disturbs the daily sleep-wake cycle in
500 the rat. *Scientific Reports.* 6: 35662
501 Toth, L.A. and Bhargava, P. (2013) Animal models of sleep disorders. *Comp. Med*. 63(2): 91-104.

Walsh, C.M., Prendergast, R.L., Sheridan, J.T. and Murphy, B.A. (2013) Blue light from light-emitting diodes directed at a single eye elicits a dose dependent suppression of melatonin in horses. *Vet. J*. 196: 231-235

Ward, R. D., Winiger, V., Higa, K. K., Kahn, J. B., Kandel, E. R., Balsam, P. D. and Simpson, E. H. (2015) The impact of motivation on cognitive performance in an animal model of the negative and cognitive symptoms of schizophrenia. *Behav. Neuro.* 129(3): 292-299.

Werhahn, H., Hessel, E.F., Bachhausen, I. and Van den Weghe, H.F., 2010. Effects of different bedding materials on the behavior of horses housed in single stalls. *J. Eq. Vet. Sci. 30*(8): 425-431.

Williams, D.C., Aleman, M., Holliday, T.A., Fletcher, D.J., Tharp, B., Kass, P.H., Steffey, E.P. and Lecouteur, R.A. (2008) Qualitative and quantitative characteristics of the electroencephalogram in normal horses during spontaneous drowsiness and sleep. *J. Vet. Int. Med*. 22(3): 630-638.

Wohr, A.C., Kalus, M., Reese, S., Fuchs, C. and Erhard, M. (2016) Equine sleep behavior and physiology based on polysomnographic examinations. *Equine Vet. J*. 48: 5-30

Zepelin, H. (2000) Mammalian sleep. In: M.H. Kryger, T. Roth, W.C. Dement (Eds.), *Principles and Practice of Sleep Medicine* (3rd edn), W. B. Saunders, Philadelphia: 82-92

Zhang, L., Zhang, H.Q., Liang, X.Y., Zhang, H.F., Zhang, T. and Liu, F.E. (2013) Melatonin ameliorates cognitive impairment induced by sleep deprivation in rats: role of oxidative stress, BDNF and CaMKII. *Behav. Br. Res. 256:* 72-81.