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1 A review of equine sleep; implications for equine welfare

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8

9 **Keywords:** equine, horse, behavior, sleep cycle, sleep quality, sleep quantity, sleep deprivation,
10 welfare

11 Abstract

12 Sleep is a significant biological requirement for all living mammals, due to its restorative properties
13 and its cognitive function in memory consolidation. Sleep is a ubiquitous characteristic amongst
14 mammalian species, however there are a number of differences between the sleep profiles of different
15 species based upon a range of biological and environmental factors. It is therefore important to
16 understand these differences given the importance of sleep to physical and psychological wellbeing
17 and thus overall welfare of the animal. This review focuses specifically on the domestic horse and
18 aims to consolidate current information on equine sleep, in relation to other species, in order to a)
19 identify what constitutes normal sleep in the horse in terms of both quantity and quality, b) identify
20 the best ways to measure equine sleep logistically and for the purpose of accuracy, c) to determine
21 whether changes in equine sleep quantity and quality reflect changes in the animal's welfare, and d)
22 the primary factors that affect the quantity and quality of equine sleep. The review then identifies
23 gaps in current knowledge and uses this information to identify and set the direction of future equine
24 sleep research with the ultimate aim of improving equine performance and welfare. The conclusions
25 from this review are also critically important in the context of current discussions around the 'social
26 license' of horse use from a welfare perspective.

27 1. Introduction

28 The primary aim of this review is to create a greater understanding of equine sleep and to discuss and
29 identify its role in equine welfare. Although a fundamental process in all mammals, sleep is not
30 commonly considered as a factor with the potential to influence animal welfare (1). For example, in
31 many of the animal welfare frameworks and guidelines, there are specific references to factors such as
32 sufficient air and light, food and water, adequate spaces for movement and contact with conspecifics,
33 but not to creating environments that allow minimum levels of species-specific sleep. In addition,
34 whilst some standards have been written to ensure the provision of species-appropriate spaces to rest
35 (e.g. Department for the Environment, Food and Rural Affairs, UK), very little information exists about
36 how to facilitate this in practice. In addition very little information exists **describing species-appropriate**
37 **spaces to facilitate rest in the domestic environment**. In this respect, more consideration needs to be

38 given to understanding species-specific sleep requirements in a domestic setting and how best to
39 accommodate these in order to help optimise animal welfare.

40 Although this review focuses primarily on the horse, many of the concepts within the review are
41 applicable to other domestic animal species and thus some of the conclusions drawn are potentially
42 generalisable to other species held within domestic, captive, farm or laboratory environments. To
43 understand the role of sleep in animal welfare requires an understanding of the normal sleep states and
44 sleep profile for the species in question, as well as how those states and profiles can be measured. The
45 review, therefore, also provides a comparative and evolutionary assessment of equine sleep to create a
46 detailed sense of the normal equine sleep profile, as well as the basic sleep requirements of the horse.
47 The different possible approaches to measuring equine sleep for applied purposes are also discussed,
48 followed by a review of what is currently known about factors affecting equine sleep.

49

50 **2. Definition, sleep stages and variation of animal sleep**

51 **2.1 Definition and different sleep stages**

52 Sleep is defined as a maintained state of quiescence characterised by relative inactivity, loss of
53 consciousness (2) and/or increased thresholds of arousal to environmental stimuli (3,4). Individuals
54 tend to adopt a distinct and sustained species-typical posture during sleep usually in a specific or
55 preferred location (3). Sleep is also characterised as a rapidly reversible state when compared to other
56 similar physiological states such as hibernation and torpor (3,5,6). Two main processes regulate the
57 occurrence of sleep; circadian rhythms organise the timing of sleep during the 24-hour cycle, whilst
58 homeostatic mechanisms determine the amount of sleep that a species requires (7,8,9).

59 Electroencephalogram (EEG) profiles have identified two primary states of sleep for a range of species,
60 non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. NREM, also known as slow
61 wave sleep (SWS), has been further divided into four stages (N1-N4, described in detail in Section
62 2.2). The transition into and out of sleep is characterised by drowsiness or quiet wakefulness and sleep
63 onset is described as a gradual process until the first occurrence of NREM N2 sleep (10). During a
64 normal sleep episode, individuals cycle between bouts of NREM and REM sleep³. Individuals usually
65 engage in behavioural rituals prior to sleep (e.g. circling the nest/yawning etc.) (11) however the
66 process of falling asleep is often irregular between individuals (10).

67 There are a number of specific behavioural and physiological correlates of NREM and REM sleep. For
68 example, the body loses muscle tone and suspends central homeostasis during REM sleep (REM
69 atonia), resulting in fluctuations in the autonomic nervous system (12). N1 and N2 NREM sleep are
70 often associated with slow eye movements and low arousal thresholds which then reduce and increase
71 respectively during the transition to N3 and N4 NREM sleep stages (see section 3). Whilst this profile
72 is common amongst the majority of mammals, there are exceptions for example, in the monotremes,
73 features of both REM and NREM sleep are merged into a single sleep-like state (13) or REM sleep
74 activity is only found in the brainstem region of the brain (14).

75

76 **2.2 Micro-arousals, wake sequences and wakefulness**

77 Sleep stages are an ever-changing dynamic process due in part to the cyclic nature of sleep and the
78 occurrence of arousing stimuli in the internal and external environment. Stages of sleep are, thus, often
79 interrupted by either micro-arousals or slightly longer wake sequences and can be broken completely
80 into a full state of wakefulness. During sleep, the brain continues to interpret information such that
81 arousal eliciting factors work against sleep promoting forces. When there is higher pressure to sleep
82 during the descending loop of the sleep cycle (N1-N4), phasic changes from sensory input often don't
83 disrupt sleep and can in fact result in deepening of SWS (15). During the ascending loop of the cycle
84 (N4 to REM), however, when there is lower sleep pressure, sensory input has a more pronounced
85 modifying and disrupting influence on sleep. These phasic changes are referred to as micro-arousals
86 (MA), defined as momentary adaptations to vigilance levels in response to internal and external sensory
87 input. Within the EEG profile, MA are seconds in duration and are associated with the emergence of
88 K-complexes (15). According to the American Sleep Disorder Association, a micro-arousal should be
89 scored when there is an increase in EEG frequency for three seconds or more and that K-complexes
90 should not be scored as arousals unless accompanied by increased EEG frequencies (16).

91 Wake sequences describe longer periods of arousal when the animal is no longer within a NREM or
92 REM state. The duration of these sequences are often species-dependent and can occur either within
93 or at the end of a sleep cycle. For example, in rodents, brief wake sequences (<300s) have been
94 described interrupting periods of NREM or REM within the sleep cycle, whilst longer disturbances
95 (>300s) have been described between sleep cycles (17).

96 The definition of full wakefulness from sleep relates to the probability of re-entering sleep within a
97 specified time period. There are a number of specific neurophysiological events that occur on entering
98 full wakefulness that are largely driven by the activation of efferent hypocretin neurons from the lateral
99 hypothalamus (18). One of the main differences between sleep and wakefulness is increased
100 sympathetic tone and decreased parasympathetic tone during wakefulness that maintains most organ
101 systems in a state of action or readiness. In humans, spontaneous awakenings lasting longer than three
102 minutes are generally acknowledged as a state of wakefulness that modifies the sleep cycle (19).

103

104 **2.3 Ecological and biological factors affecting mammalian sleep**

105 A comparison of average total sleep time between polyphasic mammalian species reveals large-scale
106 variation and several factors have been proposed to explain this (Table 1). One of the primary factors
107 is body mass which negatively correlates with total sleep time (20, 21). This is considered to reflect
108 the risk of predation for larger prey species which, due to their size, are required to sleep in exposed
109 locations²¹, and thus tend to not enter into prolonged periods of deeper stages of NREM sleep. The
110 severity of predation and safety of sleeping place are often scored from one to five using a sleep
111 exposure index; a score of one is given to a sleep site that is well-protected with minimum predation,
112 a score of five is a sleep site of high predator risk/exposure (20, 23) (Table 1). Mammalian prey species
113 sleeping in riskier locations are commonly observed to engage in lower proportions of REM sleep, as
114 this sleep stage can only be attained in the recumbent position (23).

115 Another factor influencing sleep time is the degree of species encephalization which positively
116 correlates with total REM sleep and supports the role of REM in memory consolidation (20). However,
117 this is a complex relationship due to additional influencing factors including whether the species bears
118 precocial or altricial young (24). For example, precocial species that experience longer gestation
119 periods have high levels of brain maturity at birth so that offspring can adapt quickly to the external

120 world (25). This would suggest that precocial species would have higher levels of REM sleep, however,
121 because these animals tend to live in exposed environments of high predation risk, the total level of
122 REM sleep tends to be lower (22). In contrast, neonates of less developed altricial species display larger
123 amounts of REM sleep (7) potentially as the result of being maintained in a protected safer environment
124 (24). Research has also shown a negative correlation between total sleep time and basal metabolic rate
125 (BMR) and this is explained by the hypothesis that species with greater energy expenditure relative to
126 their size are required to make a trade-off between sleep and foraging, with foraging superseding sleep
127 (24, 26). Additionally, higher BMR is linked to higher levels of restorative neurophysiological
128 processes thus reducing the need for long periods of restorative sleep (20).

129 **Table 1.**

130 Similar to most mammalian species, the horse engages in stages of wakefulness, rapid eye movement
131 (REM) sleep, and non-rapid eye movement (NREM) sleep. Periods of drowsiness or light sleep have
132 been recorded before the horse experiences NREM/REM (27). In comparison to the mammals listed
133 in Table 1, the horse is one of the species engaging in the least amount of sleep (equal rank 1 with
134 sheep and goat). This fits with its ecological and biological characteristics, a precocial species with a
135 high gestation period (rank 1), high basal metabolic rate (rank 1) and high body mass (rank 2) and a
136 sleep exposure index of five (equal rank 1 with cow, sheep and goat).

137 Additional to the factors in the table, which from an evolutionary perspective have determined animal
138 sleep duration and patterns, is the social context in which sleep is performed. Protection during sleep
139 can be provided through group living in social species (28), for example in the equine herd, individuals
140 are often observed to interchange between recumbent and standing positions as a supposed rota system
141 of group vigilance (29). These innate behavioural characteristics are observed in both free-living horses
142 (those populations receiving very little human management), and domesticated horses (those born into
143 an artificial environment) (29). This element of protection however, is not considered to increase sleep
144 quotas overall where often for large herbivorous species, the continued trade-off between risk of
145 predation and need to forage maintains a comparatively low total sleep time (30). This form of
146 synchronised social sleep may, however, still be critical to vigilance levels and thus the level of micro-
147 arousals and wake sequences the animal experiences during sleep. In the domestic environment, the
148 social context as well as several other factors (e.g. athletic training, nutrition, **housing environment**)
149 can be substantially different to the free-living environment. This will therefore have an impact on
150 sleep patterns in the horse and is discussed in more detail in section 6.

151 **2.4 Comparison of human and equine sleep stages**

152 In the awake state, human EEG patterns present as two types of waves, beta and alpha. Beta waves are
153 associated with full wakefulness and have the highest frequency (13-30Hz) and lowest amplitude (10–
154 30 μ V) (31). Beta wave forms have few rhythmic components and are thus more dyssynchronous than
155 other wave forms (32). Beta wave forms have been measured in the horse during wakefulness and
156 active states (33). During more inactive states of reported relaxation in humans, alpha brain waves
157 predominate (34). These brain waves are slower (8-13Hz) with increased amplitude and are
158 more synchronous in nature (35) with occasional bursts of high frequency (beta) wave forms associated
159 with periodic alertness (34). Alpha waves have been measured in the horse (33, 36) and are associated
160 with the behavioural markers of weight bearing on 2 fore and 1 hind limb with the head above withers
161 (36). The transition into the sleep state is associated with a further reduction in brain wave frequency
162 to a theta wave state (3-7 Hz) but with occasional presentation of alpha waves (8-13Hz) (37). This is
163 referred to as the N1 stage of NREM sleep and is often preceded by a state of drowsiness, particularly

164 in animals (38). Full sleep onset is typically considered to occur at the N2 stage of NREM, here the
 165 EEG profile is similar to N1 (theta waves, 3-7 Hz) but now also contains sleep spindles and/or K
 166 complexes with at least 1 sleep spindle or K-complex occurring per 30 seconds on a N1 background
 167 (10). In humans, the N2 sleep stage tends to predominate during periods of sleep across all ages (39)
 168 and this sleep stage is routinely observed in the horse (36, 40). The next two stages of NREM sleep
 169 (N3-N4) are collectively referred to as SWS and are characterised by slow wave oscillations (0.5-2.0
 170 Hz) of high amplitude (75mv), referred to as delta waves. The N3 stage of SWS is defined as having
 171 between 20% and 50% delta waves whereas the N4 stage contains above 50% (41). More recently
 172 within the human literature the N3 and N4 stages of sleep have been merged (42). N3 but not N4
 173 NREM sleep has been explicitly reported in horses and equine studies commonly, referred to as SWS
 174 (36, 43).

175 REM sleep is often referred to as "paradoxical" sleep because of the mixed frequency (3-30 Hz), low
 176 amplitude (10-30uV) nature of brain waves associated with this sleep stage that is often also observed
 177 during wakefulness (41). REM sleep is associated with tonic suppression of skeletal muscle tone and
 178 reflexes through inhibition of the brain stem and spinal motor neurons (44) but with episodic bursts of
 179 rapid eye movements and muscle twitches arising from ponto-geniculo-occipital brain waves (39).
 180 REM sleep has been observed in the horse and is associated predominantly with lateral recumbency
 181 (reflecting full muscle atonia) (43) and also sternal recumbency with the head propped on the floor
 182 (36). In some instances, short episodes of REM sleep occur in the horse whilst standing accompanied
 183 with complete loss of tone in the neck muscles, head dropping to just above the floor and the horse
 184 buckling forelimbs with the muzzle hitting the floor (36). REM sleep in the horse is also associated
 185 with rapid eye movements and rhythmic ear twitching (36). In order to characterise and compare sleep
 186 profiles within and between species, standard descriptive terminology of the different sleep states in
 187 accordance with previous definitions is presented in Table 2. alongside some additional terminology
 188 that may be particular to the equine sleep profile.

189 **Table 2.**

190 **2.5 Comparisons of the human and equine sleep cycle**

191 **2.5.1 General characteristics of the human sleep cycle**

192 The human sleep cycle involves predictable patterns of sleep sequences and is commonly reported to
 193 last on average 90-100 minutes, measured from the end of one REM sequence to the end of the next
 194 (47). After initial sleep onset (beyond the N1 stage), normal progression through a sleep episode
 195 follows N2 into SWS (N3-N4), followed by either N1 and/or REM. On average, 20-25% of the total
 196 sleep time (TST) is occupied by REM sleep occurring in four to six sequences (47). At the end of the
 197 first REM sequence, SWS re-emerges within subsequent cycles of sleep. As the overall episode of
 198 sleep progresses however, SWS sequences diminish in duration and are superseded by more time
 199 within N1 and N2 stages (47, 48). In this respect, sleep cycles earlier during the night are shorter (on
 200 average 70-90 minutes) than later cycles which can last between 90-100 minutes (49). Across a
 201 complete sleep episode, N1 is reported to account for 2-5% of TST, N2 accounts for approximately
 202 45-55% TST, whilst N3 constitutes 5-15% TST⁴⁶ although this varies with age (Figure 1).

203 **Figure 1.** Changes in duration of sleep stages with increasing age (50) (adapted from Ohayon et al.,
 204 2004)

205 A normal pattern of human sleep is often difficult to characterise due to individual sleep duration
 206 variability, compounded by factors such as duration of prior waking, staying up late, and waking by

207 alarm (39). In addition, internal (e.g. drugs) and external (e.g. sound) stimuli will also influence the
208 dynamic process of sleep (15). Sleep architecture also changes considerably with age. Twelve month
209 old infants typically sleep for 14 to 15 hours per day (51), which drops to 11 hours by age 5, whilst
210 adolescents require 9 to 10 hours of sleep each night (52). Differences in the profile of sleep stages
211 within a sleep episode are also apparent between young adults and the elderly (Figure 2). This is
212 especially true when comparing the increasing number of brief arousals and wake sequence as a
213 function of age (49, 53). In total, the amount of time awake after initial sleep onset (WASO) increases
214 with increasing age (Figure 2 and Figure 3) along with a decrease in duration of REM and SWS sleep.
215 Since arousal thresholds are higher during SWS and this sleep stage decreases with age, older adults
216 become prone to experience more frequent awakening during a sleep episode (52).

217 **2.5.2 Characteristics of the equine sleep cycle in comparison with the human sleep cycle**

218 Most mammalian species, including the horse, are polyphasic sleepers engaging in multiple sleep
219 episodes across 24 hours (7). This is considered to be an adaptation for increased vigilance (and thus
220 survival) but may also indicate limited ability to sustain wakefulness (54). Table 3 presents data on all
221 reported studies of equine sleep to date. This table sets out a number of sleep parameters to help define
222 the characteristics of equine sleep and the equine sleep cycle, and allow for comparison with the human
223 equivalent sleep profile.

224 **Table 3.**

225 *2.5.2.1 Total sleep time (TST)*

226 Studies of equine sleep describe on average 230.72min (± 82.87) devoted to sleep. This is in comparison
227 to an average total sleep time of 429.63 min (± 25.6) observed for humans (37, 66). Studies using EEG
228 or ECoG reported mean total equine sleep duration ranging from 172min to 262min. Meanwhile,
229 studies using behavioural observations record total sleep as between 65min to 382min, depending on
230 whether standing sleep was included and how total sleep time was defined. Differences in age of horses
231 between studies (from 6 months to 20 years), use of sampling techniques, and total sleep observation
232 time (e.g. 12 versus 24 hrs) are also likely to cause variation within reported TST between studies.
233 This latter point identifies the importance of using standardised methodology when taking a
234 behavioural approach to quantifying animal sleep (discussed in more detail in section 3).

235 *2.5.2.2 Total REM and Total NREM*

236 For horses, NREM sleep consistently accounts for the largest proportion of total sleep time (77.50%)
237 (mean 178.74 ± 87.53 min) compared to REM sleep (17.50% of total sleep time) (40.27 ± 24.15 min).
238 Although the average total NREM and REM sleep duration is greater in humans (352.86min and
239 72.92min respectively), the ratio of NREM to REM for human sleep (82% and 17%) is similar to the
240 horse (37, 66), with a larger proportion of total sleep time devoted to NREM sleep for both species. In
241 total, NREM and REM sleep constitute 95% of the total sleep time for the horse compared to 99% for
242 the human which may be indicative of greater levels of wakefulness in the horse during sleep cycles.

243 *2.5.2.3 Sleep cycle duration and REM and NREM components of the sleep cycle (sequences and* 244 *episodes)*

245 The average number of sleep cycles for human sleep has been recorded at 4.23 with a range of 2-6
246 cycles per night that last for an average of 119.70 minutes, ranging from 80.7 to 199.1 minutes (66).
247 The horse also engages in 2-6 cycles (averaging 4.1 cycles) but these are shorter on average than in
248 humans (40.74 minutes). Many equine studies only measure sleep at night and thus additional sleep

249 cycles, that are known to occur during the day for the horse, will not have been taken into account. In
 250 this respect, the average value of 4.1 cycles pertains only to the nocturnal sleep profile.

251 In the horse, the average duration of NREM sequence within the total sleep time is 6.02min (ranging
 252 from 3.37 to 9min) with the average number of sequences being 31.96 (ranging from 18 to 52). This
 253 is substantially different to humans where the average duration of NREM sequence is 67.97 mins
 254 (ranging from 62 to 77.5min) within the total sleep time. The number of these prolonged NREM
 255 sequences in humans during the total sleep time is therefore also substantially lower, with one sequence
 256 per sleep cycle and an average of 4 sleep cycles per night (66). This is reflected in recorded durations
 257 of NREM episodes which are substantially greater in humans (101.36min) compared to the horse
 258 (17.14min) (66, 43).

259 The average duration of REM sequence in the horse is 3.72min (ranging from 0.91 to 5.13min) and
 260 average number of REM sequences is 10.5 (7.11 to 14.6) during the total sleep time. This is compared
 261 to an average duration of 22.33min for humans with the average number of REM sequences equating
 262 to the average number of sleep cycles per night (4) (40). This difference in REM sequence duration
 263 and frequency illustrates the fragmented nature of equine versus human sleep (Figure 2).

264 2.5.2.4 *Wake sequences (duration and number)*

265 The mean number of wake sequences per sleep cycle is 0.25 for children, 0.77 for adults, 0.9 for elderly
 266 adults and 6.89 for the horse (Table 3). The mean duration of wake sequences for children is 1.64mins
 267 versus 2.75mins for adults, 11.56mins for elderly adults and 0.96mins for the horse. In this respect,
 268 elderly humans are experiencing wake sequences greater than 3 minutes which are thus more likely to
 269 be complete breaks in the sleep cycle with a new sleep cycle restarting after the wakefulness has
 270 occurred. Thus, whilst the number of wake sequences within a sleep cycle for the elderly adult has
 271 greater similarities to the horse compared to younger humans, the nature of the wake sequence appears
 272 to be quite different (Figure 2). The increased number of long duration wake sequences in the elderly
 273 human is considered to reflect non-functional age-related changes in the brain (68), whereas the large
 274 number of short duration wake sequences in the horse is considered to serve a much more functional
 275 role in maintaining vigilance levels against predators (69).

276 **Figure 2.** Hypnogram comparing sleep architecture of a typical young human adult, and elderly human
 277 adult and an adult horse over an eight hour sleep episode: A. the young adult experiences 5 REM
 278 sequences and 2 short sequences of wakefulness; B. the elderly individual experiences 8 disturbed
 279 REM sequences and multiple sequences of wakefulness accompanied by a distinct lack of stage 4 sleep;
 280 C. the horse experiences 13 disturbed REM sequences and multiple sequences of wakefulness
 281 accompanied by a distinct lack of stage 4 sleep (adapted from 42,70).

282 In conclusion, the horse sleeps for approximately 50% of human total sleep time for periods of
 283 nocturnal observation. Although the nocturnal sleep cycle frequency appears comparable between
 284 humans and horses, the duration and frequency of sleep stages (NREM and REM) within the equine
 285 sleep cycle is much shorter compared to the human. This discrepancy is due in part to the higher
 286 frequency of short duration wake sequences that occur within sleep cycles, and the extended periods
 287 of wakefulness that occur between the equine sleep cycle. In this sense, the conventional definition of
 288 a sleep cycle in human terms (progressive stages of NREM followed by REM) may be less applicable
 289 to the horse, particularly given that the horse can cycle through sleep onset to wakefulness and display
 290 only NREM sleep. In this sense, equine sleep demonstrates greater similarity to the elderly human
 291 sleep profile. The data from the table also does not completely capture the polyphasic nature of equine
 292 sleep as many of the studies only record or observe the horse overnight. More complete 24hr studies
 293 are still needed therefore to provide a complete picture of the equine sleep profile.

294

295 **3. Enhancing the behavioural measurement of equine sleep quantity**

296 As previously discussed (section 2.0) EEG profiles, often in combination with polysomnography
 297 (PSG), give an accurate quantification of specific sleep states and in a range of species including the
 298 horse (3), however, equipment is specialised and the use of surface electrodes often produces data loss
 299 thus producing incomplete data sets (71). The behavioural quantification of sleep has historically
 300 provided a viable alternative to EEG measurement (3) and thus, there is an argument for developing
 301 this approach for the measurement of equine sleep when EEG equipment is not available. One of the
 302 primary problems with this approach, however, is that the horse is capable of achieving various stages
 303 of sleep in multiple body positions (Table 4). For example, NREM N1-N3 sleep stages are
 304 differentiated only by the position of the eye lid and the position of the poll in relation to the withers
 305 when the horse adopts these sleep states whilst standing (36). Although NREM sleep tends to occur in
 306 standing positions, it can also occur when the horse is recumbent making the differentiation between
 307 NREM and REM sleep states more difficult. REM sleep, due to muscle atonia, can only occur in
 308 recumbent states (lateral and sternal) with the muzzle being placed on the floor in the sternal position
 309 (56). Some horses (although rare) can enter a REM sleep state in a standing position but only
 310 momentarily before muscle atonia occurs and the horse collapses bringing it out of sleep (36).

311 **Table 4.**

312 Individual horses thus appear to have different strategies of sleep with different proportions of sleep
 313 states occurring in different behavioural positions. For example, in an EEG study of seven horses (43),
 314 the quantity of total sleep in the standing position ranged from 26.4% in one horse to 65.7% in another,
 315 in sternal recumbency it ranged from 21% to 59.7% and in lateral recumbency from 1.8% to 13.9 %.
 316 By comparing EEG data with behavioural data, it may be possible to derive better estimates of sleep
 317 state from the behavioural assessment of the animal. For example, by plotting the duration of the EEG
 318 sleep state against the duration of associated behavioural state, the linear regression equation that is
 319 derived from this plot can be used to more accurately estimate the sleep state from the behaviour of the
 320 animal. To illustrate this, Figure 3 presents the linear regression of the average duration of EEG sleep
 321 states (Light sleep, N1; SWS N2-N3; REM) for 7 horses (over 4 nights) against the average duration
 322 of sleep behavioural states (standing sleep; sternal recumbent sleep; lateral recumbent sleep) (data
 323 taken from Kalus, 2014). From Figure 3a, it can be seen that both light sleep (NREM N1) and SWS
 324 sleep (NREM N2-N3) can occur whilst the horse is in standing sleep with predominantly more time
 325 spent in the latter sleep state compared to the former. The linear equations give the estimation of the
 326 time spent in each sleep state when the animal is in standing sleep (Light sleep [NREM N1]
 327 = $0.25 \times \text{Total time spent in standing sleep} - 3.13$; SWS [NREM N2-N3] = $0.75 \times \text{Total time spent in}$
 328 $\text{standing sleep} + 3.13$). Interestingly, the variation within this estimation (as indicated by the R^2 value)
 329 can be reduced by taking into consideration other behavioural features during the sleep period. For
 330 example, the number of wake sequences (periods of wakefulness during sleep cycles that are less than
 331 3 minutes) that occur within the total period of sleep increases the accuracy of the estimation. Taking
 332 the Kalus (2014) data for example, including the wake sequence data into the regression analysis
 333 marginally increases the R^2 value for Light sleep (NREM N1) from 0.19 to 0.34 and for SWS sleep
 334 (NREM N2-N3) from 0.68 to 0.74.

335 The two other primary behavioural sleep states within which sleep can occur are sternal and lateral
 336 recumbency. For sternal recumbency, all three sleep states are present as can be seen in Figure 3b.
 337 SWS (NREM N2-N3) predominates (SWS [NREM N2-N3] = $0.66 \times \text{Total time spent in sternal}$

338 recumbency – 9.85) followed by REM sleep ($REM = 0.12 \times \text{Total time spent in sternal recumbency}$
 339 $+14.97$) and then light sleep (NREM N1) ($\text{Light sleep [NREM N1]} = 0.22 \times \text{Total time spent in sternal}$
 340 $\text{recumbency} - 5.11$). Again, adding in additional behaviours into the estimation analysis can increase
 341 the R^2 value and the accuracy of the estimation. In this instance, the inclusion of the total number of
 342 wake sequences into the regression analysis marginally reduces the variation of estimation from R^2
 343 value for Light sleep (NREM N1) from 0.73 to 0.74, from 0.90 to 0.91 for SWS sleep (NREM N2-N3)
 344 and from 0.23 to 0.24 for REM sleep. For lateral recumbency, again all three states can occur as
 345 illustrated in Figure 3c, but with a more equal distribution across the three states: Light sleep (NREM
 346 $N1) = 0.36 \times \text{Total time spent in lateral recumbency} - 1.04$; SWS [NREM N2-N3] = $0.28 \times \text{Total time}$
 347 $\text{spent in lateral recumbency} + 1.24$; $REM = 0.36 \times \text{Total time spent in lateral recumbency} - 0.36$. Again,
 348 inclusion of the total number of wake sequences into the regression analysis marginally reduces the
 349 variation of estimation from R^2 value for Light sleep (NREM N1) from 0.55 to 0.57, from 0.35 to 0.36
 350 for SWS sleep (NREM N2-N3) and from 0.50 to 0.51 for REM sleep. Further EEG studies that monitor
 351 in close detail the changes in behaviour of the horse as it transitions between the three primary sleep
 352 states has the potential to increase the accuracy of behavioural sleep analysis through this multiple
 353 regression approach.

354 **Figure 3.** Linear regression analysis of average duration EEG sleep states against average duration
 355 behavioural states for 7 horses over 4 nights. Data taken from Kalus (2014). (A = Standing; B = Sternal
 356 Recumbency; C = Lateral Recumbency)

357

358 4. Measuring sleep quality

359 Whilst sleep quantity and quality are inextricably linked, they are also often dissociated and thus it is
 360 important to take separate measures of both particularly in the context of ‘sleep deprivation’ and human
 361 and animal welfare (75). It is important, therefore, to have a definition of optimal sleep quantity and
 362 quality for any given species. It is also important to identify factors that can affect sleep quality and
 363 quantity that may produce a state of sleep deprivation in the animal. In this section, we will discuss the
 364 concept of sleep quality and how it can potentially be measured in the horse.

365 4.1 General concepts of sleep quality versus quantity

366 Although the average (and thus potentially optimal) quantity of total sleep time for a range of animal
 367 species is well documented (3), sleep quality is an uncommon measurement within animal sleep
 368 research and is therefore very poorly defined for the majority of mammalian species. In humans, the
 369 subjective experience of sleep quality has been quantified using sleep continuity measures such as
 370 reduced latency to sleep onset, the number of awakenings, and duration of wakefulness after sleep
 371 onset (76), correlating with a reduction in total sleep time. Poor human sleep quality is also associated
 372 with patterns of sleep fragmentation or interruptions described as sleep that is punctuated by repeated
 373 periods of waking throughout the night (77). These can involve transient arousals (transition to brain
 374 alpha activity [2 seconds or more]) not associated with a change of sleep stage and body movements
 375 lasting 0.5 seconds or longer (37) or wake sequences (<3 minutes) (19). Reduction in both sleep quality
 376 and quantity produce a state of sleep deprivation and subsequent sleep debt (78). Sleep deprivation is
 377 defined as either a complete lack of sleep or a shorter than optimal sleep time (75), for example
 378 quantified for humans as less than six hours of sleep per night (79). A distinction is made between
 379 acute and chronic sleep deprivation based on the number of days the individual experiences less than
 380 the optimal sleep time. For example, in humans acute sleep deprivation has been defined as three

381 consecutive nights of restricted or no sleep (80) whereas chronic deprivation has been described as
382 persisting over longer periods of time e.g. fourteen consecutive nights of restricted sleep (81). In
383 animals, the former has been associated with reduced energy whereas the latter has been associated
384 with generalized inflammatory and stress responses in the brain (82) leading to the death of the animal
385 (83).

386 Due to an accumulation of hours of lost sleep relative to the daily sleep requirement at an individual
387 level, there is a need for recuperative or recovery sleep (81, 84). For example, human individuals
388 maintained on a sleep wake pattern that induced a reduction in total sleep time were described as more
389 sleepy and less alert (78). The effects were reversed via extended sleep following the sleep reduction,
390 and the individuals were described as having 'repaid the sleep debt' (78). The sleep debt can also be
391 repaid by higher intensity sleep in the form of deeper slow wave sleep, where EEG slow wave activity
392 (SWA) observed during NREM sleep is considered to represent a parameter of sleep intensity (9). In
393 this context, slow wave sleep has also been described as a function of the duration of prior wakefulness
394 (85) where it occurs closer to the point of onset of sleep during the sleep cycle (86), therefore providing
395 an efficient mechanism with which to recover the sleep debt if required. After sleep deprivation,
396 increased levels of SWA during NREM sleep are also associated with a decreased number of
397 spontaneous awakenings and an increased threshold for induced awakening (85, 87), which are
398 characteristic of deeper/more intense/higher quality sleep. REM sleep is less sensitive to sleep
399 deprivation, however sustained deprivation of REM sleep results in elevated REM sleep that is not
400 always immediate but lasts for several nights compared to SWS which tends to be elevated during the
401 first recovery night (9). To summarise, whilst acute changes to sleep cause an immediate, short-lasting
402 compensatory SWS response, only a severe deficit in REM sleep results in a rebound which is often
403 delayed and prolonged (9).

404

405 **4.2 Potential measures of sleep quality in the horse**

406 Recent human research (192) has confirmed the involvement of inflammatory dysfunction in sleep
407 inconsistency, highlighting a novel physiological measurement of sleep disturbance that could be used
408 to determine sleep quality. Additional consideration of the profile of salivary cortisol might also be
409 relevant, due to the links between concentrations upon awakening and subjective reports of poor quality
410 sleep in humans (193). Meanwhile, some behavioral studies have scrutinized equine sleep data to
411 increase the resolution beyond total sleep time that provides a more detailed profile of the generalised
412 equine sleep pattern (Table 3). For example, the average duration of NREM sequences (total time of
413 consecutive NREM sequences not interrupted by REM or wakefulness) (ranging from 3.37 to 9min)
414 and the number of these sequences (18 to 52) within the sleep profile may provide an indirect measure
415 of the quality of sleep experienced by the animal. Similarly, the average duration (ranging from 0.91
416 to 5.22min) and number of REM sequences (7.11 to 14.6) in the horse may act as an important indicator
417 for sleep quality, as has previously been demonstrated in humans (88). However, it should also be
418 noted that abnormally high levels of REM sleep might be indicative of prior REM sleep deprivation
419 but can also act as a marker of stress and depression and thus it is important to establish the range of
420 normal baseline values for any given species (89).

421 Additional measures of the equine sleep profile that may also be useful in quantifying equine sleep
422 quality is the total duration of NREM sleep and also the number of wake sequences (<3min) or micro-
423 arousals within a sleep cycle. Previous work in humans has shown that an increased number of wake
424 sequences negatively correlate with subjective sleep quality, whilst increased quantity of NREM sleep
425 positively correlates with better motor function and accuracy (89). As indicative baseline values for

426 horses the mean total duration of NREM sleep is 178.74 minutes and the mean number of wake
427 sequences within a sleep cycle was 6.89 (Table 3).

428 A potential approach to transforming these measurements of equine sleep into a metric of sleep quality,
429 is to develop an equine sleep quality index (SQI). Using the data from Table 3 and additional data from
430 Kalus (2014), one example is given below that uses some of the sleep duration parameters discussed
431 as well as the number of wake sequences. Incorporation of additional sleep parameters (e.g., average
432 number/duration of NREM sequences, EEG micro-arousal events) may further increase the resolution
433 of the index and form the basis for future research. Consideration may also be given to different
434 weightings for the different elements of the index as this research develops.

435

436 **SQI= Total NREM duration/no. of NREM wake sequences + Total REM duration/ no. of REM**
437 **wake sequences**

438 **SQI= 178.74/1.98 + 40.3/4.91 = 98.48**

439

440 In summary, many equine studies fail to report in detail the nuances of the sleep cycle, with TST
441 (NREM and REM duration combined) predominating as the primary measure of sleep quality. These
442 data are undoubtedly useful to understand if a horse is sleeping and the impact of environment on sleep,
443 however, measuring the frequency and duration of wake and sleep state sequences may provide better
444 metrics of sleep quality relative to sleep deprivation. These measures have the potential to form the
445 basis of an equine sleep quality index, that can be compiled using both behavioural and/or EEG data.

446

447 **5.0 The interrelationship between reduced welfare/exposure to stress and reduced animal sleep**

448 The central premise in the relationship between sleep and welfare (Figure 4) is that sleep deprivation
449 has the potential to diminish animal welfare but also that factors affecting welfare (e.g., physical and
450 psychological stressors, changes in environment) have the potential to affect sleep and thus further
451 affect the welfare of the animal (35). In this section, we will discuss the interplay of these factors to
452 gain a better understanding of the relationship between sleep quality and the welfare of the animal.

453

454 **Figure 4.** The interrelationship between factors of well-being affecting sleep and sleep affecting well-
455 being (red arrows indicate the pathways to reduced sleep and the interplay between increased
456 stress/reduced welfare and reduced sleep).

457

458 **5.1 Using changes in sleep as a marker for poor welfare**

459 It is often difficult to establish whether changes in sleep can be used as a marker for stress or whether
460 changes in sleep are partially or wholly responsible for the animal being stressed. It is likely, as
461 intimated in **Figure 4**, that changes in sleep are both a marker and cause of stress with the importance
462 of the latter increasing over time as the quality of the animal's sleep progressively diminishes. For
463 example **in rodents**, sleep has been used as a behavioural marker of stress alongside other standard
464 biomarkers (adrenal weight, corticosterone) in response to cage size and social stress (90). It is difficult
465 however to ascertain within this study whether it was social stress directly that was having the stress
466 effect or whether the physiological response was due to the significant reduction in sleep duration and
467 interruption. In this sense, reduced quality sleep may be a reasonable marker of stress in the first

468 instance but ultimately becomes a compounding stressor in its own right over the longer term. When
469 investigating the relationship between sleep and welfare, non-significant relationships have been
470 reported between total time spent asleep, judgement bias, and behaviour-based measures of welfare for
471 shelter dogs (91) potentially highlighting the need for more sensitive measurements of sleep beyond
472 total sleep time. As previously discussed, measurements of sleep quality such as number of
473 disturbances or micro-arousals might yield more valid results. Little research has been carried out
474 assessing how welfare-reducing factors might manifest as changes in the sleep profile in the horse.
475 However, horses performing stereotypy are reported to display different sleep profiles compared to
476 non-stereotypy animals with significantly less time ($p<0.001$) spent in REM (2.2 ± 1.7 versus 6.7 ± 1.9)
477 and N2-N3 SWS (13.8 ± 8.2 versus 29.5 ± 3.4) sleep states and significantly more time ($p<0.001$) spent
478 in light sleep (N1) (22.6 ± 4.5 versus 8.8 ± 3.4) (194). Stereotypy is associated with current and/or historic
479 states of reduced welfare (195) and the result of this study, given the welfare consequence of sleep
480 deprivation, suggest that stereotypy horses may be suffering from compounded state of negative
481 welfare.

482

483 5.2 Effect of sleep deprivation on animal behaviour and welfare

484 Sleep deprivation and disorders in humans are well documented as constituting a major risk factor for
485 psychiatric, cardiovascular, metabolic or hormonal co-morbidity and mortality (92). Sleep deprivation
486 in humans has also been described as an anxiogenic factor with major impacts on the individual's
487 welfare state (93). Sleep deprivation in animals reportedly causes serious physiologic changes
488 including a state of high caloric ingestion without weight gain, reduction in anabolic hormones,
489 opportunistic infections, and in some cases death (94). For example, evidence of immune compromise
490 in rats exists where processes underlying bacterial disease were detected early after the onset of
491 prolonged sleep deprivation with infection of normally sterile tissues preceding overt signs of
492 morbidity (95). In human studies, sleep deprivation has been shown to produce hyperalgesic changes
493 in healthy subjects, specifically slow wave sleep disruption due to its effect on the descending pain
494 inhibitory control system measured through pressure pain sensitivity (96). Experimental animal studies
495 have also evidenced the hyperalgesia effects of REM or TST deprivation which appeared to prevent
496 the analgesic action of endogenous and exogenous opioids (96). In terms of the effects of sleep
497 deprivation on the welfare of the horse, much less specific research has been carried out. Excessive
498 daytime sleepiness is known to increase risk of injury whilst cases of spontaneous equine collapse
499 linked to sleep deprivation have been observed (97). Theoretically, horses will be susceptible to many
500 of the clinical sequelae of sleep deprivation that has been observed in other species (Table 5). Further
501 research is needed to establish whether these conditions are apparent in horses and whether they are
502 associated with the sleep profile of the animal.

503

504 Table 5.

505

506 6.0 Factors reducing sleep

507 In this section we discuss the primary factors affecting sleep quality and quantity generally and also
508 specifically in the domestic horse as well as identifying clinical and non-clinical conditions for which
509 reduced sleep may be symptomatic.

510

511 6.1 Stressors

512 Both physical and psychological stressors can lead to a reduction in sleep duration and quality. Pain is
513 an example of a physical stressor that influences sleep. For example, in a meta-analysis of human
514 studies (107) using polysomnography (PSG) to quantify sleep in people with chronic pain (CP), 44%
515 of those with CP were also diagnosed with a sleep disorder, most commonly insomnia, which was
516 comparatively higher than the general population. The review also reports that in terms of sleep
517 architecture, people with chronic pain appear to spend more time in NREM N1 and experience greater
518 sleep fragmentation than healthy controls. Mechanisms underpinning the relationship between pain
519 and sleep disruption include the physical discomfort of pain, associations between CP and sleep
520 disruption in a variety of brain-based changes, and alterations to the inflammatory response by the
521 brain (critical for sleep-wake regulation) (107). Indeed in humans, chronic pain is described as
522 comorbid with sleep disruption, recognising that pain can be both cause and consequence of sleep
523 deprivation (108) as it can reduce pain thresholds thus further enhancing the influence of pain (109).
524 In large animals, conditions that induce pain such as arthritis are suggested to prevent the animal from
525 adopting a recumbent position, resulting in reduced sleep and sleep disruption (108, 109). For example,
526 chronic joint disease preventing recumbency was associated with spontaneous collapse for captive
527 elephants (112) and abdominal pain was associated with reluctance to adopt a recumbent posture in an
528 equine case study (113). However, geriatric horses and those with orthopaedic conditions tended to
529 display a profile of movement behaviour similar to non-lame horse populations which was highest
530 when at pasture (114). Geriatric horses may not choose to be sedentary due to secondary foot pain
531 associated with excessive standing, further amplified pain in large mammals with greater body mass.
532 However little evidence exists to describe this or the influence of pain on the occurrence of equine
533 sleep specifically.

534 Chronic pain, as a stress state, is one of the critical factors associated with depression in humans, and
535 the coexistence of these disorders tends to further aggravate severity of both for the patient (115). Some
536 human sleep disturbances (insomnia or hypersomnia) have been linked to states of depression (116),
537 often compounded by the experience that all efforts to initiate sleep are unsuccessful leading to ‘learned
538 helplessness’ and a further state of depression (117). Depressive patients have been reported to exhibit
539 reductions in sleep efficiency, shorter REM sleep periods (and latency), and increases in the number
540 of awakenings (118). In animals, there is evidence that sleep deprivation contributes to the development
541 of depression or anxiety-like symptoms and produces states of physiological stress (119, 120, 121).
542 Depressive-like forms of waking inactivity have been reported for horses and rodents in non-enriched
543 housing, suggested as an alternative to stereotypic behavior (122). Horses observed in their usual
544 domestic environment displayed behaviours including a stretched neck accompanied by an unusual
545 gaze, head and ear fixity, and indifference to environmental (tactile and visual) stimuli, which were
546 likened to symptoms of “depressive syndrome” (123). This atypical posture differs to “standing rest”
547 where comparatively the horse’s neck is rounder and the eyes are at least partly closed (124). Little is
548 known about the relationship between equine models of depression and sleep patterns, however horses
549 displaying established stereotypic behaviour are reported to display different nocturnal activity
550 profiles. For example, crib-biting is usually observed within every hour of the observed nocturnal
551 profile, whilst weaving horses tend to display a large peak in activity usually in anticipation of the
552 morning feed ration (125). Recumbent behaviours of stereotypic horses are reduced compared to non-
553 stereotypic horses (125, 126), suggestive of sleep deprioritization or differing sleep strategies compared
554 to non-stereotypic animals.

555 Beyond comfort, perceived safety within the environment also influences sleep. For example, human
556 sleep is sensitive to a novel environment and stimuli, described by the ‘first night effect’ (FNE) and
557 this is also observed in dogs (127). However, humans have the capacity in most instances to modify
558 the stress-inducing factors within the environment which is often in stark contrast to domesticated
559 mammalian species that lack a level of control over factors within their environment. For example,

560 moving horses from a period of turnout to overnight stabling has been shown to affect their daytime
561 behavioural profile (128) whilst nocturnal recumbency significantly increased six weeks after horses
562 were brought into an overnight stabling management regime from a period of overnight turnout (129).
563 Seminal EEG data states that horses require a period of acclimatisation to novel environments (40),
564 observed as greater levels of vigilance displayed when horses are initially stabled after a period of
565 turnout (128, 129). It seems that stabling in isolation removes the aspect of shared safety through group
566 vigilance during turnout, whilst presenting a wealth of novel (auditory and other) sensory stimuli.

567

568 In animals, the nature of the psychological stressor becomes an important factor relative to its effect
569 on sleep. For example, in rats and mice, the occurrence of sleep after stress appears to be highly
570 influenced by situational variables including whether the stressor was controllable and/or predictable,
571 whether the individual had the possibility to learn and adapt, and by the relative resilience and
572 vulnerability of the individual experiencing stress (130). In this respect, deeper or longer NREM sleep
573 reportedly follows acute social stress (131, 132), whilst stress experienced in response to restraint is
574 followed by a selective increase in REM sleep (87, 133). Similar findings have been reported in canine
575 studies where stress-inducing experiences resulted in increased sleep (134). The increase in sleep states
576 reported in animals post-stress contrasts with sleep reductions often observed in humans, where stress-
577 based memories of past events as well as worries and expectations can disrupt and reduce human sleep.
578 In that respect, compared to some animals, the human brain has the capacity to turn a single acute
579 stressor or previous life event, or even one situated in the future, into a persistent and chronic stress
580 state (130). Other psychological stressors in humans have also been reported to reduce the quality of
581 sleep through increased levels of sleep fragmentation (135). Sleep deprivation can also further sensitize
582 the individual to stressful stimuli and events (136) thus further compounding the problem. In horses,
583 changing the animal's sleep environment from pasture-kept groups to single housed stabling has been
584 reported to significantly reduce total sleep time (129). This suggests that the psychological stressor of
585 changing the social environment can have a significant impact on sleep in the horse. Again, further
586 work identifying the exact aspects of psychological stress in the horse that affect equine sleep is needed.

587

588 **6.2 Environment**

589 Light is one of the most important environmental factors affecting sleep across a range of species. The
590 sleep-wake cycle is driven by a central clock, the superchiasmatic nucleus (SCN), and in most
591 mammals, by changing concentrations of melatonin due to light exposure (137). Photoentrainment of
592 sleep to circadian rhythms is usually mediated by photoreceptors that detect changes in the quantity
593 and quality of light over the 24 h dawn/dusk cycle (138). The sleep-wake cycle is vulnerable to changes
594 in the timing of circadian rhythms (phase shifting) (139) via exposure to bright light at specific points
595 during the light-dark cycle, even during sleep. Because of the increased use of artificial light within
596 society, humans tend to spend less time in the dark which has been described as influential in the shift
597 from biphasic to monophasic sleep patterns in humans (140). This includes exposure to artificial light
598 at night (ALAN), which could be considered an environmental stressor due to the fact that it has been
599 shown to disrupt the biological clock via suppression of melatonin (141). Prolonged exposure to ALAN
600 induces adverse effects on mood and productivity (142), and in laboratory rodents has been associated
601 with reduced anxiety-related behaviour including more time spent in the open (143). The latter is
602 considered a maladaptive response specifically for urban dwelling prey species (143). Changes in
603 exposure to light and associated phase shifts (waking earlier or later) are important to humans, enabling
604 them to adjust to travel across time zones or facilitating adaptation to night shift work or early
605 awakening (144). However, sleep disruption can be a biproduct of these phase shifts, for example in

606 the form of 'jet lag' (145). Domesticated horses are often housed in situations that include artificial
607 light, although the way in which this acts as a zeitgeber for equine sleep is little understood. A recent
608 study specifically examined the effect of overnight light on sleep behaviour in horses and reported a
609 significant reduction in sternal recumbency linked with the REM sleep state (56). Interestingly
610 breeding mares are routinely exposed to artificial light to manipulate the breeding cycle in the northern
611 hemisphere horse racing industry (146) but very little is known about the impact of this procedure on
612 quality of sleep for those animals. The use of red light at night has been advocated for use within equine
613 husbandry as a means to minimize circadian disruption (147). Competition horses are regularly
614 travelled internationally although little is known about the effects of changing time zones and rates of
615 adaptation relative to sleep and performance.

616 Non-photic zeitgebers for sleep include physical and social activity. Experimental studies on the effects
617 of exercise for human sleep patterns have described increased total sleep time (TST), prolonged REM
618 latency, decreasing REM sleep and increasing SWS sleep (148). Physically-active individuals also
619 report less daytime tiredness, better subjective sleep and fewer sleep problems than sedentary
620 individuals (149, 150). The effects of physical exercise on sleep are known to depend upon the time
621 the exercise is performed (151), fitness and the intensity of the exercise (152), and other exogenous
622 and endogenous factors linked to the general well-being of the participant (153). Daily routines and
623 social rhythms are also linked to good human sleep, for example, self-reported good sleepers have
624 more daily activities, earlier daily scheduling of their social rhythms, social rhythms characterized by
625 greater regularity, and are involved in more activities with active social engagement than poor sleepers
626 (154). Overall, exercise has been described as a robust zeitgeber of sleep acting via skeletal muscle
627 clocks (155) that have an important role in regulating the mammalian circadian system generally (156).
628 In horses, groups of animals will demonstrate both rest and locomotory synchrony (157, 158) and this
629 can be significantly affected by stabling and social conditions. For example, horses at pasture
630 demonstrate synchronised ultradian rhythmicity in patterns of locomotion that are much weaker when
631 the horses are stabled (159). This strongly suggests that, for the horse, there is a state of endogenous
632 circadian periodicity that acts irrespective of light and social cues (158). Research investigating the
633 circadian 24-h expression of exercise relevant genes in equine skeletal muscle has concluded that
634 metabolic muscle capacity is influenced by scheduled exercise, with significant interactions between
635 circadian time and exercise for specific muscle genes (160). On the basis of these results, it has been
636 suggested that optimal performance may be achieved when competition and scheduled training times
637 coincide (160). Little is known, however, about how this might result in phase shifts for sleeping and
638 further investigation is necessary to understand how this and overlying social factors affect the
639 occurrence of equine sleep.

640 Other non-photic entrainment factors/ zeitgebers for sleep include temperature and humidity. Sleep
641 and rest in many mammalian species are associated with a reduction in core body temperature (CBT)
642 (163), a thermoregulatory process whereby heat is redistributed from the core to the outer layer of the
643 body. The CBT rhythm is suggested to be able to entrain peripheral pacemakers around the body and
644 can affect normal sleep patterns. For example, increasing distal skin blood temperature via exercise
645 (164) is considered to be one of the factors that can reduce the latency of sleep onset (163). Sleep is
646 also highly susceptible to environmental heat, as demonstrated in rats (165), and in cows heat stress is
647 known to reduce lying time that can subsequently impact on sleep levels (166). Meanwhile, recent
648 work by Yadhapalli et al (167) suggests that sleep-promoting circadian clock neurons are inhibited by
649 heating and excited by cooling in *Drosophila melanogaster* and evidence that these neurons are
650 continuously integrating temperature changes to coordinate the timing of sleep and activity. Horses
651 tend to sleep less during higher (57) and longer during lower (168) ambient temperature when
652 theoretically core heat redistribution is harder and easier respectively. However, this also tends to
653 coincide with changes in daylight season and thus it is difficult to identify the predominating sleep

654 affecting factor. According to Duncan (1985) (169), free-living Camargue horses adopt recumbent
655 positions more so in spring with a higher prevalence of standing alert and walking during the summer
656 although these behavioural patterns are also thought to be driven by availability of forage (specifically
657 crude protein) and the presence of biting flies. During the autumn and winter months these horses also
658 increased the proportion of time spent resting whilst standing but with decreased time spent in a
659 recumbent posture, correlating with low ambient temperatures and increased rain (169). All in all,
660 equine sleep-related behavioural patterns appear closely associated with environmental seasonal
661 fluctuations with temperature being an important factor in this respect. However, limited research
662 exists specifically on the direct effects of ambient temperature on equine sleep and CBT, which is of
663 particular interest since many horses have their coats clipped, are fitted with rugs and experience
664 different climates due to international travel.

665 Non-zeitgeber environmental factors affecting sleep also pertain to whether the environment is safe
666 and comfortable and facilitates species-specific sleep postures. Humans commonly sleep in preferred
667 sleep sites (e.g., bedrooms) on surfaces designed to provide support and comfort during recumbency
668 (e.g., mattresses) and these sites/surfaces may be shared. Many aspects of sleep quality can be affected
669 by perceived (dis)comfort of the sleeping surface (e.g., 170), but also by the presence of co-sleepers
670 (e.g., 171). In addition, the relative merits of different human sleep postures (prone, supine and lateral)
671 are also discussed relative to sleep quality (e.g., 172). Some animal species sleep only at specific sites
672 (173), others may utilise multiple sites with specific characteristics. In non-human primates, Anderson
673 (1998) (174) identified influential factors associated with comfort and the selection of sleep sites, these
674 included thermal comfort, noise reduction and postural demands during sleep. In cows, management
675 factors such bedding type (175) and stall design (176), can significantly influence lying time which is
676 known to impact on the sleep quantity (177). The domesticated horse often has access to pasture at
677 night offering a larger area from which to select sleeping sites although little is known about preferred
678 sites and their influence on sleep quantity and quality. Anecdotally, horses that are stabled overnight
679 are reported to display recumbency in a preferred area of the stable, which is often different to standing
680 sleep sites. Significantly longer bouts of recumbency have been reported for horses kept in stables with
681 larger surface areas, suggesting that this factor influences motivation to adopt recumbent positions
682 (178, 179). In this respect, a larger surface area might facilitate manoeuvrability which is essential to
683 achieve recumbent positioning and critically important to enable the horse to effectively achieve REM
684 sleep. Within the stable, the characteristics of the sleep surface have also been shown to influence
685 sleep-related behaviour. For example, straw as a bedding substrate is consistently associated with
686 higher proportions of recumbency as part of the nocturnal time budget when compared to wood
687 shavings (60, 180, 181), and when compared to other bedding substrates including peat moss/ shavings
688 mix and crushed wood pellet (62). The depth of the bedding substrate used within the stable is also
689 noted to have a significant effect on nocturnal behaviour, where lower depths (<10cm) of bedding
690 appear to significantly reduce the occurrence of recumbent behaviour regardless of bedding substrate
691 (57, 56).

692 The level of stimulation from the environment may also influence sleep due to varying levels of arousal
693 and alertness. In humans, an example of a hyper-stimulating environment would be an Intensive Care
694 Unit (ICU), with evidence of very poor sleep occurring during stays in ICU due to noise, critical illness
695 itself, and treatment events throughout the day and night (182). Noise is generally accepted as
696 unwanted audible acoustic phenomenon (183). The human auditory system continues to scan, evaluate
697 and react to environmental sounds even whilst asleep where more meaningful noise events are more
698 likely to cause arousals from sleep than less meaningful events (184). The depth of the sleep phase,
699 background noise level and individual characteristics affecting sensitivity to noise are known to
700 determine whether or not noise will disturb sleep (185, 186, 187). Other factors include the type of

701 noise (e.g., continuous, intermittent, impulsive), noise intensity, noise frequency, noise spectrum, and
702 noise interval (e.g., duration, regularity, expected) (183). It is currently unclear how many additional
703 noise-induced awakenings are acceptable/ without consequences for sleep recuperation and health,
704 especially given the large inter-individual differences in susceptibility to noise. Prey species typically
705 tend to remain vigilant for the rest of the night, even after initial adaptation to the nocturnal
706 environment, following one awakening elicited by a spontaneous/ startling stimulus (3). However,
707 continuous auditory stimulation provided overnight (e.g., music) can have a masking and relaxing
708 effect in animals (61, 188). For example, in horses, overnight music appeared to facilitate increased
709 displays of biologically significant behaviours including lateral recumbency and the behavioural
710 benefits continued beyond the enrichment period (61).

711 Conversely, hypo or low levels of stimulation can also have a dramatic impact on sleep. Low levels
712 of stimulation (often connotated with boredom (189) leads to lethargy and mental fatigue that may
713 result in the animal sleeping earlier than usual or resting more, as the environment offers no
714 opportunities to keep them awake or tire them out (189, 190). In this sense, increased total sleep time
715 is not always an indicator of positive welfare. Several studies (50, 149, 191) have shown that sleep
716 quality is related to daily activity level, such that poor sleep quality arises from inactivity or proneness
717 toward sedentary lifestyles. Horses displaying depressive-like forms of waking inactivity may be
718 mistakenly observed as standing at rest or standing asleep due to the general similarities in the
719 behavioural ethogram (123). However, they may in fact not be achieving species-specific optimal sleep
720 due to the hypo-stimulating environment.

721 In summary, whilst most horse management systems seek to provide optimal husbandry conditions,
722 the domestic stable environment potentially creates a number of challenges from a sleep
723 quantity/quality perspective. Whilst some research has been carried out on the impact of some of these
724 factors (e.g., bedding and light) and how sleep can be improved in the stable environment (e.g., music),
725 much more research is needed to further investigate these and other factors (e.g., exercise, social
726 contact, changing environments and perceived threat) as well as sleep outside of the stable environment
727 (e.g., at pasture). **The impact of regular environmental changes for competition horses, travelling
728 nationally and internationally, also needs due consideration.**

729

730 **7. Discussion and Future Directions**

731 Understanding the evolutionary function of sleep has been widely regarded as one of the greatest
732 challenges for ethological research. Researchers have identified variation in sleep duration in a range
733 of species, with some suggested factors linked to the major forces driving the occurrence of sleep,
734 including risk of predation (the sleep exposure index), gestation period and neonatal body mass, body
735 mass, encephalization, and basal metabolic rate. One of the primary aims of this review was to
736 establish, through a review of the literature, a detailed profile of normal equine sleep. We provided a
737 summary table of all equine studies to establish both normal sleep quality and quantity that will be a
738 useful reference tool for establishing baseline levels of quantitative and qualitative metrics of horse
739 sleep. The table, however, also highlighted that the majority of studies commonly reported total sleep
740 time and that there were a limited number of studies that measured a) the different stages of sleep and
741 b) sleep across the 24hour period. We recommend that future studies should focus on determining
742 what 'normal' equine sleep is, through 24hour sleep profiles that describe the duration and frequency
743 of NREM/ REM cycles, or sequences of wakefulness, which would yield novel information providing
744 a deeper understanding of equine sleep quantity and quality. In addition, to better understand levels of

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745 variation between horses, more research is needed into the effects of variables such as age, sex and
746 breed on the different measurements of equine sleep.

747 Technologically, there is now an opportunity to improve the accuracy of sleep quantification in animals
748 through mobile wireless EEG and polysomnography (PSG) equipment. This will also greatly increase
749 the level of equine EEG sleep data that, to date, has come from a limited number of sources over limited
750 observation periods with a lack of precision measurements of sleep quality (e.g., NREM/REM cycles,
751 wake sequences). Further EEG studies that monitor in close detail the changes in behaviour of the horse
752 as it transitions between the three primary sleep states also has the potential to increase the accuracy
753 of behavioural sleep analysis. In this review, we demonstrated that EEG data can be used to refine the
754 behavioural analysis of sleep through a multiple regression approach. Further EEG studies with
755 simultaneous detailed behavioural monitoring of equine sleep will further refine this multiple
756 regression methodology. Moreover, the inclusion of automated measures of behaviour (e.g., movement
757 data loggers, vision motion analysis) alongside EEG sleep data has the potential to automate animal
758 sleep scoring with high levels of accuracy. In the meantime, although behavioural measurements of
759 sleep lack the precision of EEG or PSG, these measurements are easily accessible alternatives that can
760 achieve valid measurements of sleep including sleep fragmentation, and reasonably accurate inferred
761 measures of sleep stages. The review also provided a strong rationale for developing an equine sleep
762 quality index, with a particular emphasis on assessing wake sequences/sleep fragmentation, in order to
763 better assess factors affecting sleep in the horse.

764 During the review, primary factors affecting sleep were explored under the categories of physical (pain)
765 stressors, psychological (perceived safety, social isolation, hypo-stimulation) stressors, and aspects of
766 the environment (light, bedding substrate, physical and social activity, noise and temperature and
767 humidity). It became clear that whilst there was a strong relationship between environmental stressors,
768 sleep and welfare, it was not always clear as to the direction of the relationship, for example, whilst
769 reduced sleep quantity/quality may initially be a marker of stress, it can also become a compounding
770 stressor in its own right over the longer term. In this respect, much more research is required to
771 disentangle the relationship of reduced sleep as a marker of stress versus reduced sleep acting as
772 stressor. Additional sleep-affecting factors that still need to be investigated in the horse include
773 emotional state, social environment, the influence of light on circadian control of sleep, levels of
774 exercise and nutritional factors. There also unanswered questions in relation to training and competition
775 schedules, for example, do regular exercise schedules help promote sleep and is there an optimal time
776 to exercise relative to optimising sleep? Furthermore, do animals under intense training schedules sleep
777 more than when they are not, and how might we ensure we facilitate sleep to support this relationship?
778 Little is also understood about how core body temperature acts as a cue for sleep and rest patterns,
779 especially in comparison to social rhythms of group housing or turnout. For example, is it possible that
780 clipping and rugging horses could result in a phase shift in sleeping patterns? Again, these questions
781 provide a huge opportunity to extend the currently limited field of equine sleep research.

782 In addition to assessing factors that affect equine sleep, compensatory mechanisms, that exist for short
783 term sleep reductions in a range of animal species, is not well defined in the horse. It is also not known
784 at what point sleep deprivation becomes chronic and how this impacts the welfare of the horse
785 particularly in the context of spontaneous collapse. Research has also identified that reduced sleep
786 quantity and quality affects cognitive (e.g., memory) function and motor performance in a range of
787 animal species but very limited research has been carried out in this area in the horse. Again, further
788 research into these areas will help identify the levels of sleep disturbance that the horse can tolerate
789 from both a performance and welfare perspective.

790 *The authors declare that the research was conducted in the absence of any commercial or financial*
 791 *relationships that could be construed as a potential conflict of interest.*

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1249 **Tables**

- 1250 Table 1. Comparison between sleep profiles and biological characteristics of various bi-hemispheric
1251 species, ordered by high to low sleep exposure indices (3, 20, 23 Allison and Cicchetti, 1976; Lesku et
1252 al., 2008; Campbell & Tobler, 1984)

Species	NREM (hrs/day)	REM (hrs/day)	Total sleep (hrs/day)	Brain mass (g)	Ave. Body mass (g)	Ave. proportion brain/body mass	BMR (cm ³ O ₂ h ⁻¹)	Gestation period (days)	Sleep exposure index*
Horse (<i>Equus caballus</i>)	2.98	0.67	3.85	534.0	260000	0.21%	65000.0	337.0	5
Cow (<i>Bos taurus</i>)	3.2	0.8	4.0	460.0	272000	0.17%	46240.0	280.7	5
Sheep (<i>Ovis aries</i>)	3.3	0.6	3.8	100.0	30000	0.33%	10200.0	146.3	5
Goat (<i>Capra aegagrus</i>)	4.7	0.7	3.8	115.0	29000	0.4%	6840.0	163.9	5
Pig (<i>Sus scrofa</i>)	6.4	1.9	8.4	180.0	75000	0.24%	8250.0	117.0	4
Dog (<i>Canis familiaris</i>)	7.1	1.6	10.7	70.0	14000	0.52%	-	62.0	2
Cat (<i>Felis silvestris</i>)	10.0	3.2	13.2	28.4	3260	0.87%	2314.6	63.9	1.5
House mouse (<i>Mus musculus</i>)	11.9	1.3	12.8	0.4	21	1.90%	69.7	21.2	1.33
Human	6.1	1.9	8.0	1320.0	62000	2.13%	14700.0	280.1	1

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<i>(Homo sapiens)</i>									
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1253 *Sleep exposure index concerns a measure of predation risk based on vulnerability associated with
 1254 sleep site. The index ranks relative exposure of a given species at its typical sleep quarters in the wild,
 1255 where 1 = low risk e.g. caves/ burrows and 7 = high risk e.g. open water (Lesku et al., 2008)

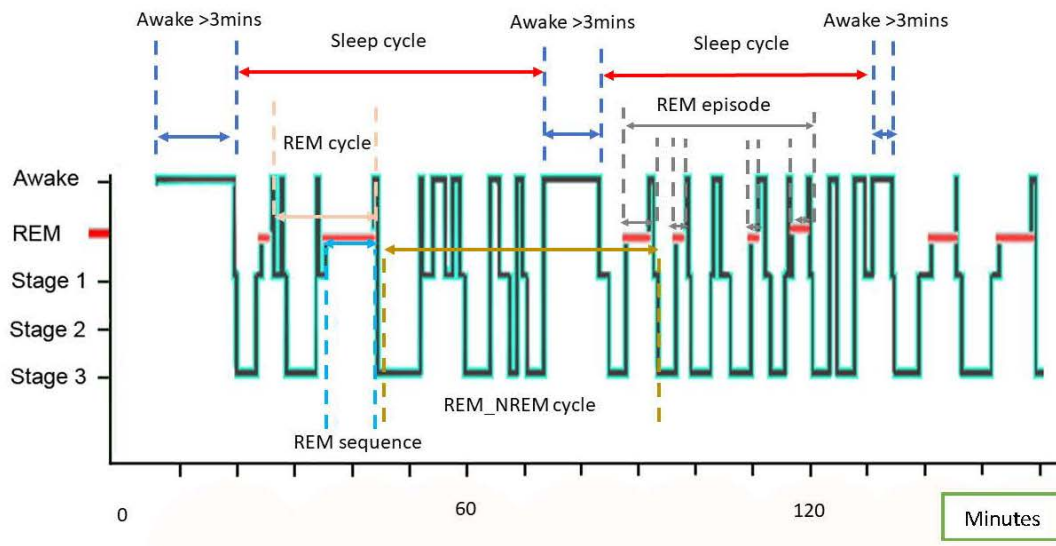
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1257 Table 2. A glossary of terms and standard definitions of sleep states (19, 45, 46) (Charles, 1980, Merica
 1258 and Gaillard 1986, Mascetti 2016) with visualization of specific terms using an example equine
 1259 hypnogram.

Term	Definition
Sleep onset	An episode of sleep lasting >1minute and containing at least 1 min of sleep other than N1 NREM (Merica and Gaillard 1986).
Sleep cycle	The interval from sleep onset (see definition above) to the start of a period of wakefulness that is greater than 3 minutes (Merica and Gaillard 1986), containing sequences of REM, NREM and wakefulness.
Epoch	A short interval of arbitrarily defined length (usually 20-60 seconds) of sleep stage normally determined from a polygraphic sleep recording (Charles, 1980).
Micro-arousal	A sudden transient elevation of the vigilance level due to arousal stimuli or to spontaneous vigilance level oscillations incorporating low-voltage fast-rhythm electroencephalographic (EEG) arousals and high-amplitude EEG bursts (Halasz et al. 2004)
Sequence	A consecutive series of epochs in the same sleep stage (Charles, 1980) e.g. A REM sequence is a series of consecutive epochs of REM sleep uninterrupted by any other sleep stage or state
Episode	A series of consecutive sequences of the same stage of sleep or the same state which may be interrupted for a short time by another sleep stage or state (Charles, 1980) e.g. REM episode is a series of consecutive sequences of REM sleep which are separated by less than 15 minutes of NREM sleep or 3 minutes of wakefulness
Sleep episode (duration)	Portion of the sleep-wake cycle from sleep onset to last epoch of sleep, which may include sequences of wakefulness (measured by the number of minutes from sleep onset to the end of the last sleep epoch)

REM-NREM cycle (length)	A general term used to describe cyclic alteration between REM and/or NREM sleep measured in units of time which must be clearly defined e.g. 'the end of one REM episode to the end of the next REM episode' and whether the cycle analyzed began with REM or NREM.
Somnolence	A state of desire for sleeping/ being drowsy/ ready to fall asleep.
Torpor	A state of decreased physiological activity usually involving reduced body temperature and metabolic rate that enables the animal to survive periods of reduced food availability.
Uni-hemispheric	Sleep is induced in only one cerebral hemisphere whilst the other remains awake, resulting in asymmetric eye closure and sleeping postures (Mascetti 2016).
Bi-hemispheric	Sleep involves both cerebral hemispheres, characterized by closure of both eyes and symmetric body muscular hypertonia or atonia.
Monophasic	Sleep occurs in one long period, usually during the night.
Polyphasic	Episodes of sleep that occur during the day and/or night.
Polysomnography	Multiple physiological measurements taken to measure sleep including Electroencephalography, Electrooculography, Electromyography, Electrocardiography, breathing frequency and body temperature.
Zeitgeber	A rhythmically occurring natural phenomenon which acts as a cue in the regulation of the body's circadian rhythms.

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Table 3. Characterising equine sleep within stabled behavioural profiles including as a percentage of Total Sleep Time (TST) and Total Observed Time (TOT) (NM = no measurement)

Study	Number of horses	Mean age (age range)	Hours recorded (from/to)	Mean total Sleep time	Mean total NREM duration	Mean total REM/lateral recumbency duration	Mean duration of NREM sequences (Mean No. Of NREM sequences/TST)	Mean duration of REM sequence (Mean No. Of REM sequences/TST)	Mean duration of NREM episode* (Mean no.) *defined as ...	Mean duration of sleep cycle (Mean no. [range])	Mean number of wake sequences** (<3min) within a sleep cycle	Mean duration wake sequence** with in a sleep cycle
(Wohr et al., 2016) (55)	7	Adult horses	7hrs at night	210.0min 50% TOT	40.0 min 65% TST 9.52% TOT	30.0min 5%TST 7.14% TOT	NM	NM	NM	NM	NM	NM
Greening et al., (2021) (55)	10	14.9 years	24hrs	311.8min 21.7% TOT	236.4min 76.1% TST	104.0min 23.9% TST	NM	NM	NM	NM	NM	NM
(Chung et al., 2018) (57)	15	Adult horses	24hrs (8am-8am)	65.0min 4.5% TOT	57.0min excl stand sleep 88% TST 4% TOT	8.0min 12% TST 0.5% TOT	NM	NM	NM	NM	NM	NM
(Dallaire and Ruckebusch, 1974b) (58)	5	(6 months to 6 yrs)	12hrs (8am-8am)	199.5min 27.2% TOT	151.5min 75.9% TST 21% TOT	48.0min 24.1% TST 6.7% TOT	6.48min	4.0min	NM	40.78min (5.5)	NM	NM

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(Dallaire and Ruckebusch, 1974a) (59)	3	(6 months to 6 yrs)	12hrs (18.30pm -6.30am)	189.3min 26.3% TOT	145.7min 77% TST 20.3% TOT	43.6min 23% TST 6.1% TOT	NM	NM	NM	NM	NM	NM
(Greening et al., 2013) (60)	10	7.3yrs	12hrs (7pm-7am)	382.0min 53% TOT	355.0min 93% TST 49% TOT	27.0min 7% TST 4% TOT	NM	NM	NM	NM	NM	NM
(Hartman and Greening, 2019) (61)	7	11.7yrs (6-16yrs)	10hrs (8.30pm-6.30am)	299.0min (scans at 2min intervals) 49.8% TOT	265.0min (scans at 2min intervals) 88.7% TST 44.1% TOT	33.8min (scans at 2min intervals) 11.3% TST 5.6% TOT	NM	NM	NM	NM	NM	NM
(Kalus, 2014) (43)	7	14.1yrs (8-20yrs)	7hrs (10.30pm -5.30am)	203.0min 51% TOT 203/420=48.3%	131.1min 65.5% TST 131.1/203=64.6%	31.3min 15.5% TST 31.3/203=15.4%	5.22min (25.88)	2.38min (7.11)	17.14min (3.04)	40.7min (2.63)	6.89	0.96 min
(Kwiatkowska-Stenzel et al., 2016) (62)	8	(4-13yrs)	48hrs (x3 12.30pm-4.30am)	321.3min 33.5% TOT	265.3min 82.6% TST 27.6% TOT	56.0min 17.4% TST 5.8% TOT	NM	NM	NM	NM	NM	NM
(Williams et al., 2008) (36)	6	(4-13yrs)	*12hrs (8am-8am)	166.4min	158.2min (95.1% TST)	8.16 (4.9%TST)	3.37 min (52)	0.91 min (14.6)	NM	NM	NM	NM

Ruckebusch (1975) (63)	4	4yrs	At night	218.0min	181.0min 83% TST	37.0min 17% TST	9.0min (18)	4.8min	NM	NM	NM	NM
Ruckebusch et al., (1970) (64)	2	unknown	12hrs (8pm-8am)	262.1min 36.4% TOT	212.42min 81% TST 29.5% TOT	49.7min 19% TST 6.9% TOT	NM	5.02 min (11.3)	NM	NM	NM	NM
(Ruckebusch, 1972) (65)	3	Adult horses	10hrs (overnight)	172.0min 28.7% TOT	125.0min 72.7% TST 20.8% TOT	47.0min 27.3% TST 7.8% TOT	NM	5.22min (9)	NM	NM	NM	NM
Mean	7 horses	10.4 years	15.83 hours	230.72min (24.3% of average TOT)	178.74min (77.5% of average TST)	40.27min (17.5% of average TST)	6.02min (31.96)	3.72min (10.5)	17.14mins	40.74min (4.1 [2-6])	6.89	0.96min
SD	3.57	4.64	1.41	82.87	87.53	24.15	4.41	1.72	NA	0.06	NA	NA
Human (Le Bon 2002) (66)	78	27.8 (5-45yrs)	7hrs 50min	433.26min 91.6% TOT	350.72min 74.1% TOT 80.9% TST	73.84min 15.6% TOT 17.04% TST	NM	NM	101.36 mins	119.7 mins (4.23 [2-6])	NM	NM
<i>Human – children</i> (Feinberg 1974) (67)	21	13.8 (11.8-16.2yrs)	NM	NM	NM	NM	77.5 min	22.7min	NM	NM	0.25	1.64min

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Human – adult (Feinberg, 1974) (67)	13	31.5 (26.2-43.3yrs)	NM	NM	NM	NM	62.0min	24.0min	NM	NM	0.77	2.75min
Human – aged (Feinberg, 1974) (67)	9	77.3 (67.4-95.8yrs)	NM	NM	NM	NM	64.4 min	20.3 min	NM	NM	0.9	11.56min
Human – old Carskadon et al., 1982) (37)	24	(63-86yrs)	48hrs (10pm-8am)	426min	355min 83.33% TST	72min 16.9% TST	NM	NM	NM	NM	NM	3.1min
Mean	29 people	37.6 years	48 hours	429.63min 25.6% TOT	352.86min 82.13% TST	72.92min 16.97% TST	67.97min	22.33min	101.36mins	119.7mins (4.23 [2-6])	0.64	4.76min
SD	28.04	27.54	NA	5.13	3.03	1.30	8.34	1.88	NA	NA	0.34	4.57

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*recording stated as 'night'

Mean total **NREM** duration = The total duration of NREM (in behavioural studies anything that is not lateral recumbency/ this relates to sternal recumbency but sometimes stand sleep, hence differences e.g. Chung and Greening et al)

Mean total **REM/lateral recumbency** duration = The total duration of REM (in behavioural studies = the duration of lateral recumbency)

Mean duration of **NREM** bout = Mean duration of bouts of NREM

Mean duration of **REM** bout (Mean no. per **NREM-REM epoch**) = Mean duration and the number of occurrences of REM within a NREM-REM sleep epoch

Mean duration of **NREM epoch** (Mean no.) = Mean duration (and number of) NREM from onset of sleep to onset of next wakeful period >3min with no occurrences of REM

Mean duration of **NREM-REM epoch** (Mean no.) = Mean duration (and number of) from sleep onset to onset of next wakeful period >3min including occurrences of REM

1277 Table 4. Behavioural markers of different EEG sleep states in the horse. Ticks (☑) indicate behavioural
 1278 states required to be in the sleep state. Crosses (☒) indicate behavioural markers that cannot occur
 1279 during the sleep state. Dashes (~) indicate behavioural states that may or may not occur during the
 1280 sleep state (72, 73, 74, 36) (Dallaire and Ruckebusch 1974, Hale and Huggins 1980, Dallaire 1986,
 1281 Williams, Aleman et al. 2008).

Stage of Sleep	Behavioural Markers								
	Leg resting	Eye lid partially shut	Ears non-vertical	Eye lid completely shut	Poll below withers	Sternally recumbent	Muzzle on the floor	Laterally recumbent	Ears or eyes twitching
NREM N1- N2	~	☑	☑	~	~	~	~	~	☒
NREM N3 (SWS)	~	☑	☑	~	☑	~	~	~	☒
REM	☒	☑	☑	~	☒	~	~	~	~

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1283 Table 5. The after effects of sleep deprivation in Human and rodent models (98, 99, 100, 101, 102, 37,
 1284 103, 104, 105, 106)

Sequalae	Species	Author
Impaired visual perception	Human	Plomhause et al., (2014) (98)
Reduced capacity to engage in tasks requiring simple sustained concentration/attention	Human	Lim & Dinges (2010) (99)
Impaired decision making including more high-risk strategies and reduced concern for negative consequences of these	Human	Kilgore et al., (2006) (100)
Impaired memory consolidation	Rodent	Graves et al., (2003) (101)

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Negative effects on vigilance and simple reaction time	Human	Herscovitch and Broughton (1981) (102)
Increased daytime sleep propensity/ micro-episodes of sleep leading to lower capabilities and efficiency of task performance and to increased number of errors.	Human	Carskadon et al., (1982) (37)
Poor memorization & schematic thinking, which yields wrong decisions (less evidence in non-human species)	Human Rodent	Harrison & Horne (2000) (103) Leenaars et al., (2020) (104)
Emotional disturbances such as deteriorated interpersonal responses and increased aggressiveness	Human	For review see Fairholme and Manber (2015) (105)
Changes to pain perception, specifically hyperalgesia	Rodent	For review see Lautenbacher et al., (2006) (106)

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1286 **Figure headings**

1287 Figure 1. Changes in duration of sleep stages with increasing age (50) (adapted from Ohayon et al.,
1288 2004)

1289 Figure 2. Hypnogram comparing sleep architecture of a typical young human adult, and elderly human
1290 adult and an adult horse over an eight hour sleep episode: A. the young adult experiences 5 REM
1291 sequences and 2 short sequences of wakefulness; B. the elderly individual experiences 8 disturbed
1292 REM sequences and multiple sequences of wakefulness accompanied by a distinct lack of stage 4 sleep;
1293 C. the horse experiences 13 disturbed REM sequences and multiple sequences of wakefulness
1294 accompanied by a distinct lack of stage 4 sleep (adapted from 43,70).

1295 Figure 3. Linear regression analysis of average duration EEG sleep states against average duration
1296 behavioural states for 7 horses over 4 nights. Data taken from Kalus (2014). (A = Standing; B = Sternal
1297 Recumbency; C = Lateral Recumbency)

1298 Figure 4. The interrelationship between factors of well-being affecting sleep and sleep affecting well-
1299 being (red arrows indicate the pathways to reduced sleep and the interplay between increased
1300 stress/reduced welfare and reduced sleep).

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