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

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7

8 **Abstract**

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

25 Wilcoxon Signed-Rank and paired t-tests determined differences in memory task parameters  
26 between treatments. Comparing Treatment Bedding with Control Bedding conditions, horses spent  
27 on average significantly less time in lateral REM ( $0.34 \pm 0.12$  versus  $0.46 \pm 0.13$ hrs;  $p= 0.032$ ) and  
28 sternal NREM ( $0.64 \pm 0.10$  versus  $0.80 \pm 0.12$ hrs;  $p= 0.007$ ), and significantly more time in standing  
29 NREM ( $3.69 \pm 0.76$  versus  $3.17 \pm 0.77$ ;  $p= 0.024$ ). Only sternal REM was significantly affected during  
30 the Treatment Light condition compared to control conditions ( $0.53 \pm 0.07$  versus  $0.67 \pm 0.11$ ;  $p=$   
31  $0.031$ ). Interactions between day and treatment were apparent for specific sleep stage behaviours  
32 indicative of acclimatisation. No significant effects ( $p>0.05$ ) of Treatment Light or Bedding conditions  
33 were detected for performance during the spatial memory test. Overall, horses exposed to sub-  
34 optimal conditions tended to display significantly less time in recumbent sleep stages (NREM and  
35 REM) and increased time in a standing NREM stage. The impact of reduced sleep on equine  
36 cognition requires further study.

37

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

39

## 40 **1.0 Introduction**

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

48 2004), increased levels of anxiety and aggression, and depletion of glycogen stores along with  
49 changes in appetite (McEwen, 2006).

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

59 The horse is an example of a large prey species that engages in less sleep compared to other  
60 mammalian species for the previous reasons identified (Siegel, 2005). On average the horse achieves  
61 3.82hrs for total sleep time (2.88 hrs NREM and 0.63 hrs REM) within a 24hr period (Greening and  
62 McBride, in preparation). However, unlike other large herbivores, the horse sleeps for relatively  
63 small amounts of time in the recumbent position tending to achieve the majority of sleep whilst  
64 standing (average 21% vs. 79%, respectively) (Dallaire, 1986). This can be reduced further if the  
65 horse is not habituated to the environment (Ruckebusch *et al* 1970). Whilst the horse is able to  
66 achieve NREM sleep in both standing and recumbent positions, REM sleep can only be effectively  
67 achieved during recumbency due to the muscle atonia that occurs within this sleep stage  
68 (Ruckebusch *et al* 1970). Thus, reluctance of the horse to enter the recumbent position within the  
69 stable can have a welfare and performance effect due to a reduction in REM sleep.

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

73 and/or straw pellets (21.6 minutes) (Pedersen et al., 2004; Greening et al., 2013; Werhahn et al.,  
74 2010) with bedding depth also being important in this respect (Pedersen et al., 2004 Werhahn et al.,  
75 2010; Modena and Greening, 2018). Although no research has been directly carried out on the effect  
76 of lighting conditions on equine sleep stages, light (between 3 and 10 lux) is known to affect  
77 melatonin production in the horse (Walsh et al., 2013). Thus, artificial lighting within the stable  
78 environment will undoubtedly have an effect on the animal's ability to enter into stages of sleep.  
79 Traditional practice is that artificial lights are turned off within the stable environment overnight,  
80 however, in some instances this may not always be the case. In addition, late-night checks on horses  
81 involving lights being turned on could affect melatonin cycles and subsequently, sleep patterns.  
82 The aim of this study was to determine the effects of altering the environment (bedding depth and  
83 light) on the duration of different sleep stages (NREM and REM) in the stabled horse. In addition, to  
84 determine the functional and welfare consequences of potential sleep deprivation, the study also  
85 assessed the effects of bedding depth and light on performance within a spatial memory task.

86

## 87 **2.0 Materials and Methods**

### 88 *2.1 Animals*

89 Ten school horses (6 geldings, 4 mares; mixed breeds; average age 14.9  $\pm$  2.4 years; average height  
90 163.5  $\pm$  7.4cm, none displaying stereotypic behaviours) were observed in their usual 3.6m x 3.6m  
91 stable, experiencing the same feeding schedule (three forage rations presented morning, midday  
92 and evening) and similar amounts of exercise, all stabled on the same yard at Aberystwyth  
93 University. During the study, subjects were routinely stabled for 24 hours Monday to Friday then  
94 turned out to pasture for eight hours on both Saturday and Sunday according to their normal  
95 routine. As standard practice in all stables, approximately two thirds of the stable floor surface was  
96 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

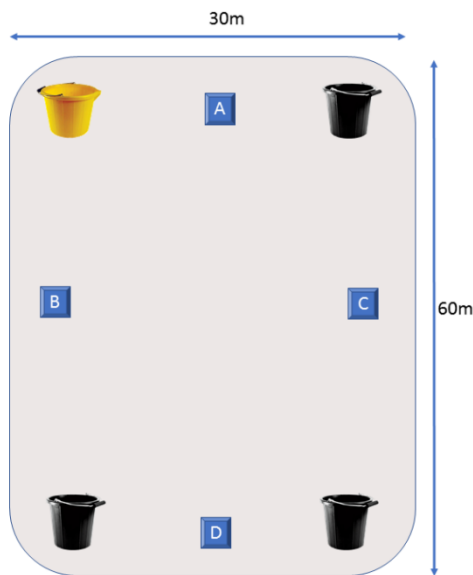
137 Table 2. Definition of equine behavioural sleep stages (adapted from Hartman & Greening, 2019)

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).

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



156  
157 Figure 1. Arena set up for the spatial memory text. A, B, C, D denotes starting position for horse  
158 release. The position of the coloured buckets was pseudorandomly ordered to appear in different  
159 corners for each trial, whilst the food reward was maintained within one corner location of the  
160 arena.

161  
162 During the training session, each horse was first led around the inside of the arena to inspect and eat  
163 from all the buckets, which contained food (pre-training phase). The horse was then led around the  
164 outside of the arena for 5 minutes whilst food was placed in the designated bucket. The first trial



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

187

## 188 *2.5 Data analysis*

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

191 linear model with bedding (control and treatment) and lighting (control and treatment) set as  
192 within-subject factors. To assess the effects of light and bedding conditions on the overall duration  
193 of sleep stage, the duration data over the 6 days of treatment were averaged and the data tested  
194 using repeated measures general linear model with bedding and light set as within-subject factors.  
195 To assess the effects of light and bedding conditions on the total amount of sleep, the duration data  
196 over the 6 days of treatment for each sleep stage were also averaged and the data tested using  
197 repeated measures general linear model with bedding and light set as within-subject factors. To  
198 assess changes in sleep stage over time during the treatment period (from days 1 to 6), data were  
199 also assessed using repeated measures general linear model but with days (1, 2, 3, 4, 5 and 6),  
200 bedding and light set as within-subject factors. Pairwise comparisons at each time point were made  
201 using a Bonferroni correction for multiple comparisons (uncorrected p value x no. of pairwise  
202 comparisons [6]). Violations of sphericity were tested using Mauchly's test of sphericity and  
203 appropriate corrections applied (Greenhouse-Geisser, Huynh-Feldt) where required.  
204 For the analysis of the spatial memory task, each variable and derived variable was analysed to  
205 statistically evaluate differences in performance between the first and the second memory test. The  
206 mean testing latency data for the control treatment significantly deviated from a normal distribution  
207 ( $D(9)=0.306$ ,  $p=0.015$ ) and the Wilcoxon signed-rank test was therefore used to analyse this variable  
208 of the memory data set. The remaining data sets (mean training latency, mean difference latency,  
209 correct percentage of training responses, correct percentage of testing responses, difference in  
210 percentage of correct responses) met the assumptions for parametric statistical analysis and were  
211 analysed using a paired t test. Interobserver variation was tested using Kendalls' coefficient of  
212 concordance and gave a value of (Kendall's  $W_a= 1$ ; chi squared =12;  $df= 3$ ;  $p =0.007$ ). All statistical  
213 analyses were carried out using SPSS 25 and statistical significance was set at  $p \leq 0.05$ .

214

## 215 **Results**

216 **Sleep**

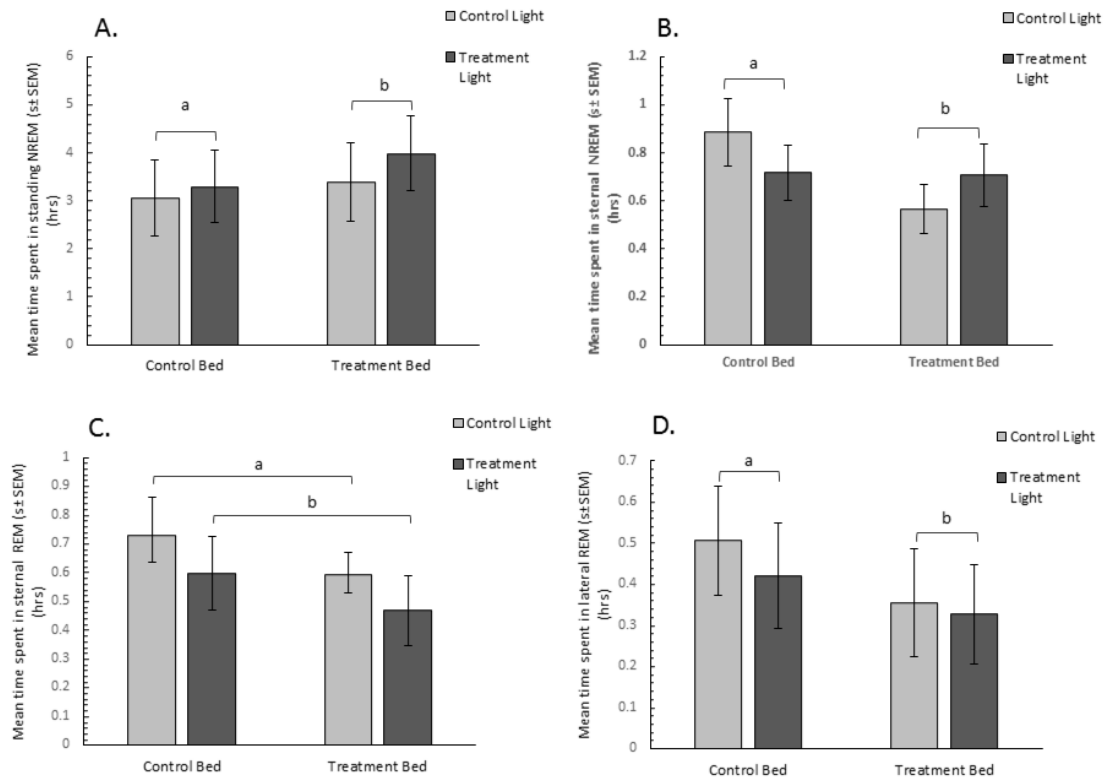
217 Mean Duration

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

224

225 There was no significant effect ( $F(1,9)=3.54$ ,  $p=0.09$ ) of Treatment Bedding ( $4.43 \pm 0.79$  hrs) versus  
226 Control Bedding ( $3.98 \pm 0.81$  hrs) or Treatment Light ( $4.35 \pm 0.76$ ) versus Control Light ( $3.95 \pm 0.84$  hrs)  
227 ( $F(1,9)=2.27$ ,  $p=0.17$ ) on the total amount of NREM sleep. There was no significant effect  
228 ( $F(1,9)=4.31$ ,  $p=0.07$ ) of the Treatment Bedding ( $0.87 \pm 0.11$ ) versus Control Bedding ( $1.13 \pm 0.12$  hrs),  
229 nor for the Treatment Light ( $0.91 \pm 0.11$ ) versus Control Light ( $1.09 \pm 0.11$  hrs) ( $F(1,9)=4.24$ ,  $p=0.07$ )  
230 on the total amount REM of sleep. However, horses spent significantly more time in standing NREM  
231 sleep for the Treatment Bedding (5cm) compared with the Control Bedding (15cm) ( $3.69 \pm 0.76$   
232 versus  $3.17 \pm 0.77$ ;  $F(1,9)=7.303$ ,  $p=0.024$ ;) (Figure 2A.). Horses also spent significantly less time in  
233 sternal NREM sleep ( $0.64 \pm 0.10$  versus  $0.80 \pm 0.12$  hrs;  $F(1,9)=12.238$ ,  $p=0.007$ ) (Figure 2B), and  
234 significantly less time in lateral REM ( $0.34 \pm 0.12$  versus  $0.46 \pm 0.13$  hrs;  $F(1,9)=6.450$ ,  $p=0.032$ )  
235 during the Treatment Bedding week compared with the Control Bedding (Figure 2D). Horses spent  
236 significantly less time in sternal REM during the Treatment Light compared to the Control Light ( $0.53$   
237  $\pm 0.07$  versus  $0.67 \pm 0.11$ ;  $F(1,9)=6.511$ ,  $p=0.031$ ) (Figure 2C). No statistical difference was found in  
238 the duration of sternal REM between Control and Treatment Bedding ( $F(1,9)=2.317$ ,  $p=0.162$ ), nor in  
239 the amount of time spent in lateral REM ( $F(1,9)=0.758$ ,  $p=0.407$ ), sternal NREM ( $F(1,9)=0.037$ ,  $p=$   
240  $0.853$ ) and standing NREM ( $F(1,9)=2.981$ ,  $p=0.118$ ) between the two light conditions. There were  
241 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, ( $F(1,9)=0.946, p=0.356$ ).

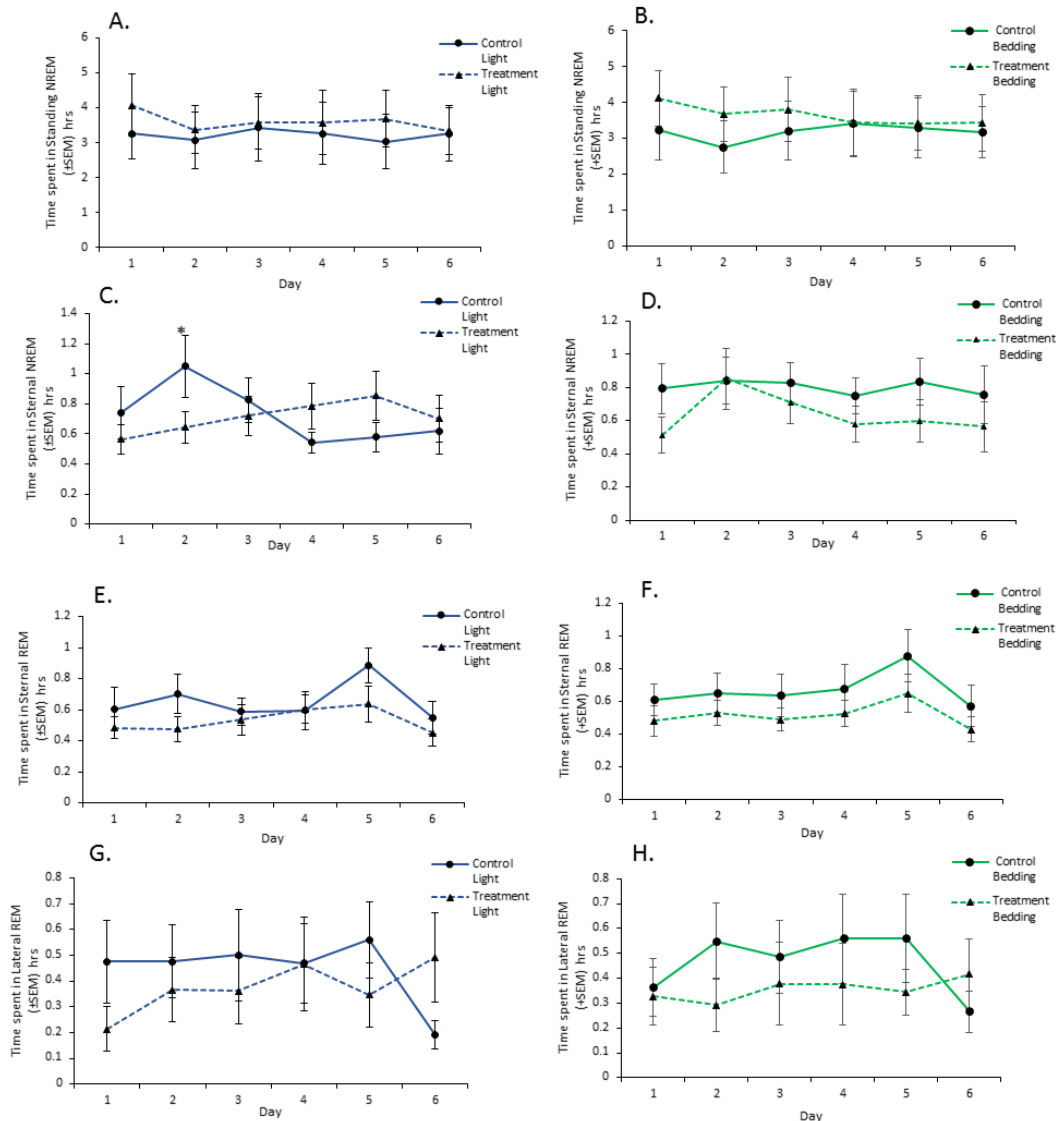


244  
 245 Figure 2. Mean ( $\pm$ SEM) ( $n=10$ ) time spent in the 4 different sleep behaviours (A. standing NREM, B.  
 246 sternal NREM, C. lateral REM and D. sternal REM over 6 days of each treatment (Control Bedding  
 247 [15cm], Treatment Bedding [5cm], Control Light (lux 2), Treatment Light (lux 180). Significant  
 248 differences between treatments are indicated by different subscripts ( $p \leq 0.05$ ).

249  
 250 Changes over time

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

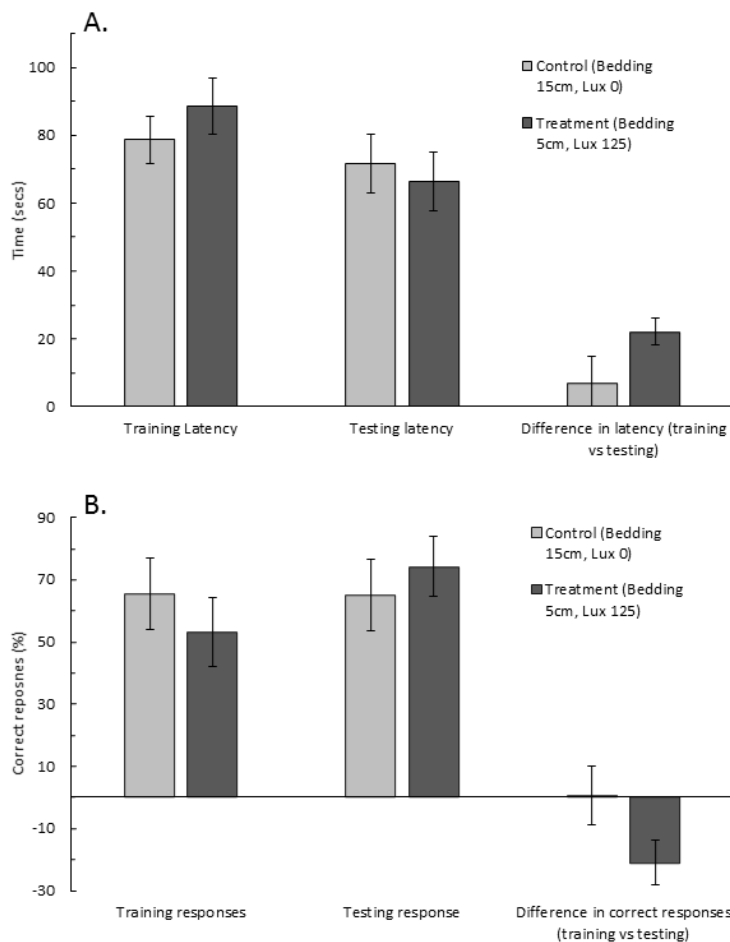
257 conditions for Day 2 ( $p=0.025$ ) but no significant difference between Treatment Light conditions for  
 258 other days. For Lateral REM, there was no significant interaction for the Treatment Light  
 259 ( $F(1,9)=3.049$ ,  $p=0.056$ ) with day nor for Treatment Bedding ( $F(1,9)=1.613$ ,  $p=0.221$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



260  
 261 Figure 3. Mean ( $\pm$ SEM) ( $n=10$ ) time spent in the 4 different sleep stages: standing NREM (A,B),  
 262 sternal NREM (C,D), lateral REM and (E,F), sternal REM (G,H) over 6 days of each  
 263 Treatment (Control Light [lux 2], Treatment Light [lux180]), Control Bedding [15cm], Treatment  
 264 Bedding [5cm]). \* Significant differences between treatments at each time point (Day)( $p \leq$   
 265 0.05).  
 266

267 **Memory**

268 A full set of memory test results was obtained for 9 out of the 10 horses. One horse completely  
269 disengaged from the task during the second set of memory testing by standing still at the starting  
270 point inside the arena. There was no statistical difference between the combined control condition  
271 (15cm bedding/lux 2) and the combined treatment condition (5cm bedding, lux 180) for training  
272 latency ( $t(8) = 0.855, p = 0.417$ ), testing latency ( $Z = -0.415, p = 0.678$ ) (figure 4A.) and mean  
273 difference latency ( $t(8) = 2.064, p = 0.073$ ). There was no statistical difference between the  
274 combined control and treatment conditions for the percentage correct training responses ( $t(8) =$   
275  $0.684, p = 0.513$ ), percentage correct testing responses ( $t(8) = 0.632, p = 0.545$ ), or difference in  
276 percentage of correct responses ( $t(8) = -1.791, p = 0.111$ ) (Figure 4B).



277

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

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

284

## 285 **4.0 Discussion**

### 286 4.1 Baseline sleep values and variability

287 Horses slept on average for a total of  $5.2 \pm 0.88$  hours/day during the Control conditions week (15cm  
288 bedding, lights off), which is higher than has been previously reported for total sleep time (TST) for  
289 horses (Zepelin, 2000; Wöhr et al., 2016). Whilst the use of behavioural observations to quantify  
290 equine sleep stages comes with a risk of over or under estimating time spent in each sleep stage, the  
291 equine sleep positions observed in this study aligned strongly with the descriptions and  
292 characteristics described in the EEG study by Williams et al. (2008). In the present study, REM sleep  
293 during the control period represented on average 23.9% of TST (1.23hrs/day), which falls within the  
294 range of 15% to 27.9% of TST for REM sleep previously reported for stabled horses (Kalus, 2014;  
295 Williams et al. 2008; Dallaire and Ruckebusch, 1974a). NREM sleep during the control period (76.1%  
296 of TST; 3.94hrs/day) was also similar in percentage of TST to previous EEG data (Kalus, 2014). The  
297 present study observed horses for 24 hours compared to previous studies of stabled horses that  
298 tend to report data from nocturnal observations only. Whilst most sleep occurs at night, stabled  
299 horses do rest during the day (Ruckebusch, 1972), and in the present study several horses engaged  
300 in sternal NREM, sternal REM and even lateral REM during the daytime period. Only 70% of horses  
301 achieved at least one bout of REM sleep every day/night indicating inter-horse variability in sleep  
302 profiles. Previous work has suggested that sleep variability in horses may be due to differences in  
303 age, diet, daily exercise or their ability to adopt a recumbent position (Crowell-Davis, 1994; Duncan,  
304 1980; Dallaire and Ruckebusch, 1974b; Ruckebusch et al., 1970). In this study, the variation in age

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

#### 312 *4.2 NREM sleep*

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

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



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

336

### 337 *4.3 REM sleep*

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

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

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