APPLIED QUANTIFICATION OF EQUINE SLEEP BEHAVIOUR AND ENVIRONMENTAL FACTORS AFFECTING ITS OCCURRENCE.

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Published work and a critical commentary submitted in partial fulfilment of the requirements of the University of the West of England, Bristol for the degree of Doctor of Philosophy by publication (DPhil)

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Paper 1: Greening, L. and McBride, S, D. (2022) A review of equine sleep; implications for equine welfare. *Frontiers in Veterinary Science: Animal Behaviour & Welfare*. 9, p.916737, doi: 10.3389/fvets.2022.916737.

Paper 2: Greening, L., Shenton, V; Wilcockson, K; Swanson, J. (2013) Investigating duration of nocturnal ingestive and sleep behaviours of horses bedded on straw versus shavings. *Journal of Veterinary Behaviour; clinical applications and research*. 8 (2), pp. 82-86, https://doi.org/10.1016/j.jveb.2012.05.003.

Paper 3: Hartman, N. & Greening, L. (2019) A preliminary study investigating the influence of auditory stimulation on the occurrence of nocturnal equine sleep related behaviour in stabled horses. *Journal of Equine Veterinary Science*. 82, p. 102782, https://doi.org/10.1016/j.jevs.2019.07.003.

Paper 4: Greening, L., Downing, J, T., Amiouny, D., Lekang, L. & McBride, S. (2021) The Effect of Altering Routine Husbandry Factors on Sleep Duration and Memory Consolidation in the Horse. *Applied Animal Behaviour Science*. 236, p. 105229, https://doi.org/10.1016/j.applanim.2021.105229.

Paper 5: Greening, L. Allen, S. and McBride, S, D. (*IN PRESS*) Towards an objective measurement of sleep quality in non-human animals; using the horse as a model species for the creation of sleep quality indices. *Biology Open*.

I confirm that no parts of the submission have been submitted for another academic award.

CONFIRMATION OF TRAINING

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And finally, I dedicate this body of work to my wonderfully clever, funny and beautiful daughter, Daisy, as an example of what can be achieved through dedication and hard work. Smash those glass ceilings and rise on high!

ABSTRACT

This thesis includes published academic work with a critical commentary submitted to fulfil the requirements of the University of the West of England, Bristol for the degree of Doctor of Philosophy by publication (DPhil). Five peer reviewed journal articles are submitted (appendix 1), alongside a number of studies that have been presented at conference but remain unpublished (appendix 2), spanning ten years of research into the topic of equine sleep behaviour from 2013 to 2023. Paper 1 offers an overview of existing research in the field of equine sleep and proposes two novel measurements. Paper 2 demonstrates no significant differences were detected when comparing sleep behaviour on two different bedding substrates, whilst Papers 4 and 5 demonstrate how bedding depth and artificial lighting can have a significant effect on quantity and quality of sleep behaviour respectively. Meanwhile Paper 3 provides evidence that playing music overnight can influence the nocturnal behavioural profile. Evidence from these studies highlights how equine sleep is sensitive to factors within the environment, based on equine management practices. The critical commentary aims to position these studies to show case what is currently known about equine sleep and the factors which influence it within the domestic environment, whilst recognising limitations and offering opportunities for further research in this field. To support the development of this field of research and attract funding to support this work in the future, effort must be made to ensure measurements are reliable and accurate in order to enable greater exploration of the relationship between sleep, welfare and performance.

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GLOSSARY OF TERMS AND ABBREVIATIONS

AWIN = Animal Welfare Indicators

BORIS = Behavioural Observation Research Interactive Software

CCTV = Closed Circuit Television

DEFRA = Department for Food and Rural Affairs

EEG = Electroencephalography

Lateral recumbency = Subject is lying on its side (either lateral thoracic area parallel to and in contact with the ground), legs extended, head is on the floor and immobile other than for ear/eye movement (Plate 1).

Plate 1. Lateral recumbency (Authors own)

NREM = Non-Rapid Eye Movement (sleep)

N1-3 = stages of NREM sleep state

PSG = Polysomnography

Recumbency = lying down

REM = Rapid Eye Movement (sleep)

Standing sleep = Subject is standing immobile, with the altanto-optical (poll) below the withers (with or without a resting hind limb) (Plate 2).

Plate 2. Standing sleep (Authors own)

SQI = Sleep quality index

Sternal recumbency = Subject is lying with the sternum in contact with the ground and legs folded beneath the body, the muzzle may or may not be in contact with the ground (Plate 3).

Plate 3. Sternal recumbency (from Greening et al., 2021; Paper 4)

SWA = Slow wave activity (within NREM sleep)

SWS = Slow wave sleep

CHAPTER ONE

Aims of the critical commentary

The critical commentary aims to position the studies appearing within the bibliography and Appendix 1 to show case what is currently known about equine sleep and the factors which influence it within the domestic environment, whilst recognising limitations and offering opportunities for further research in this field. Table one demonstrates how, subsequently, the critical commentary and the body of work presented here for assessment demonstrate the skills requirements of the UWE Doctoral Descriptors.

Table 1. Meeting the UWE Doctoral Descriptors.

Doctoral Descriptors	**Chapters**	**Papers**
Has conducted enquiry leading to the creation and interpretation of new knowledge through original research or other advanced scholarship, shown by satisfying scholarly review by accomplished and recognised scholars in the field;	1 to 4	1 to 5
Has demonstrated a critical understanding of the current state of knowledge in the field of equine sleep research;	2 to 5	1, 4 & 5
Has shown the ability to conceptualise, design and implement a project for the generation of new knowledge at the forefront of the discipline or field of practice including the capacity to adjust the project design in the light of emergent issues and understandings;	3 & 4 (Appendix 2)	1 to 5
Has demonstrated a critical understanding of the methodology of enquiry;	3 & 4	1 & 5
Has developed independent judgement of issues and ideas in the field of research and / or practice and is able to communicate and justify that judgement to appropriate audiences;	2 to 5 (Appendix 2)	1 to 5
Has critically reflected on their work and evaluated its strengths and weaknesses including understanding validation procedures	2, 3 & 4	1 to 5

Sleep is described as a vital and cyclic mechanism (Lefter et al. 2022) and ubiquitous to all mammalian species (Campbell & Tobler, 1984; Siegel, 2008), although relatively little is known about equine sleep compared to similar herbivorous ungulates. Sleep has multiple functions that are beneficial to health and welfare. For example, in the US, the majority of older adults presenting with sleep disturbances also describe at least one major mental or physical disorder (Foley et al. 2004). The research on animal models of sleep disorders provides a good basis for clinical diagnosis and treatment of depression (Li et al. 2022) highlighting biological similarities between mammals and how sleep is broadly experienced across species (Rattenborg et al. 2017). Increasing evidence shows how sleep problems not only commonly co-occur with other morbidities, but also play a causal role in the development of these condition (Hale, Troxel and Buysse, 2020). Rather than a distinct entity, sleep has been described as "manifestation of the process of metaregulation, which enables efficient moment-to-moment integration between internal and external factors, preceding history and current homeostatic needs" (Vyazovskiy, 2015). According to our knowledge about sleep therefore, the importance of it in maintaining general wellbeing appears critical. However, the importance of sleep in human wellbeing has only recently been fully acknowledged (Espie, 2022), with a paucity of research available on animals kept in captivity, despite the survival-critical nature of sleep behaviour.

Investigating how to provide environments that promote sleep for stabled horses could therefore be considered a proactive step towards providing opportunities to meet the needs of these animals relative to the environment within which it is kept. Currently, little industry guidance is available for horse care givers on the topic of horse sleep, despite the many environmental factors that exist which are largely beyond the control of the horse. The discussion around the studies presented within this critical commentary therefore aims to expand knowledge and understanding of equine sleep, and offer insights for the industry and horse care givers to optimise husbandry practices, looking at environmental factors which influence sleep. Discussions around measuring sleep are also presented offering recommendations and ideas for future research. Specific objectives included:

- Conducting a comparison of equine sleep profiles against other mammalian species;
- Highlighting key features of equine sleep to evidence what we know and where opportunities for further research exist;
- Exploring how changes to sleep could impact upon welfare;
- Determining how core environmental factors influence sleep, specifically lighting and bedding;
- Propose a novel sleep quality metric to investigate nuances observed within equine sleep;
- Use critical awareness and experience gained through research to make recommendations for future research in this field, and;
- Use findings to make preliminary recommendations about equine husbandry and management.

CHAPTER TWO

Chapter two introduces the functional importance of animal sleep to demonstrate a critical understanding of the current state of knowledge. The chapter helps to demonstrate effective communication and justification of independent judgement of issues and ideas in the field of equine sleep research. The inclusion of reference to research from the body of work to be assessed evidences the conduct of enquiry that lead to the creation and interpretation of new knowledge through original research, shown by satisfying scholarly review of accomplished and recognised scholars in the field.

2.1 Introducing the importance of sleep

Sleep can be described as consolidated periods of resting behaviour, further defined (see Tobler, 1995) as:

- Occurring in a specific sleeping site;
- Individuals adopting a typical body posture;
- Individuals engaging in physical quiescence;
- Individuals demonstrating an elevated arousal threshold;
- Being a rapidly reversible state; and
- Eliciting a rebound effect when not enough sleep is achieved.

There is evidence of the occurrence of sleep states across a range of mammalian species (Campbell & Tobler, 1984; Tougeron and Abram, 2017), some nematodes (e.g. *Caernorhabditis elegans;* Raizen et al., 2008) and arthropods (e.g. *Drosophilia melanogaster*, Shafer and Keene, 2021), with species-specific differences. For example, slow wave brain-wave activity occurs uni-hemispherically in cetaceans without evidence of the occurrence of rapid eye movement sleep (Lyamin et al., 2004). Where it is less practical to measure brain wave activity to infer sleep, behaviour is used as a proxy measure following the definition of Tobler (1995). Sleep-like states have been reported in crayfish, octopuses, some frogs, green iguanas, and box turtles (Jha & Jha, 2020). Meanwhile, elephants in the free-living environment have been observed to go without sleep for up to 46 hours (Gravett et al. 2017). The evidence behind the idea that some animal species, including bullfrogs, dolphins and honey bees, do not sleep is strongly contested on methodological grounds (Cirelli and Tononi, 2008), thus the occurrence of sleep or sleep states across different taxa suggests a universal need for it. This thesis will only focus on mammalian sleep; as regulated by a circadian process linked to the light-dark cycle, and homeostatic processes that regulate the need for sleep against wakefulness (Borbély and Achermann, 1999).

Mammalian sleep typically occurs in two different states; rapid eye movement (REM) and non-rapid eye movement sleep (NREM) of which the latter is further divided into three stages from light to deep sleep. The quality and quantity of sleep can have a number of consequences (Lefter et al. 2022). For example, sleep has been described as a state that optimizes memory consolidation, compared to the waking state which is optimized for encoding of memories (Rasch and Born, 2013). Deep sleep, described as NREM stage 3, is essential to enable neuroplasticity implicated in learning through consolidation of declarative (long term) and procedural (skills development) memory (Fattinger et al. 2017; Gorgoni et al. 2013). REM sleep deprivation can affect an individual's ability to complete a complex task and increased REM sleep is often observed following a learning task (Rasch and Born, 2013). More recent research has shown that even a brief period of waking rest facilitates memory consolidation for humans similar to the benefits seen from sleep (Tucker et al., 2020). This perhaps is very similar to the phase of drowsiness that some domestic ungulate species display (Ruckebusch, 1972), and may account for the comparatively low levels of REM sleep these species achieve within 24 hours. However, little evidence of the influence of sleep on memory consolidation and learning currently exists for a wide variety of animal species when compared to human and rodent studies.

Physiologically, a bidirectional relationship exists between sleep and both the immune system, and emotional regulation (Besedovsky et al. 2019; Vandekerckhove and Wang, 2018). Sleep also modulates neuroprotection of the brain by facilitating filtering of cerebrospinal fluid to remove waste, and during the sleep state optimal levels of noradrenaline are maintained (Mehta et al. 2002). Fear responses in prey-species usually involve noradrenaline and numerous animal studies highlight how different experimental stress models effect sleep in complex ways (Sanford et al., 2013). However, it would be interesting to understand the influence of fear-eliciting stimuli in terms of subsequent sleep behaviour such as performance/trainability for working animals such as the horse. Sleep is also positioned to serve a pivotal function in removal of metabolic waste products from the central nervous system (CNS) via the glymphatic system, however the basic mechanisms driving CSF flow during sleep remain to be elucidated (Hauglund et al. 2020).

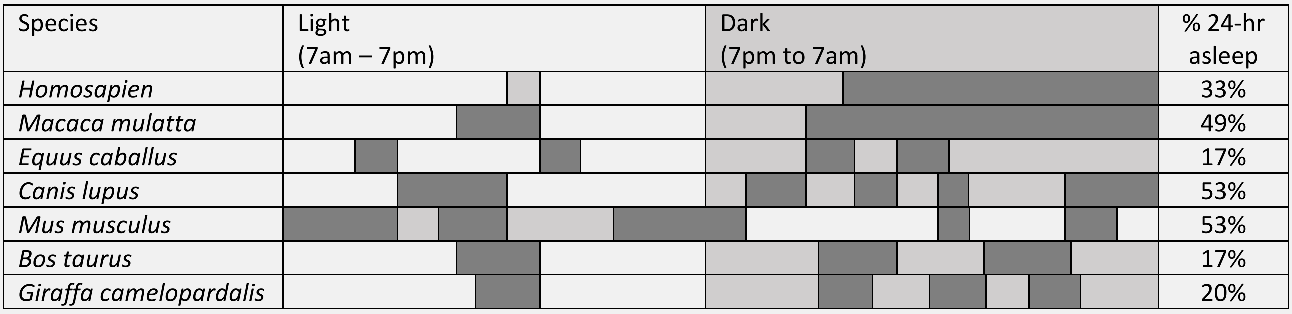
Sleep therefore appears to be a functional behaviour. Although little resolute evidence exists proving the occurrence of sleep outweighs the beneficial outcomes of waking behaviours such as nutritional fulfilment, protection, and successful reproduction (Krueger et al. 2016), the motivation for sleep nonetheless takes priority over biologically significant behaviours. Sleep also occurs despite the reduced responsiveness to potential threat. Thus, it is agreed that sleep has an adaptive purpose (Rasch and Born, 2013) and performs functions for the brain that are incompatible with a state of wakefulness (Rattenborg et al. 2017).

Sleep deprivation involves a reduction in total sleep time induced by a preventative factor e.g. constant handling in rodents (e.g. Lemons et al. 2018). Sleep deprivation in humans is associated with mood disruption (Zhao et al. 2019) and increased risk of obesity (Kim et al. 2015). Prolonged periods of complete and chronic sleep deprivation in rats has been shown to result in death (Everson et al. 1989). Sleep disruption on the other-hand is described by interruptions or awakenings during sleep episodes that can also reduce total sleep time (Gorgni et al. 2013) elicited by a range of factors, for example novel auditory stimuli in the sleep environment (Wells and Irwin, 2008). A number of negative consequences have been associated with sleep disruption for example, long term consequences in humans include the occurrence of hypertension, dyslipidemia, cardiovascular disease, weight-related issues, metabolic syndrome, type 2 diabetes mellitus, and colorectal cancer (Medic et al. 2017). Disruption to sleep typically results in reports of poor sleep which is usually described subjectively in terms of quality by humans (Harvey et al. 2008). Objective quality measures are less common but important as sleep quantity does not always equate to sleep quality. For example, there is a mechanism that facilitates higher quality sleep, identified as intense slow wave activity (SWA) that occurs within the brain during early onset NREM sleep (Borbély, 1982), which enables sleepers to engage in deeper and more intense sleep more quickly following sleep onset. This may enable the sleeper to achieve good quality sleep in a relatively low total sleep time. In addition, a range of genes affect different components of sleep, including sleep duration, have been identified (Webb and Fu, 2021) such as a mutation in DEC2 gene which leads to familial natural short sleep behaviour trait (Hirano et al. 2018). People with the mutation are described as elite sleepers as they able to function effectively during the day after less than the recommended eight hours of sleep (Dong et al. 2022), and in this situation low sleep would not equate to poor quality sleep. These findings are largely based on studies of humans and rodents, and there is little understanding of whether these mutations could exist as wild-type for large prey species of animal such as the horse.

Whilst an increased understanding of the genetic mechanisms of sleep will help to uncover the underlying mechanisms of sleep regulation (Webb and Fu, 2021), the increasing awareness of the biological significance of sleep to optimize human health (Czeisler, 2013; Medic et al. 2022) has resulted in the elevation of sleep in recent years to the same level as nutrition and exercise. Sleep is now described as one of three key pillars of human health (Quan et al. 2008). The focus on good sleep hygiene aims to avoid changes in sleep away from the optimal, which can influence the response thresholds of emotional systems that influence how an individual perceives its environment (Coria-Avila et al. 2022). The growing awareness of the need for good sleep hygiene was first promoted by Dr Nathaniel Kelitman in 1939 (Espie, 2022). More recently in the UK, five principles of good sleep health were developed to encourage people to Value, Prioritise, Personalise, Trust, and Protect their sleep (Espie, 2022). These principles are intended to educate the public about sleep health and to support evidence-based self-management of sleep, developed as part of the '*Every mind matters*' campaign run by the National Health Service in the UK (Mental Health Foundation, 2020). Such an awareness has yet to fully filter down into approaches to animal management in the domestic environment. Good sleep hygiene is described by optimising night time routines and sleep environments, although for a variety of domestic animal species these elements are largely overlooked beyond an anthropomorphic understanding of sleep (for example in dogs, Fomina et al., 2023). Furthermore, in a recent review of conventional laboratory rodent housing, conditions were considered largely inadequate to meet behavioural needs, resulting in poor welfare indicators such as impaired sleep, and were deemed to influence the outcomes of the studies that the rodents were taking part in (Cait et al. 2022). It is likely that the welfare of domestic animals could be improved through consideration of their managed environment relative to animal sleep hygiene and their species-specific sleep needs.

Human sleep differs from other mammalian species; for example, humans confidently engage in consecutive hours of unconsciousness during sleep, whilst in contrast most prey species, including the horse, need to maintain a level of alertness throughout a 24-hour period (Lima et al. 2005). To further facilitate efficiency of sleeping, humans in some cultures are largely monophasic sleepers compared to the polyphasic sleep patterns exhibited in most mammalian species (Table 2) (Zepelin et al. 2005). The effects of reduced human sleep (quantity and quality) have been reported for non-human mammalian species (Greening and McBride, 2022; Appendix 1, Paper 1), likely because mammalian neurobiological systems important for ensuring optimal wellbeing are also involved in controlling and inducing sleep through homeostatic processes and circadian rhythms (Motamedi-Fakhr et al., 2014). However, it is important to remember that the majority of research into animal sleep has been conducted under artificial conditions such as laboratory housing or in zoos, with or without the attachment of sleep measuring equipment such as accelerometers, due to a number of the issues with studying free-ranging animals for example accessing sleeping sites to accurately observe sleep behaviour (e.g. Reinhardt, 2020). The occurrence of normal or natural behaviour as observed by free-living counterparts is often used as a benchmark in welfare assessments of animals in the captive/domestic environment (Greening, 2019). Thus, the lack of information about sleep behaviour of animals in free-living environments may hamper human attempts at facilitating sleep for those animals living in captive/domestic environment.

Table 2. A representative and comparative schematic of sleep profiles for different species, including the proportion of total sleep time, over a 24-hr period (developed from Campbell & Tobler, 1984; Carskadon & Dement, 2011; Hsieh et al., 2008; Kalus, 2014; Takeuchi & Harada, 2002; Tobler and Schwierin, 1996; Wang et al., 2020). Dark segments indicate periods of sleep. Light grey segments indicate likely occurrence of sleep. White segments indicate wakefulness.



Species	Light (7am – 7pm)	Dark (7pm to 7am)	% 24-hr asleep
Homosapien	☐ ▨ ☐	▨ ■	33%
Macaca mulatta	☐ ■ ☐	▨ ■	49%
Equus caballus	☐ ■ ☐ ■ ☐	▨ ■ ▨ ■ ▨	17%
Canis lupus	☐ ■ ☐	▨ ■ ▨ ■ ▨ ■ ▨ ■	53%
Mus musculus	■ ▨ ■ ▨ ■	■ ☐ ■ ☐ ■ ☐	53%
Bos taurus	☐ ■ ☐	▨ ■ ▨ ■ ▨	17%
Giraffa camelopardalis	☐ ■ ☐	▨ ■ ▨ ■ ▨ ■ ▨	20%

Competition between biologically significant systems exists, to maintain homeostasis whilst responding to increasing homeostatic pressure to sleep. For example, not sleeping could potentially enhance welfare by satiating hunger motivation via ingestion behaviour. However, poor sleep quality in humans has been found to result in greater hunger/higher disinhibited eating, especially in relation to palatable foods (Blumfield et al. 2018). Changes in motivational states related to palatable food intake can in turn mediate sleep quality due to changes in eating behaviour (Binks et al. 2020; Mendoza et al. 2010). Thus, whilst poor sleep can result in a diminished welfare state, changes to sleep behaviour can further perpetuate a state of poor welfare (Kalirathinam et al. 2019; Greening & McBride, 2022). A complex interplay therefore clearly exists between sleep and welfare.

2.2 Sleep in relation to animal welfare

The term animal welfare can be broad in its application, but largely considers normal biological functioning, the emotional state of an animal and its ability to express normal behaviour (WOAH, 2008). The definition can be further simplified to the notion of 1) good health and 2) animals having what they want (Stamp Dawkins, 2021). The field of animal welfare science provides evidence through research that informs ethical decisions around the roles that animals play in human society, helping to ensure humans acknowledge their ethical responsibilities to captive and free-living animals (Appleby et al. 2011). Legislation aims to ensure minimum requirements for the care of animals in order to avoid poor welfare (Mellor et al. 2020), and this tends to align with the Five Freedoms developed as part of the Brambell Report (1965), which is today recognised by the World Organisation for Animal Health (OIE, 2019). Sleep has been described as an instinctive behaviour (Roth and Roehrs, 2000), due to chemical, circadian and homeostatic regulation. Individual animals will therefore engage in sleep as and when the body requires it, and the environment permits it. Human caregivers therefore have a duty of care to provide an environment that is conducive to sleep to maintain optimal welfare states. This is reflected in the Five Freedoms with reference to the freedom from discomfort that describes how animal housing should facilitate rest. However, sleep is not explicitly considered within the freedom to express normal behaviour. The Five Freedoms inspired the formulation of the Five Domains Model (Mellor and Reid, 1994), that aimed to facilitate systematic and structured approaches to the assessment of animal welfare, and facilitate consideration of how the affective state is influenced by both sensory input and cognitive assessment. The fifth domain, 'Mental State' maintains consideration of evidence following assessment of the physical/functional domains to infer associated subjective or affective experiences. The model can be used to determine the extent to which a balance of positive and negative experiences have been achieved, such that the animal has a 'life worth living' (Mellor, 2016), as the domestic environment can present barriers that prevent an animal from indulging in satiation of self-directed goals. This is particularly important where the animal is experiencing aversive conditions that are unavoidable and within which the animal cannot take the required remedial action themselves. However, the absence of discomfort or aversive experiences could make the environment hypo-stimulating rather than positive (Mellor et al. 2020). The Five Domains model discusses survival-critical negative affects, generated by sensory inputs that register imbalance within the internal functional state of the animal, aligning with "genetically embedded mechanisms linked to the survival of the animal" (Mellor et al. 2020). That is to say, the environment should present some form of challenge and evoke negative affective states as these motivate behaviours that provide opportunities for animals to have positive experiences (Mellor, 2017). Positive affect is experienced through different forms of comfort (Mellor, 2016) and for sleep this has been described as being well-rested in conditions that are conducive to sleep (Mellor et al. 2020). Conditions within equine husbandry systems to promote sleep include but are not limited to those which enable the animal to feel safe enough to adopt a recumbent posture, provide a comfortable sleep site in terms of the space and surface available, and utilise appropriate lighting levels, and/or access to other horses (Greening and McBride, 2002; Paper 1; Appendix 1). Despite inter-individual differences in susceptibility to sleep loss are evident, a sleep debt will increase with increasing hours of sleep loss (Van Dongen et al. 2003), resulting in changes to daytime sleep latency and behavioural alertness. To facilitate optimal welfare states, it therefore seems important to consider whether the physical environment provides conditions conducive to rest and sleep (Domain 2), resulting in a positive affective experience through a level of comfort associated with being well-rested (Domain 5) (Mellor, 2016; Mellor & Burns, 2020). The notion of what 'well-rested' is however requires further investigation due to species-specific differences and the limitations in human understanding of animal rest/sleep due to the study environments from which our knowledge has been gathered.

Updates to the model in 2020 involved renaming domain four ‘Behavioural Interactions’ to enable consideration of animal sentience and agency in response to variable and sometimes unpredictable environmental conditions (Mellor et al. 2020). Environmental interactions, such as experiences during human-animal interactions such as training, could also be used to determine whether the animal was in a positive or negative state. This is important relative to sleep because waking events particularly stressful ones (Langford and Cockram, 2010), such as poor training through the incorrect application of the principles of reinforcement (McGreevy et al. 2018), negatively impact upon sleep. With the correct application of reinforcement, operant conditioning requires that the animal learns how to operate within its environment to get what it wants and is the sort of learning that largely underpins animal learning and training (McGreevy et al. 2018). The International Society for Equitation Science has explicitly described learning principles for horse trainers and owners to encourage effective and correct use of operant conditioning (International Society for Equitation Science, 2023). Although it provides the animal with control over its decisions, the process of learning can itself be stressful whilst the animal learns the desired outcome. As stress influences sleep, Domain 4 is an important consideration relative to the animal’s ability to achieve comfort through rest. Animal welfare assessment is therefore complex, having evolved from simply thinking about the functioning or fitness of an animal, to consideration of the animal’s nature or ‘telos’ (Appleby and Sandøe, 2002) linked to whether the animal subjectively experiences positive welfare or not (Browning and Veit, 2022).

Although the Five Domains model has been posited as a tool with which to assess welfare (Mellor et al. 2020) the model is not adopted within existing welfare assessment tools that are available to help evaluate the welfare state of animals. The Welfare Quality® protocol is used to assess farm animal welfare (Blokhuis et al. 2010), and is based largely on the Five Freedoms. Four main welfare principles are assessed with more detailed criteria appearing under each principle, utilising resource-based and animal-based measures. Under the principle of good housing is the welfare criteria of comfort around resting (Blokhuis et al. 2010), which involves measuring time needed to lie down. There is however no consideration of whether the animals have slept or for how long they maintained a recumbent position (Welfare Quality®, 2009). There is also little consideration of the level of agency the animal has in choosing where it rests/sleeps. Recent discourse around the way in which robotic milking systems do not necessarily result in liberation of cows as claimed in promotional materials (Holloway et al. 2014), highlights how farmers manipulate the environment and fail to recognise opportunities for positive affective experiences, based on true agency.

Based largely on the principles of the Welfare Quality® protocol, the Animal Welfare Indicators (AWIN) and equine AWIN project aimed to develop science-based indicators using animal-based measures, rather than looking at resource availability (Dalla Costa et al. 2016), however the assessment protocol did not take into direct consideration resting and comfort. As part of the project, the AWINHorse app alongside I-Watch Turkey and AWIN Sheep apps, were developed to increase efficiency and transparency of the welfare assessment process (Estevez et al. 2017), which for horses included a resource-based measure for comfort around resting e.g., a measurement of the proportion of horses with satisfactory box dimensions and sufficient, clean bedding (standards defined by the app). However, unlike the Welfare Quality protocol, it did not include indicators that would signal whether the horse had achieved a recumbent position (animal-based measure), nor whether the animal appeared well-rested. Recumbency has not yet been validated as a reliable measure for assessing comfort around resting, as it can also be indicative of injury/illness (Belling, 1990), and there are limitations associated with measuring recumbency during (often brief) on-farm assessments (Dalla Costa et al. 2014). Lateral recumbency is the preferred posture for REM sleep in horses, however it has not been established to what extent horses adopt this posture whilst injured or ill, or in equine models of depression (Fureix et al. 2012). Horses cannot achieve REM sleep whilst standing, due to muscle atonia associated with this state (Williams et al. 2008). Thus, lack of recumbent behaviour has been described as REM sleep deprivation and is linked to the occurrence of spontaneous collapse (Fuchs et al. 2016). Determining what a restful laterally recumbent posture looks like for the purposes of equine welfare assessments could therefore be beneficial. Additional or alternative measurements could involve an assessment of excessive daytime sleepiness, that could involve the multiple sleep latency test (Dement and Carskadon, 1982), or behavioural markers during exercise such as excessive tripping/stumbling/lethargy. More recently it was suggested that the use of the Horse Grimace Scale post-exercise could be used to study tiredness (Ijichi et al. 2023) highlighting how existing measures could be used more innovatively. Physical measures of sleep are discussed in more detail in section 2.4, but for on-farm assessment carry some limitations in their application. Meanwhile, refinements to the equine AWIN welfare assessment are required, for example using data from a larger reference population, consideration of different horse housing and husbandry conditions between different establishments, and the need to develop and validate indicators of adequate rest and sleep (Dalla Costa et al. 2016).

Beyond AWIN, there is a lack of clear or explicit guidelines on the extent or type of housing required to facilitate specific equine behavioural needs linked to rest and sleep, or for how to measure recumbency (discussed further within this chapter in section 2.4). One recent review on the use of time budgets to measure animal welfare did associate decreased lying time with inappropriate environmental conditions, stress, and pain (Auer et al. 2021). There are however practical limitations associated with the use of time budgets in welfare assessment, based largely on the amount of time required to analyse video footage. Given that sleep is ubiquitous to all mammals (Frieberg, 2020) and the evidence that poor sleep or sleep deprivation can negatively impact on health and wellbeing (Motamedi-Fakhr et al. 2014), the lack of explicit inclusion of a measurement of sleep within welfare measurement protocols appears to be a critical omission. The Five Domains model could offer better guidance in this sense, enabling human care givers an opportunity to review how they are providing environments conducive to positive affective states such as rest/sleep. The innovative use of existing measurements and/or the establishment of behavioural states and measurements of these to evidence the occurrence of rest/sleep seem critical to include in the future or to redevelop existing welfare assessment tools.

2.3 Equine sleep

A detailed understanding of what is normal behaviour for a species under optimal conditions is critical for animal welfare assessment (Harvey, Ramp and Mellor, 2022). The following section aims to present a summary of equine sleep, derived from Greening and McBride (2022) (Appendix 1; Paper 1) an overview of the factors that influence the occurrence of sleep reviewing existing research in this field, and current perspectives on sleep within the equine industry.

2.3.1 Characteristics of normal equine sleep

Horses engage in a similar number of sleep cycles to humans, however the duration of cycles and the sleep states that occur within them differ. Whilst humans commonly cycle through NREM to REM, a sleep cycle for the horse can be composed entirely of NREM sleep but they also seem capable of cycling straight into REM sleep. Domestic horses sleep for approximately 50% of the average human total sleep time, between 3 to 4 hours within a 24-hr period, of which 40 minutes on average is devoted to lateral recumbency or REM sleep. Although total sleep time for horses is less than that of humans, the proportion of REM (~15%) and NREM (~85%) sleep within this is very similar (Greening and McBride, 2022; Appendix 1, Paper 1). The horse can achieve NREM sleep whilst recumbent or whilst standing due to the passive stay apparatus, however due to the muscle atonia that occurs during REM sleep a recumbent posture is necessary to successfully engage in this sleep state. To overcome reduced sleep opportunities, horses can utilise compensatory mechanisms such as standing sleep (Greening et al. 2021; Appendix 1, Paper 4). Potentially the use of slow wave activity (SWA) to increase intensity of sleep during standing sleep may also occur (Tobler, 1995) but little is understood of this compensatory mechanism for horses currently. Standing sleep however cannot compensate for REM sleep deprivation due to the homeostatic nature of sleep, thus some horses are subject to partial and full collapse when they engage in REM sleep whilst standing (Williams et al. 2008; Fuchs et al. 2016).

2.3.2 Environmental influences on equine sleep

Sleep occurs in response to biological cycles that are controlled by the superchiasmatic nucleus (SCN) in mammalian species (Morris et al. 2021). A number of cues influence the SCN as a result of the changes between light and dark over the course of the day (light, temperature, resource availability) that act to synchronise physiology (Roenneberg et al. 2007). The occurrence of sleep is also regulated by a number of ecological factors, for example, in the free-living environment the inverse relationship between time spent resting and the time spent foraging (Duncan, 1985). As an obligate grazer and an herbivore, the horse forages for 55% to 75% of the daytime budget and 53% of the night time-budget (Kiley-Worthington, 1990; Ermers et al. 2023). This helps to maintain optimal gastrointestinal functioning but compresses available time for sleep. The interplay between the occurrence of sleep and the vulnerability experienced during sleep (Allison and Cicchetti, 1976) is also described as influencing the duration of total sleep time. As a large prey species, the horse has evolved sensory perception and a behavioural repertoire that have played a pivotal role in the success of the species (McGreevy, 2004). Because the horse is largely always visible to predators in a free-living situation, the horse has evolved to survive on relatively little REM sleep thus avoiding the vulnerable position of recumbency for prolonged periods of time (Lesku et al. 2006) and adopting these recumbent postures largely between midnight and dawn (Houpt et al. 1986). Thus, the sleep site becomes an important consideration, compared to a small omnivore species who can more easily find shelter locations within which to nest and sleep safely (Schradin, 2005). There is a lack of agreement over whether sleep only occurs when there is a sentinel (Belling, 1990), however it is reasonable to suggest that recumbency is unlikely to occur as a group activity (McGreevy, 2004). For horses bred into the domestic environment, risk of predation is reduced, however the instinctive strategy of comparatively less REM sleep than the majority of mammals is persistent (Campbell and Tobler, 1984) and is readily observed in the equine sleep profile (Greening & McBride, 2022, Appendix 1; Paper 1). Seasonality has also been associated with the occurrence of rest behaviour for example, feral New Forest ponies are reported to spend less time resting during the winter daytime compared to the summer daytime (Tyler, 1972) and Camargue horses engage in more stand-resting at night during the wintertime (Duncan, 1985).

Traditional management in Western cultures for horses used in leisure and competitive riding usually involves single stable housing of varying duration, with less emphasis on outdoor housing (Dai et al. 2023). Horses are subject to decisions made by their human caretakers about the environment they live in that are highly influential on the occurrence of sleep which in some situations means that horses lack control to adjust their environments to achieve sleep (Figure 1). Forage rations vary in the way they are managed, although optimally forage should be available *ad libitum*, as the horse evolved to forage extensively. In an attempt to preserve the night time forage ration for stabled horses, a small holed hay-net can be employed however the main resting period between 01.00 and 04.00 hr can be shortened as a result (Ellis et al. 2015) and in this sense requires further investigation to determine if this constitutes a sleep reduction that requires compensatory sleep during the day. In stables or barns, horses can be bedded down on a variety of substrates, all of which influence comfort in different ways. The primary aim of Papers 2 and 4 (Appendix 1) were to assess the effects of bedding on the equine sleep profile and it was found that both bedding substrate and depth of substrate were linked to differences in the duration of recumbent behaviours (Greening et al. 2013; Greening et al. 2021), supporting the findings of similar studies (e.g., Pedersen et al. 2004; Werhahn et al. 2010). The size of the stall (e.g., Kjellberg et al. 2022) has been reported to influence the occurrence of recumbency. With the introduction of the concept of social stabling, different stable systems have been designed to facilitate social interaction such as the Big Box ©. Observations of horses in these systems suggest the behavioural profile is similar to that of free-living horses (Marliani et al. 2021), however observations were conducted in the daytime only and knowledge of the sleep profile beyond lying behaviour is not reported.

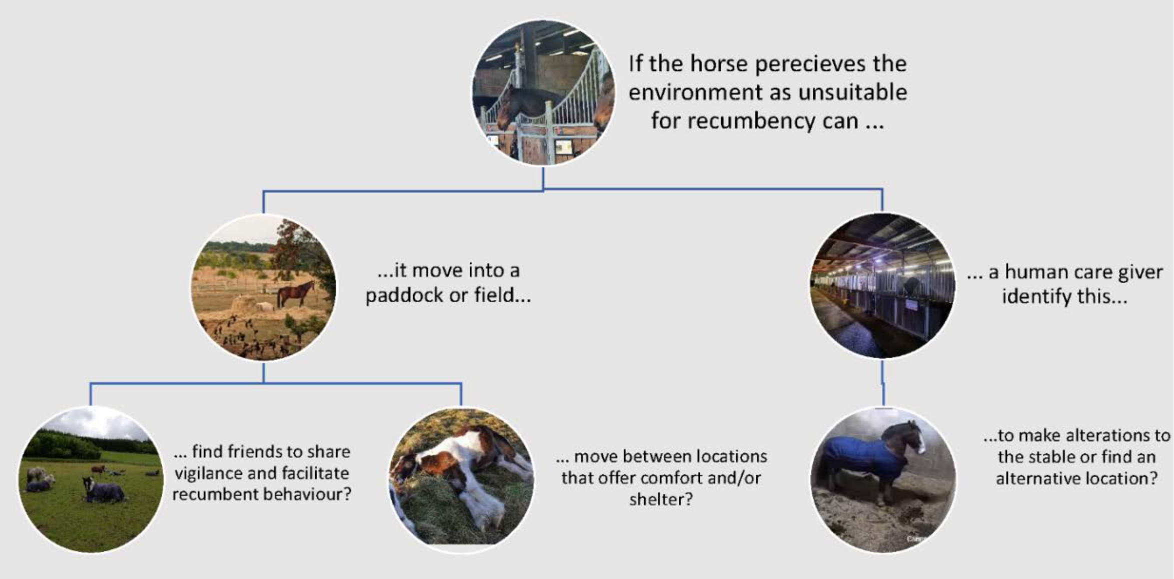


Figure 1. Differences in agency around obtaining a recumbent posture to facilitate sleep within the two predominant environments for domestic horses.

Group turnout is the most ecological and welfare-optimising approach to keeping horses (Hendersen, 2007). Horses in a paddock paradise system were recorded as not lying down at all, explained due to the amount of forage available such that grazing took priority (Marliani et al. 2021). The majority of sleep, specifically lying behaviour, was observed when horses were returned to the stable (a Big Box ©). Studies consistently report that stabled horses spend more time resting and less time foraging than when at pasture (Noble, 2023), however it is unclear about how access to shelter during turnout could affect that. Access to shelter during turnout seems pertinent relative to the need for a safe site for resting. The routine and duration of exposure to light during turnout should also be considered due to the association between light (specifically low-intensity blue wave light) and circadian and circannual physiology in horses (Murphy et al. 2019). Within a barn or stable, horses are subject to the lighting conditions that human care-givers implement, which could influence circadian control of sleep (Murphy et al. 2019), for example sleep profiles changed such that horses displayed more standing sleep when lights were left on overnight (Greening et al., 2021, Appendix 1; Paper 4).

A hypo-stimulating environment can also dramatically change total sleep time but in this instance, increased sleep would not be an indicator of positive welfare, especially where depressive-like forms of waking inactivity are mistakenly reported as standing sleep (Greening and McBride, 2022, Appendix 1; Paper 1). Thus greater time resting as reported for stabled horses does not necessarily indicate an improved welfare state *per se*. Individual profiles should be ascertained and compared between different conditions to determine whether these are affecting sleep specifically. Particular care should be taken to differentiate between standing awake, standing in drowsiness, standing sleep and the fixed posture described by the equine model of depression (Fureix et al. 2012). A hypo-stimulating environment can manifest both in the stable and during turnout, but rather than observing waking inactivity in the stable as a result, this could be observed through horses waiting at the gate that may involve different motivational systems in response to environmental stimuli.

Further influential factors including increased vigilance due to acclimatisation to novel surroundings e.g. a horse travelling to stay overnight at a competition venue, or lost group vigilance due to individual stabling after a period of turnout. The latter has been shown to affect sleep profiles, through increased standing sleep and reduced recumbency (Dallaire, 1986; Fewings and Greening, 2019, Appendix 2), but little is known about how competition horses adapt in new environments. Due to the homeostatic pressure associated with achieving sleep, increasing duration of sleep over successive nights might not necessarily be associated with a reduced state of arousal and more to do with increasing pressure to engage in this survival-critical behaviour. Little is known about how the company of other horses in neighbouring stables influences sleep and whether this presents as a comforting factor i.e. through perceived shared vigilance, or a disruptive factor i.e. due to movement and noises (e.g. coughing) that individual horses display during the night. The influence of social enrichment such as mirrors may offer some comfort for horses where physical contact between stables is prevented however, as recumbency is not considered a group behaviour, this could also negatively impact on sleep/rest.

In addition to environmental challenges, age and stressors such as injury/disease also influence sleep. For example, older horses engage in recumbency less than their young counterparts (Kelemen et al. 2021), whilst brain waves of the foals differ in their morphology from those previously reported for adult horses (Zanker et al. 2021). Horses with mild osteoarthritis spend more time in recumbency than those with severe osteoarthritis (Oliveira et al. 2022) suggested to be as a result of the pain associated with the condition. Gastrointestinal health issues can ensue if continuous gut fill is not enabled (Ermers et al. 2023) and a high prevalence of equine gastric ulceration syndrome (EGUS) and equine glandular gastric disease (EGGD) in a variety of equine disciplines is well documented (Tamzali et al. 2011). Human research indicates how individuals with functional gastrointestinal disorders experience changes to sleep patterns (Bouchaoucha et al. 2018), although it is difficult to determine the cause and effect of the sleep disturbances due to the unique interplay between the two (Khanijow et al. 2015). An association between gastrointestinal health conditions and the occurrence of sleep however has not yet been investigated.

Horses with stereotypic behaviour also display different nocturnal behavioural profiles, specifically crib biters who engage in the cribbing behaviour inside of every hour of observation (Clegg et al. 2008). Stereotypic horses appeared to engage in recumbency less frequently than non-stereotypic horses although differences were not statistically different. Neurophysiologic dysfunction is reported as the cause of crib-biting, described as changes within the dopaminergic system, specifically lower dopamine (DA) receptors (D1) in the caudate nucleus and higher DA D1 and D2 receptors in the nucleus accumbens (McBride and Parker, 2015). Dopamine-containing neurons are largely involved in the regulation of sleep and waking (Monti and Monti, 2007; Monti, 2011). The extent to which up- and down-regulation of how these receptors may influence the occurrence of sleep in horses has not yet been considered for horses. Stereotypic behaviour in animals has been likened to obsessive compulsive disorder (OCD) in humans (Wickens and Heleski, 2010), and the role of dopamine in the pathophysiology of OCD has recently been discussed (Koo et al. 2014). OCD patients also report altered sleep patterns, including significantly greater difficulty falling asleep and reduced total sleep time (Paterson et al. 2013). The role of stereotypy in sleep modulation deserves further investigation, for example it would be interesting to understand whether reduced sleep impacts daytime functioning of stereotypic individuals, whether they develop coping mechanisms through standing sleep/drowsiness, or could be genetically ‘elite sleepers’.

Horses within the domestic environment clearly experience a range of challenges, some of which are specific to achieving sleep (Greening and McBride, 2022). However, little is known about horses in a free-living environment to understand natural sleep profiles, thus stabled horse sleep behavioural profiles are largely referred to which is a limitation in our knowledge of normal sleep behaviour. As a minimum, the design and planning of features of the domestic environment should aim to meet the behavioural needs of the horse, including recumbent postures to facilitate sleep and choice over sleep sites, whilst avoiding factors known to negatively impact upon the occurrence of sleep, to best sustain positive equine welfare.

2.3.3 Stakeholder recognition of the importance of sleep

The endemic consideration of sleep within human health is increasing our knowledge and understanding of sleep. However, this does not appear to be reflected amongst horse owners and their knowledge of equine sleep. In a small-scale survey of horse owners in the UK (N = 582), over a quarter of respondents to a questionnaire investigating perceptions of horse sleep admitted to never considering the amount of sleep their horse achieved, with 55% of respondents not attributing lethargy in the ridden horse to a lack of sleep (Greening et al., 2020, Appendix 2). A number of lay articles exist, offering strategies to help riders with ‘lazy horses’ during ridden work (e.g. Clark, 2021; Maynard, 2022). One possible explanation for this potential misinterpretation of behaviour is the paucity of information available for horse owners about the importance of sleep and/or management practices to optimise equine sleep hygiene, compared to the information available promoting human sleep but there is evidence of how this is changing. This could largely be due to the lack of research in the field of equine sleep to increase knowledge and understanding of equine sleep. At the time of writing, the National Equine Welfare Council is redeveloping materials to add to its online advisory notices, and here exists an opportunity to educate horse owners on the topic of horse sleep, utilising an increasing knowledge base and encouraging others to do more research in this field (Hall, 2022 – personal communication). Although the British Horse Society does not discuss the importance of sleep on its website, it does highlight how bedding can be used to facilitate lying behaviour (BHS, 2022). The DEFRA Code of Practice for the Welfare of Horses, Ponies, Donkeys and their Hybrids (2017) also contains no mention of sleep or rest, nor does it identify the importance of an environment that facilitates recumbent behaviours. Furthermore, despite the fact that sleep is recognised as one of the foundations of human athlete performance (Halson and Juliff, 2017), reviews of equine performance and emerging issues in equestrian practice largely fail to recognise the importance of sleep explicitly to the equine athlete (e.g., Parkin and Rossdale, 2006; Williams and Marlin, 2020). The lack of empirical evidence to demonstrate associations between equine performance and sleep beyond generic consideration within health and welfare could be one of the reasons for this. The existing limitations of measuring sleep profiles of competition horses on their home yard compared to the competition yard are mainly due to the measurements of sleep that can be collected. For example, behavioural observations are less invasive but require the use of CCTV equipment that needs to be erected at both sites which is expensive and often impractical outside of an experimental set up. The limitations of measuring equine sleep are discussed in detail in section 2.4.

The equine industry is currently responding to increasing public pressure linked to the welfare of horses both within and outside of equestrian competitions (Furtado et al., 2021) and the use of the horse in sport is increasingly being called into question. During a panel discussion considering the use of horses in sport at the 18th International Society for Equitation Science conference, concerns were raised about the kinds of topics and questions that the public poses which industry and indeed equine scientists do not currently have answers for (Marlin, 2022). However, sleep did not factor into this discussion. Evidence to promote the species-specific equine sleep profile and environmental considerations necessary to help management systems facilitate optimal sleep to promote positive welfare is therefore paramount. Raising the profile of equine sleep and encouraging sustained research in this area is also crucial to ensure this component of wellbeing is considered as a central tenet in any ethical framework, especially in response to the increasing threats to equestrianism's social license to operate (Douglas et al. 2022; Campbell, 2021).

2.4 Measuring equine sleep

Horse sleep was seminally studied by Ruckebusch in the 1960-70s (Williams et al. 2008), who was affiliated with the Laboratoire de Physiologie, Ecole Nationale Veterinaire in Toulouse. Ruckebusch utilised implanted electrocorticography and concurrent electromyography to establish duration of the states of sleep and wakefulness during the circadian cycle in farm animals, including horses (e.g., Ruckebusch et al. 1970). The sample population comprised three Pottok stallion ponies that were bred at the laboratory, making results difficult to transpose to the broader horse population. The implanted electrodes used to measure brain wave activity were able to produce very accurate output due to the close proximity to neurones, however they also raise ethical and welfare-related questions. More recently, surface electrodes have been used to measure brain wave activity (e.g., Williams et al. 2008). Further developments in the field of electroencephalography (EEG) mean that multiple physiological measurements can be taken simultaneously, described as polysomnography (PSG) (e.g. Zanker et al. 2021).

Reliability and validity of physiological measurements of animal sleep using EEG can however be affected in different ways. Human interpretation of EEG output relies upon identification of the predominance of brain wave activity characterful of a sleep state occurring within 30 second epochs. This requires an expert scorer to manually analyse and annotate each night which can take two hours per night and is subject to considerable inter- and intra-scorer variability (Sridhar et al. 2020). As a monophasic sleeper, human studies can be conducted during a block of time that requires participants to tolerate placement of the leads and attachments to help them stay in place. For polyphasic sleep species, data collection must occur across 24-hr requiring attachment of equipment throughout this period. However, contact between the skin and the electrode can be affected by sweat and dirt and interference from superficial facial muscle movement due to the location of electrodes. Thus, animal EEG research is fraught with issues such as electrodes becoming dislodged, and cross-talk or noise within the data which reduces the quality of the EEG output. The need to shave hair to increase contact can also be off-putting to horse owners due to the chance that the hair grows back a different colour (Zanker et al. 2021). Due to the nature of horses, working around the head to fit the surface electrodes for the EEG could be difficult. In a study using EEG with cows, a period of habituation to the equipment was required both in attachment and wearing the equipment (Hunter et al. 2021).

EEG measurements of sleep are complimented by additional physiological measures within studies utilising PSG, but in equine studies the reported results largely focus on brain wave activity and the nuances in attaching equipment to ensure accurate data output (e.g. Zanker et al. 2021). A protocol for the use of EEG in equine studies would be useful to avoid studies experiencing the same pitfalls. In an unpublished study looking at heart variability (HRV) and sleep profiles in a group of horses, the authors detected variation in HRV that they found was associated with sleep states with some specific nuanced profiles linked to REM sleep (Ash, 2021). However, the girth with which the HRV equipment was attached needed to be very tight to the horse in order to ensure HRV could be read. This was uncomfortable for the horse to wear over a 24-hr period, and thus HRV data was incomplete for comparison against the occurrence of sleep states. These impracticalities in animal sleep research therefore reinforce the need for an accurate alternative method (Table 3). Behavioural observations via video recordings, are a common alternative sleep measurement to EEG in animal studies and behavioural quantification of sleep has historically been considered a viable alternative to EEG measurement (Campbell & Tobler, 1984).

Table 3. A summary evaluation of different methods available for measuring sleep

Method of measuring sleep	Strengths	Weaknesses
Electroencephalography	Quantitative data, automatically detects and records brain wave activity that can be used to identify sleep states.	In animal studies, contact between electrode and skin can be affected by 1) movement of superficial facial/ear muscles, 2) sweat, 3) dust, 4) head rubbing shaking, which affect quality of data output. Animal often requires habituation to equipment before data collection can begin. Human interpretation of EEG output every 30 seconds = open to human error/ labour intensive. Currently expensive and inaccessible. Data anomalies may require confirmation of sleep states through behavioural video data.
Polysomnography	Multiple quantitative measurements recorded automatically including brain wave activity, eye movement, respiration and muscle activation.	As above but in addition, large volumes of data that must be reviewed. Currently expensive and inaccessible. Further practical limitations for application on animals.
Behaviour videos	Non-invasive observations of behaviour that capture duration and frequency of sleep states. Does not require expensive EEG equipment.	Requires development of a robust ethogram to ensure accurate data is collected. Camera angle can result in blind spots. Open to error through human interpretation of behaviours. Labour intensive.
Actigraphy	Does not require time for behavioural observation. Does not require expensive EEG equipment.	Cannot differentiate between different sleep states. Has been shown to overestimate rest episodes if data is not corrected (e.g. removing duration of lying behaviour <4mins). Only some models/makes can differentiate between different forms of recumbency.
Heart rate	Does not require time for behavioural observation. Can be measured in a variety of ways in human research.	Can be affected by physical factors such as age/ fitness. For use in horses, needs to be attached tightly to facilitate contact between electrode and skin which can be uncomfortable over long periods of time.
Heart rate variability	Highly predictive of sleep states/ cycles.	Complex measure, requires statistical modelling not just visual observation. Still learning what 'normal' patterns are. Similar to actigraphy, need to discard short behavioural sequences to avoid inaccurately recording lying behaviour.
Respiratory rate	A variety of measurements in human research e.g. thermal sensors, oximetry, inductance plethysmography. Increasing use of contactless approaches e.g. laser spectroscopy.	Much of the equipment in human is less transferable to animal research. Increasing interest in contactless measures and wearable measures (e.g. Inertial Measurement Units) but little work exists validating these measures.

Behavioural sleep studies can utilise observed behavioural states via video recordings (Plate 4) or from accelerometer data (e.g., Kelemen et al. 2021). Sleep is inferred, specifically in standing and recumbent states. Whilst horses can sleep standing they tend to adopt recumbent positions to achieve REM sleep because of associated muscle atonia (Williams et al. 2008). However, horses can engage in slow wave sleep both whilst standing and during recumbency, and REM sleep can occur during both sternal and lateral recumbency (Dallaire and Ruckebusch, 1974). The challenges presented to video analysis due to the ability of the horse to sleep in multiple positions are discussed in more detail in Chapter 3, but the problems with only using generic behavioural states must be considered as a limitation when using accelerometers. More recently regression models have been developed (Greening & McBride, 2022, Appendix 1; Paper 1) but are yet to be tested.

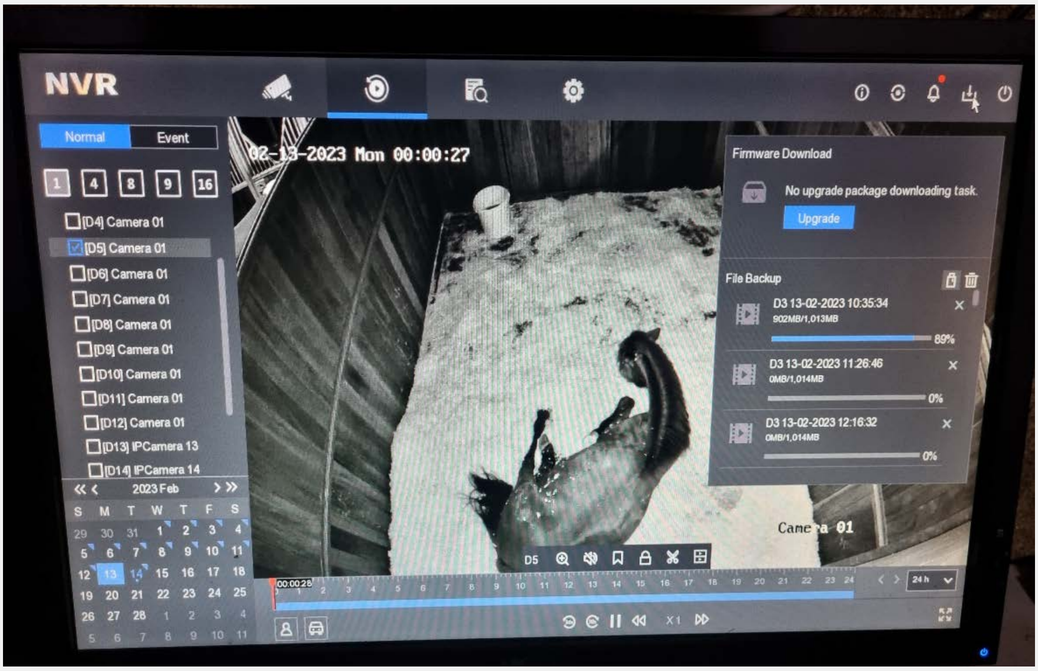


Plate 4. An example of CCTV video footage

The main tool in behavioural research is the ethogram, which facilitates standardisation of descriptions and definitions of each behaviour being observed. These should include detail of the movement (or lack of movement) of body parts, usually with a clear start and end point. A functional ethogram attempts to provide functional interpretation of behaviour units (Pierard et al. 2015), but usually ethograms simply enable measurement of the phenotypic behaviour displayed in response to (internal and external) stimuli. Ethogram variations have been employed in a number of equine sleep studies which makes comparison of findings difficult. For example, Oliveira et al. (2022) measures drowsiness, which is little accounted for in existing publications. Meanwhile, Zanker et al. (2022) measures standing without a separate category for standing sleep, which could result in over- or under-estimation of the occurrence of sleep. Such variation may be due to the lack of a standardized sleep ethogram; a tool which would facilitate comparison between studies and make interpretation of findings easier. Where behavioural data are collected, the duration of observational periods can vary, for example Bradshaw-Wiley and Randle (2023) observed horses across 48 hours but not for consecutive hours with a random generator subsequently used to select hours for observation. This could have skewed results, where the majority of the hours observed were not between midnight and 4am when the greater proportion of recumbent behaviour is commonly displayed. A longer observational period, especially utilising continuous focal sampling, ensures observations are not simply a snap-shot within one or two nights for example, Chung et al. (2018) observed each horse for 72 hours. However, the time required to analyse video data can be extensive (see chapter three). The majority of behavioural sleep research measures cumulative duration of behavioural/inferred sleep states, generating duration of total sleep time. Within the review by Greening and McBride (2022, Appendix 1; Paper 1), existing EEG data (Kalus, 2014) were re-analysed looking at the cumulative duration of awakenings during sleep that on average described 5% of sleep time that was unaccounted for. That is to say, the time spent in different sleep states did not align with the total time spent sleeping, thus the blunt measure of sleep quantity to determine whether optimal sleep has been achieved would not account for the frequency of awakenings which could affect the quality of sleep achieved. Total sleep time therefore can be reviewed relative to sleep quantity and quality (Figure 3) offering opportunities to expand knowledge in this emerging field, via a variety of tools to measure sleep states.

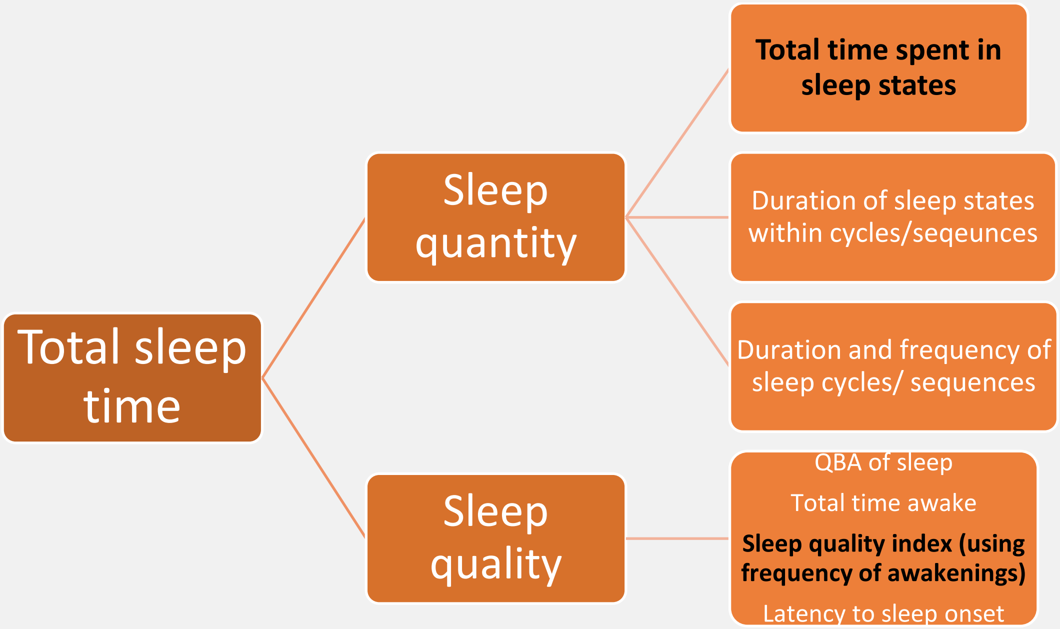


Figure 3. Visualising some of the metrics available with which to measure sleep (*black text denotes those used in equine sleep research within the last 20 years*).

Qualitative behavioural assessment also exists as a tool with which to measure the quality of an animal's demeanour (Yon et al. 2019) that could be used to determine not only how the animal appears in their sleep state but also potentially what their demeanour is following reduced or disrupted sleep. Considering the survival-critical negative affect linked to a lack of opportunities to sleep, future studies may consider measurements of 'motivational priorities', in recognition of animal telos, and how a variety of inputs are used by animals on an individual basis to make sensitive judgements about their own interests (Swanson, 2010).

CHAPTER THREE

Within this chapter, the approaches taken to the studies presented for assessment will be discussed, based upon a critical appreciation of the reliability and validity of study designs and through my own development as a researcher. The chapter offers an opportunity for critical reflection involving evaluation of the approaches taken, to demonstrate a critical understanding of the methodology of enquiry.

3.1 Personal epistemology

As a lifelong learner, I subscribe to the notion that knowledge is not always certain, and that learning doesn't just happen (Bath and Smith, 2009). During the four years of writing the review paper (Greening and McBride, 2022; Paper 1) I developed a sustained, structured and systematic approach to seeking out knowledge and information on the topic of sleep, utilising research on a variety of mammalian species from an objectivist epistemological viewpoint. Prior to this, I had learnt about sleep *ad hoc* through student project supervision and whilst conducting my own small scales studies. Writing the review enabled me to focus in on and explore a wealth of literature that furthered my understanding of the complex topic of sleep in general.

Listening to authority voices in my institution, and wider, led me to believe that behavioural measurements of sleep lacked objectivity compared to gold standard EEG equipment. In this sense I was a passive receiver of knowledge which has been linked to gender differences in the way in which men and women develop their beliefs about the nature of knowledge (Belenky et al. [1986] in; Schommer-Aikins [1994]). I continued to validate the lack of support for behavioural research against the fact that little research on equine sleep existed beyond EEG studies. However, I began to realise through desk-based secondary research that in fact, behavioural science is a valid alternative to EEG measurements when observing animal sleep, due to the issues including difficulty gaining a full 24 hr+ EEG data profile and the subjectivity linked to human interpretation of EEG output. Indeed historically, EEG output has been mapped against behavioural data to provide context where data is unclear (Campbell & Tobler, 1984). Taking a relativist ontological stance, I realised that equine sleep could be studied using more than just one measurement to broaden our knowledge and understanding in this area.

With self-assurance from studying and teaching within the field of equine behaviour for two decades, balanced by a critical awareness of the limitations of using ethograms and behavioural data, I devoted myself to elevating the profile of behavioural sleep research, recognising how important it could be for horse welfare. I feel my research journey has progressed in a similar way to the seven stages of the reflective judgement model (King and Kitchener, 1994), which started by thinking only about the knowledge I received through to quasi-reflective thinking (Paper 2; Greening et al. 2013 and Paper 3; Hartman and Greening, 2019), culminating in reflective thinking (Paper 1; Greening and McBride, 2022 and Paper 4; Greening et al., 2021) that facilitated innovative thought and reasoning on the subject (Paper 1; Greening and McBride, 2022 and Paper 5; Greening et al. 2023).

3.2 Methods

The experimental studies presented here for assessment use deductive reasoning which is mainly associated with a positivist approach to research that facilitates hypothesis testing (Williamson et al. 2002). These studies aimed to measure outcomes (changes to sleep behaviour) based on exposure to variables within a given time-frame and thus utilised a cohort study design. Although descriptions of observable behaviour are often considered qualitative in nature (Taylor et al. 2016), a quantitative strategy to null hypothesis significance testing was employed such that the ethogram(s) of sleep behaviour enabled quantitative data collection, as either duration or frequency of occurrence of sleep behaviour.

Paper 2 (Greening et al. 2013) utilised an observational one-shot design that has also been employed in a similar way in published sleep studies (e.g., Bradshaw-Wiley and Randle, 2023; Chung et al. 2018) to compare the influence of different environments on the nocturnal behavioural profile. Paper 2 adopted a quasi-experimental approach, using two groups of horses housed on the same yard and a non-equivalent control group, observed under their normal housing regime. The dependent variable, sleep, was measured using continuous focal sampling, with bedding substrate as the independent variable. Such an approach offered high external validity however, it was difficult to account for confounding variables that may have affected internal validity (Privitera and Delzell, 2019) for example, there was no control group and horses in each group were not match-paired. To overcome this, in subsequent studies a repeated-measures cross over design was utilised

(Paper 4; Greening et al. 2021 and Paper 5; Greening et al. 2023) that enabled the animals within the study to act as their own control. The randomized allocation of animals to the two experimental groups also helped reduce the likelihood that underlying variables skewed data for each experimental group (Johnson and Besselsen, 2002). The potential for carry over effects was addressed by including a wash-out period between experimental conditions.

Paper 3 (Hartman and Greening, 2019) also employed a washout period within the reversal (ABA) design of their study, where A was the control phase, B was the phase where music was played over night, and the second A phase was the post-intervention control period. Horses in Paper 3 acted as their own control, with the dependent variable (sleep) manipulated by exposure to music played (or not) at night (independent variable). This approach aimed to reduce variability between individual horses to help strengthen internal validity and within the statistical grouping, thus reducing the number of animals required for adequate statistical power (Byiers et al. 2012). To further address the potential order effects, the second A phase was staggered such that observations were not conducted over consecutive nights. Usually, a minimum of three baseline data points is required to establish dependent measure stability (Kazdin, 2010) but in this study researchers gained baseline data during two nights of observation that may have affected interpretation of results. For analysis, the B phase was separated into two (B1 and B2) to measure the immediacy of effect, where an immediate change from one phase to another provides a compelling argument for the functional relationship between independent and dependent variables (Privitera and Delzell, 2019). Significant differences were detected between B1 and B2, as well as A phases, suggesting that music was influential on nocturnal behaviour.

Paper 1 (Greening and McBride, 2022) highlights how existing equine sleep research employs sample sizes from two to fifteen. Sample sizes in the studies presented here ranged from seven to ten. The smaller sample size in Paper 2 (Greening et al., 2013) may have resulted in a type II error, such that the lack of significance was because the study was not powerful enough to find a difference (Eng, 2003). Limitations included the relative lack of previous studies with which to conduct a power analysis or to determine effect size. In all cases where the sample size is minimal, it is possible to increase precision by repeating the measurements taken from each individual (Eng, 2003) as observed in paper 3 (Hartman and Greening, 2019) where observations were conducted over a number of nights. As previously discussed (Chapter Two) there is a large amount of inter-individual variation in sleep patterns, as well as intra-individual variation in profiles between nights. Papers 2 and 3 report small sample sizes in part due to the novel nature of these studies, but also in accordance with the challenges acknowledged with acquiring sufficient sample sizes for applied research in the equestrian field (Williams and Tabor, 2017). The mean data from these two studies report relatively large standard deviation, likely as a result of the differences in individual horse sleep profiles within each study cohort, resulting in underpowered results. The same degree of inter-individual variation has also been observed in bovine sleep research (e.g., Ternman et al. 2018). Recognising the limitations in terms of underpowered results, assumptions were made based on existing research about desired effect size to facilitate a power analysis for Paper 4 (Greening et al. 2021) which was the same for Paper 5 (Greening et al. 2023), to confirm an appropriate sample size ($N \geq 10$). Data were subject to statistical tests of difference using non-parametric tests (Paper 2; Greening et al. 2013, and Paper 3; Hartman and Greening, 2019) or general linear modelling (Paper 3; Hartman and Greening, 2019, Papers 4; Greening et al., 2021, and 5; Greening et al. 2023) to determine statistical significance, all with an alpha level of 0.05. Effect sizes were calculated *post hoc* to address the issue of underpowered results, where Paper 2 returned medium to large effect sizes and Paper 3 returned only small effect sizes.

All experimental studies involved convenience sampling due to the lack of access to experimental populations of domestic horses in the UK. The lack of a research population in combination with the cost and practicalities of mounting cameras in multiple stables (potentially across multiple sites) is a limitation to equine sleep studies, and it can affect whether a study is adequately powered. Convenience sampling is typically seen as a poor representation of the general population however, the horses used in the studies were all used for leisure riding and/or riding lessons without elite competitive experience, which helps to increase transferability of results. Horse populations in the different studies were housed at three different locations; Hartpury Equestrian Centre, Aberystwyth University yard, and a private yard in Gloucestershire such that the data collected at different venues/with different horses ensured that the body of work was not a case study of the same population of horses at the same venue in each publication. However, the populations of horses in each study were all used for leisure or within riding schools at University establishments, therefore results cannot be easily transposed to horses used for competition. The range of uncontrolled variables for each study must also be carefully considered when interpreting and comparing results (discussed in more detail in Chapter Four, section 4.3).

Paper 3 (Hartman and Greening, 2019) measured the number of scans every two minutes, as an approximation of the percentage of time of different behavioural states for each individual, which was within the gold standard ethological method of scoring behaviour every 3–5 minutes for ≥ 72 hours (Yon et al. 2019). The remaining studies employed a continuous focal sampling method to assess duration of behavioural states exhibited by all horses during the observed periods, providing a more complete profile of behaviour. All of the studies involved retrospective review of video data which enables events to be repeatedly viewed and behavioural occurrences to be viewed in slow/fast motion or freeze-frame (Bateson and Paul, 2021). Being able to manipulate video footage in such a way helps to increase the accuracy of the data collected, for example where transition between behaviours is rapid, or to accurately determine the occurrence of a behaviour that was short duration. Retrospective recording also overcomes the potential for extraneous environmental cues to disrupt the observer whilst coding. Videos were reviewed using VLC Media Player typically at normal speed or x1.5/ x2 speed and were able to rewind videos as required. Individual observers conducted provisional training with the ethogram in an attempt to overcome inaccuracies within observational data recording, as a result of expectation bias, coding system complexity and agreement checking (Harris and Lahey, 1982). Where a large group of individuals was involved in video data analysis (Paper 4; Greening et al.,2021), standardised training sessions were provided and inter-observer reliability tests were conducted. On average, an hour of video footage took between ten and twenty minutes to analyse using continuous focal sampling. Thus, 24 hours of footage could between four and eight hours to analyse; much more than the time reported to review a single night of human sleep EEG data (Sridhar et al. 2020).

The total number of hours in the observational period largely depended upon the aims of the study, generating on average 3000 hours of footage to review, ranging from 120-hrs (Paper 2; Greening et al. 2013) to 5,760-hrs (Paper 4; Greening et al. 2021) (Table 4). The snapshot of data collected for each horse in Paper 2 (Greening et al., 2013) from 7pm to 7am was used to develop an average profile of nocturnal behaviour for comparison between two groups. The duration of the observational period in Paper 2 did not consider the intra-individual differences in the equine nocturnal profile from night to night. Intra-individual variation in sleep patterns occur due to the circadian and homeostatic processes which govern sleep, as much as environmental influences (Kalus, 2014). It is therefore important to gain information about the normal sleep pattern of an individual horse. Thus, consecutive 24-hr observation using focal observation and continuous sampling within a cross-over design were adopted (Paper 4; Greening et al. 2021 and Paper 5; Greening et al. 2023) to increase the validity of the results.

Table 4. Comparison of approaches to observation and sampling within the collection of work for assessment.

	Hours observed/ horse	**N**	**Days of observation**	**Study design**	**Sampling method**	**Data collected**
Paper 2; Greening et al., (2013)	12-hrs	Ten	One	Observational one shot	Continuous focal	Duration of behavioural states
Paper 3; Hartman and Greening (2019)	90-hrs spanning ten hours each day	Seven	Nine	ABA with washout period	Instantaneous focal (every 2-min)	Number of scans of behavioural states
Paper 4; Greening et al. 2021	576-hrs spanning 24 hrs each day	Ten	Twenty-four	Repeated-measures cross-over with washout periods	Continuous focal	Duration of behavioural states
Paper 5; Greening et al. 2023	576-hrs spanning 24 hrs each day	Ten	Twenty-four	As above	Continuous focal	Frequency of awakenings

The narrative literature review (Paper 1; Greening and McBride, 2022) is the only work presented for consideration that is not empirical. The article synthesized an average sleep profile from the results of existing equine sleep research and generated two novel measurements of sleep. The first was a sleep quality metric, identified as a consequence of the comparison between human and horse sleep profiles within the review. Looking at objective measurements of human sleep quality, the number of awakenings (a period of arousal not full wakefulness during a sleep episode) was selected as it has been found to correlate with subjective sleep quality (Rosipal et al. 2013). Comparing duration of awakening in humans and rodents, the human metric (>3 minutes = wakefulness) as proposed by Merica and Gaillard (1986) was adapted rather than the rodent measurement (>5 minutes, Simasko 2009). Looking at EEG data in the form of a hypnogram from the work of Kalus (2014), awakenings within sleep met this criterion. There is opportunity to investigate the application of these indices using >5 minutes as the criteria as per the rodent data, but validation is required to show that the horse does not break into full wakefulness beyond between three and five minutes as this would end the sleep episode rather than interrupt it. The sleep quality index (SQI) score is a product of the cumulative duration of total sleep/ different sleep states divided by the number of awakenings within the sleep state and aims to differentiate between good quality (high score) and poor quality (low score) sleep. Whilst developing the index equation, a false score of zero was obtained when a horse experienced zero sleep disturbance because zero was the denominator in the calculation. The term +1 was added to all equations to address this. The SQI has been tested (Paper 5; Greening et al. 2023) utilising existing data (Paper 4; Greening et al. 2021). The use of SQI scores in other species is advocated by both Paper 5 and Paper 1.

The second novel measurement, a regression equation, has yet to be tested. It aims to offer a method by which to differentiate between different sleep states occurring during different behavioural states. Equations were developed by plotting utilising existing equine EEG data from Kalus (2014) (sleep state duration) against different behavioural states. In future, it would be useful to plot residuals to check they followed normal distribution. Repeating this exercise with behavioural data would also be useful to determine whether similar patterns can be distinguished to EEG data. Thus, Paper 1 (Greening and McBride, 2022) offers a ‘where we are now’ synopsis alongside novel measurements, both of which could be useful to better understand the occurrence of equine sleep. The additional synthesis of existing data behind the review article offers enhanced justification for and underpins key aspects of the sleep ethogram. The review also offers opportunity for refinement to existing approaches to equine sleep research, considering how best to accurately and reliably measure sleep behaviour, across 24-hrs, with an increased appreciation of confounding factors, and within the context of the horse as a prey species and as an obligate herbivore.

3.3 Ethics

All the horses in the experimental studies were observed to engage in recumbent behaviour for at least one night during study conditions, which is an important consideration due to the issues surrounding sleep disturbance and (specifically REM sleep) deprivation (Fuchs et al. 2016). All studies received ethical approval from either Hartpury University or Aberystwyth University. Ethical considerations during or after experimental phases involved asking horse care-givers in the studies to monitor the daytime behaviour of horses, although none reported any excessive sleepiness or tripping/stumbling that could suggest rest had been negatively affected. Paper 4 (Greening et al. 2021) and Paper 5 (Greening et al. 2023) involved modification to the environment through the reduction of bedding substrate. Prior to this study, the effects of bedding on sleep had not been previously revealed. To date, however, there is still little empirical evidence of the outcomes of these changes beyond behavioural changes. Thus, in line with the legal minimum requirements outlined in the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes Prior (Home Office, 2014) bedding material and sleeping space were available to all horses throughout each of the studies.

CHAPTER FOUR

The publications presented within this thesis offer insights into sleep behaviour of horses and how the environment could be manipulated to best facilitate optimal sleep quality/quantity to enhance equine welfare. However, as with all research, there are limitations to the accuracy and application of results which are explored within this chapter, to provide evidence of critical reflections on the key findings and learnings from the presented work.

4.1 Behavioural profiles – what is normal sleep?

The analysis of the findings from thirteen published behavioural and EEG studies on equine sleep of horses kept in stables in Paper 1 (Greening and McBride, 2022), were used to create an average equine sleep profile. These data were taken from a range of studies from different countries using different types of horses from different equestrian disciplines thus the profile can be considered representative of the general horse population. However, the accuracy of the data may also have varied due to the length of time that horses were observed (average 16-hr out of 24-hr) with a minimum of 7-hr (Wohr et al. 2016 and Kalus, 2014) to a maximum of 24 hr (Greening et al. 2021). It is widely recognised that studies enabling measurement over a longer period of time produce better accuracy scores and a greater representation of true values (Wilder et al. 2021), which is particularly advantageous when researching a species that is a polyphasic/opportunistic sleeper which often rests during the day. Thus, the number of hours of observation increased from papers 2 and 3 to papers 4 and 5 to ensure more accurate behavioural profiles were obtained. All future equine sleep research should therefore aim to capture behaviour data over a 24-hr period and over multiple days.

Using data from published research, the review paper also highlighted how the mean total sleep time of horses could be divided into mean proportions of the occurrence of NREM (77.5%) and REM (17.5%) (Greening and McBride, 2022; Paper 1). Measuring the median of these may have been a better measure of the central tendency of the group as it is not skewed by exceptionally high or low characteristic values. Some of the experimental groups in Paper 2 (Greening et al. 2013) and Paper 3 (Greening and Hartman, 2019) did not achieve the average proportions of sleep as derived from the average sleep profile (Table 5).

Table 5. Proportion of REM and NREM sleep achieved, calculated from total sleep time reported within the presented body of work for assessment.

Study	**Proportion of REM**	**Proportion of NREM**	**Total sleep time (hrs/mins)**	**Observed (hrs)**
Paper 2; Greening et al., (2013)	Straw = 12% Shavings = 1.4%	Straw = 88% Shavings = 98.6%	Straw = 6hr 23m Shavings = 5hr 30m	12
Paper 3; Hartman and Greening (2019)*	With music = 6% W/out music = 6%	With music = 94% W/out music = 94%	With music = 4hr 42m W/out music = 5hr 36m	10
Paper 4; Greening et al., (2021)*	Control = 24% Treatment = 14.5%	Control = 76% Treatment = 85.5%	Control = 5hr 16m Treatment = 5hr 48m	24

* Denotes significant differences were detected between groups ($P < 0.05$)

These discrepancies could have occurred because standing sleep and sternal recumbency were used in combination to determine the occurrence of NREM sleep. For example, the ethogram in Paper 3 (Hartman and Greening, 2019) did not distinguish between the behavioural states ‘standing’ and ‘standing sleep’. The ethogram in Paper 4 (Greening et al. 2021) included a state of REM during sternal recumbency and looked specifically for the occurrence of ‘standing NREM sleep’ rather than including a category for ‘standing’ that likely prevented over-estimation of sleep. This highlights the importance of developing an ethogram that is capable of distinguishing between behaviours within which different sleep states can occur (e.g. stand awake vs. stand sleep). The horse population in papers 4 and 5 were also able to see and touch their neighbours due to different stable design to horse populations in papers 2 and 3. Although there is no published evidence to explain how the presence of a sentry affects equine sleep, this could be a valid area for research considering how both stable design and sleep states differed, specifically the low proportion of REM (for further discussion, see limitations section 4.3).

The regression equations proposed in Paper 1 (Greening and McBride, 2022) demonstrate how different sleep states can exist within different behavioural states. The profile of standing NREM sleep met expectations, such that there was zero occurrence of the REM sleep state, and a positive linear relationship detected between NREM sleep and duration of standing behaviour. However, zero REM sleep was also apparent during lateral recumbency, whilst light sleep and SWS all occur during this behaviour. The equations also suggest that lateral recumbency does not always result in REM sleep but sternal recumbency does, and it could be suggested that these horses did not feel suitably comfortable in this posture to assume the vulnerable state of REM. The limitations in the accuracy of inferring sleep from generic behavioural states such as standing without a separate category for standing sleep, or without separating sternal from lateral, must be considered with the increasing use of behavioural observations and accelerometers for 24-hr monitoring of horses each day (Van Herbruggen et al. 2020). Behavioural data derived from video footage however can provide superior information such as limb, head, eye and ear movement that better predict if the animal is awake or asleep during different behaviours. The ethograms within the studies presented here have increased the number of sleep-inferring behavioural categories from three (Paper 2 and Paper 3) to five (Paper 5), to include sleep and wake states within behaviours. However, with an increasingly complex ethogram comes increasing risk of reduced reliability of observer codes/scores (Pierard et al. 2019). Recognising the increased reassurance that rigorous observer training offers, complemented by inter-observer reliability testing, when comparing Papers 4 and 5 with 2 and 3, these elements are strongly recommended for future behavioural research in this area.

The perceived lack of standardised agreement over descriptions and sleep terminology, determined through writing Paper 1 (Greening and McBride, 2022), is also a persistent issue. For example, within EEG research, there has been some hesitation over the prescription of human sleep states to describe equine sleep states, due to the similarities in the EEG profile for wakefulness and REM sleep (Kalus, 2014). Equine studies using PSG do not tend to report the findings of electrooculography or electromyography, which would offer different measures with which to determine sleep states beyond brain wave activity. Utilization of this physiological data could help to overcome some of the difficulty experienced when trying to accurately determine when the horse is transitioning from wakefulness to light sleep. Similar issues in determining between lighter stages of human NREM sleep states have been overcome using measurements of heart rate variability in humans (Fujiwara et al. 2019). Seminal equine research highlights specific differences between the state of drowsiness and the state of sleep (Ruckebusch, 1972) but behaviourally this particular state has not been effectively investigated. For example, Oliveira et al. (2022) explicitly recommended the use of their ethogram which utilised a category of ‘drowsiness duration’ to describe behaviour that had previously been categorised as ‘standing NREM sleep’ in Paper 4 (Greening et al. 2021). Increased drowsiness was described by Oliveira et al. (2022) as a result of reduced recumbency, whilst Paper 4 described increasing standing sleep as a trade-off for a reduction in the occurrence of recumbency, suggesting these behavioural descriptions may indeed be the same. It is important to distinguish sleep from rest, which is simply a state of reduced activity without loss of consciousness or greatly reduced responsiveness (Siegel, 2008). The functional significance of drowsiness versus deeper stages of NREM sleep are unknown and has largely gone unrecorded in existing equine sleep research. It is unknown for example to what extent this state can be utilised during homeostatic recovery of sleep. Further investigation into the behavioural profile of drowsiness could help to enhance the accuracy of future ethograms but simultaneous quantification of sleep states would be required.

Paper 1 (Greening and McBride, 2022) highlighted that stabled horses engage in greater levels of wakefulness during sleep cycles than humans and proposed a novel sleep quality index (SQI) scoring system to measure this. Paper 5 (Greening et al. 2023) trialled the SQI to determine its effectiveness as a measurement of sleep quality in non-verbal animal species. Data indicated the SQI could be used to determine sleep disturbance, as significant differences were established for SQI scores between treatment and control conditions. These patterns differed between sleep states and varied when compared to the differences determined for sleep quantity data, indicating that sleep quality could be affected differently by manipulation of environmental factors. Further investigation is warranted to determine whether the criterion and measurement used for awakenings is appropriate for the horse. Such work may well be hampered by the difficulty in distinguishing between wakefulness, drowsiness, REM sleep and light sleep in both EEG and behavioural data. The function of wakefulness during sleep also deserves consideration in relation to levels of vigilance within stables and how wakefulness can be influenced by different environmental designs as previously discussed in this chapter. For example, lying bouts can be stopped if horses are forced to stand by another horse in shared shelter spaces (Kjellberg et al. 2022) and similar recumbence patterns have been related to social rank in cattle (Galindo and Broom, 2000). Researchers therefore need to recognise the occurrence of arousals/wakefulness that disturb the sleep states. In itself, being able to measure sleep disturbances is important due to the negative outcomes associated with disturbance in a range of species (e.g., Fabbri et al. 2021; Jakubcakova et al. 2012; Mauss, Troy and LeBourgeois, 2013).

The findings from the body of work presented for assessment provide data that supports an average profile of equine sleep. However, recommendations can be made to increase the reliability and accuracy of data collected, particularly through the use of other physiological measurements, for example heart rate (variability), respiratory rates, or electroencephalography. Matching brain wave activity against behavioural states would help to validate the ethogram and develop agreed standardised terminology. Meanwhile, behavioural data could help to further validate EEG outputs due to the novelty of attaching this type of equipment to horses. Recommendations about validation are made in section 4.4.

4.2 Environmental influences on equine sleep

Manipulating the environment results in statistically significant changes to sleep profiles, for example in Paper 4 (Greening et al. 2021) and Paper 5 (Greening et al. 2023), the depth of straw bedding and environmental lighting significantly affected sleep quantity and quality respectively. When looking at behaviour states inferring REM sleep specifically, deeper beds encouraged longer duration recumbency and thus increased the likelihood of REM sleep being achieved. The importance of comfortable sleeping sites has been reported in other species including tufted capuchin monkeys (Di Bitetti et al. 2000), and elephants (Holdgate et al. 2016), and a straw bed reportedly offers more comfort for horses due to increased recumbency time observed when compared to other substrates (Werhahn et al. 2010; Köster et al. 2017). Although paper 2 (Greening et al. 2013) reported no statistically significant differences in the nocturnal behavioural profiles of horses bedded on straw compared to those bedded on shavings, this outcome has been discussed in Chapter 3 and earlier in this chapter. Paper 2 may have benefitted from including effect size (Farrar et al., 2023) as behavioural profiles were consistent with similar bedding-related research (Werhahn et al., 2010; Köster et al., 2017), where straw resulted in a greater proportion of the observed time budget in recumbency. Whilst a straw bed is also beneficial in terms of meeting the ethological feeding needs of horses (Baumgartner et al., 2020), straw pellet bedding has been associated with higher neutrophil percentage in the lower airway of health horses compared to wood pellet and peat, highlighting how it might not be appropriate for horses with respiratory conditions (Mönki et al. 2021). Historically, there are also concerns around the association between colic and husbandry practices that involve straw bedding (Thorne et al. 2005). A pilot study looking at depth of bedding for horses on a shavings bed, underlaid with rubber matting (Modena and Greening, 2019, Appendix 2) found horses with 5cm beds were significantly (P=0.046) less likely to engage in lateral recumbency compared to when kept on a bed 10cm deep. Similar results from Paper 4 (Greening et al. 2021) using a straw bed therefore suggest that the depth of bed, regardless of substrate, is the influential factor.

Paper 4 (Greening et al. 2021) is the first to evidence the impact of bedding depth on equine sleep behaviour and it is recommended that minimum requirements for this element of husbandry are promoted to ensure optimal equine welfare. Using this knowledge, I recommended a minimum standard bedding depth (10cm) to the Yard Manager of Hartpury Equestrian Centre to best facilitate recumbent postures and therefore sleep. The recommendation was first put into practice, in line with veterinary advice with administration of phenylbutazone, for a horse that was displaying partial collapse behaviour and had not been recorded in a recumbent position during our observations. The horse was observed to lay down as soon as these changes were made and the minimum bed depth at Hartpury was subsequently implemented. Although observing recumbency doesn't confirm the occurrence of sleep, the partial collapse behaviour also ceased suggesting environmental alterations were beneficial in this case. Future equine sleep studies should also be careful to report how horses are provided with environments meeting minimum standards (e.g., bedding depth) that may influence sleep. Environmental manipulation resulting in zero recumbency for a number of consecutive nights, and that is different to the normal behavioural profile of the individual horse, could be considered a sleep deprivation causal factor. Horses that are not lying down should in all cases be reported to the care giver which may be reason to withdraw these horses from the study population.

The results of Paper 4 (Greening et al., 2021) and Paper 5 (Greening et al., 2023) provide evidence that artificial light can influence the occurrence of sleep behaviour. In these studies, fluorescent tube lighting was 'warm white' with primary spectral peaks of 490 nm, 550 nm and 625 nm, the with the first two peaks being within the spectral sensitivity range of the horse (Carroll et al. 2001). Changes between light and dark cycles provide strong cues for the onset of sleep, and these are mediated by cells using signals from the superchiasmic nuclei (SCN) to stay in rhythmical phase (Murphy, 2019). In human research, exposure to room light (<200 lux) before bedtime suppressed melatonin and resulted in later melatonin onset when compared to individuals exposed to dim light (<3 lux) (Gooley et al. 2011). The H_1 for Paper 4 (Greening et al., 2021) predicted that horses may achieve less sleep when lights were left on overnight, due to the way in which light suppresses melatonin secretion. Horses in these studies were accustomed to a routine in which fluorescent lights (180 lux) were turned off (2 lux) at 8pm. Whilst no overall differences in total sleep time were detected, looking at different sleep states, significantly more NREM and significantly less REM sleep was observed under treatment light conditions compared to control light. Significant interactions between treatment light and day were also detected by sleep quality scores in Paper 5 (Greening et al. 2023). In retrospect, measuring the timing of sleep episodes and REM sleep within the 24-hr period would have yielded sleep onset metrics that may have provided more meaningful data for comparison. However, the effects of suppressed melatonin on REM sleep are more likely mediated by other mechanisms associated with sleep onset, such as changes to core body temperature (Dijk and Cajochen, 1997). Although more research is required, changes to duration of equine sleep states seem indicative of changes in circadian responses due to exposure to artificial light. Light intensity could therefore become another consideration when designing a stable and associated husbandry practices that promote equine sleep behaviour in the future.

The use of music has been proposed to provide masking (Rørvang et al. 2020) and relaxing effects for zoo animals (Wells and Irwin, 2008), and horses (Neveux et al. 2016), similar to the suggested effects of Paper 3 (Hartman and Greening, 2019). Significant differences in the nocturnal equine behavioural profile, with a longer-term affect apparent in the second A phase, were reported. However, the classical music was only played between 8pm and 1am on request of the yard manager whereas horses tend to adopt recumbent positions more so between midnight and dawn (Houpt et al. 1986). Furthermore, the study lacks some validity as observations were only carried out overnight so it is unknown whether horses were able to repay any sleep debts incurred at night during quiet periods on the yard in the day. Finally, the study lacked power to fully support H_1 acceptance. Further research is warranted over 24-hr however, considering how music affected nocturnal profiles that may indicate how it countered novel auditory stimuli. This could have functional relevance during periods of acclimatisation to novel stabling. For example, the lack of shared vigilance and/or the occurrence of novel stimuli within individual stabling has been linked to the need for a period of acclimatisation when husbandry practices change from turnout to stabling (Ruckebusch et al. 1970; Fewings & Greening, 2016, Appendix 2), and music may offer an additional factor within the nocturnal environment which could help horses adjust to overnight stabling.

The papers presented within this thesis cover common elements within equine husbandry including lighting, the sound environment and bedding, for stabled horses. Further consideration in future studies is required to look at various influential external factors and how these intersect and/or impact upon sleep, studied in the stable and in the field, ideally including free-living horse populations. These are discussed as limitations to the published work in the following section.

4.3 Limitations

The quasi-experimental nature of Papers 2 to 5, mean that results could be influenced by a number of confounding variables. For example, all of the studies presented for assessment were conducted during the autumn and winter seasons of the Northern hemisphere where temperatures remain below 15℃. Chung et al. (2018) observed different equine sleep profiles during the monsoon season of the Southern hemisphere in temperatures averaging 29℃, highlighting how transferability of results beyond colder months is questionable. Thus, seasonal norms should be established and reflected upon when interpreting equine sleep profiles, especially in the domestic environment. Environmental temperatures may also be a contributing factor, considering that a reduction in core body temperature acts as a strong cue for sleep onset (Szymusiak, 2018). Horse sweating is reported to be significantly different in rugged vs. non-rugged horses indicating reduced efficiency using evaporative heat loss as a cooling mechanism (Padalino et al. 2019). All of the horses in Papers 2 to 5 were rugged according to normal practice and individual requirements, for example whether clipped or not. It is possible rugged horses in the reported studies may have experienced issues with latency to sleep onset due to the way rugging may affect core body temperature, making transferability of results to un-rugged horses difficult. Due to the complex interaction between circadian control of sleep, melatonin secretion and core body temperature, sleep onset may also have been affected by rugging and/or affected the results of studies looking at the influence of artificial lighting. More research is warranted to establish how season, environmental temperatures and rugging practices (rugged vs. no rug) affect the sleep profile through the core body temperature zeitgeber. Additional zeitgbers such as exercise and food on sleep should also factor into studies as potentially confounding variables in their own right. For example, in a pilot study investigating the influence of sleep on affective state, it was proposed that decreased sleep may have affected appetite of study horses and thus increased goal-directed motivation using food as a reward (Matthews et al. 2021, Appendix 2). Currently, little is known about the interaction between forage or forage availability and sleep profiles.

Horses in all the studies presented here were accustomed to the stable in which they were observed, including variables such as bedding substrate/depth, for more than five months offering reassurances that they were acclimatised to their environment. None of the horses were reported to display any stereotypic behaviour, which has been shown to influence the nocturnal behavioural profile (Clegg et al. 2008). Horses within the sample population of Greening et al. (2021) were able to engage with each other both physically (i.e. touching noses) and visually as each stable was separated by bars, and thus enabling some fulfilment of telos due to the social nature of horses. This was not the case for horses that participated in the studies published by Greening et al. (2013) and Greening et al. (2019) and the sleep profiles (Table 5) for these horse populations were very different. A pilot study (Hadley and Greening, 2014, Appendix 2) comparing nocturnal behaviour of horses stabled with and without bars between stables found differences in the orientation of the horse within the stable and duration of recumbent behaviour. Stable design therefore appears to influence the occurrence of equine sleep, and should also be taken into consideration in future research especially with the increasing interest in ‘social stable’ designs. The influence of the previous social history of the horse also requires exploration. Although the majority of research into early life experiences focus on how this affects behavioural interactions with human handlers, more affiliative behaviours are recognised from group-housed horses (Søndergaarda and Ladewig, 2004; Hausberger et al. 2008). A horse that has not learnt to be a horse may struggle to engage in positive social interactions with other horses in confined spaces (e.g. the stable) and this may influence how much rest the animal achieves.

Another limitation to current knowledge about sleep is an appreciation of the how the superior equine sensory systems could influence sleep. Whilst to some extent, paper 3 investigated an aspect of auditory perception, there are so many elements of the five sense to consider. Evidence is growing in the field of sound environments in zoos (e.g. Rose et al. 2020) but very little is known about the sound environment of a yard overnight. For example, there are high frequency sounds horses can detect that could inadvertently be making the nocturnal environment noisy beyond our perception. Within the video data observed for the studies presented here, there appear to be times when sleep is interrupted because of some stimuli external to the stable and these could be auditory or even olfactory in nature. Being able to record the sound environment at night would provide insight into future sleep quality data. Individual differences in sleep profiles can be due to a plethora of variables. Although the studies presented here largely controlled for age, sex, feed rations, and workload, and reported no stereotypic behaviour within sample populations, further individual differences require consideration. For example, prior stabling and/or social experiences considering stable design might influence the way in which the horse feels able to rest in a confined space such as the stable. Short sleep duration is reported as a risk factor for the development of obesity in humans (Beccuti and Pannain, 2011), such that future studies may wish to consider body condition or BMI scores of horse study populations. Very little is known about whether sleep apnea occurs within obese horses and may be an area for future research. Clinical conditions such as pars pituitary intermedia dysfunction (PPID) may also be implicated in sleep disruption due to the association with Parkinson’s Disease (PD) (Fortin et al. 2020). In humans, sleep disturbances are common in PD and comprise the entire spectrum of sleep disorders (Stefani and Högl, 2020). Different types of medication can also influence the occurrence of sleep. For example, elderly humans sleep complaints are often comorbid with medical and psychiatric illness and the medications involved in treating these (Ancoli-Israel et al. 2008), whilst different beta blockers result in different self-reported sleep quality scores (Yilmaz et al. 2008).

The use of behavioural data requires some degree of verification. Earlier studies employed just one observer and would have benefitted from consensus through more observers and inter-observer reliability testing. The ethogram utilised in Papers 4 and 5 has been used successfully to train multiple researchers to correctly identify behaviour based on clear, detailed descriptions resulting in high inter-observer reliability scores. Papers 4 and 5 could have conducted intra-observer reliability tests also to further increase reliability of results. The ethograms used in the studies presented here have evolved over time. Initially recumbency (both sternal and lateral) were linked to the occurrence of sleep however in later studies the ethogram was further refined using behavioural data provided in seminal EEG studies (Williams, Aleman et al. 2008; Dallaire and Ruckebusch 1974; Dallaire 1986). Specifically, this resulted in the inclusion criteria for standing sleep, the addition of a sternal REM sleep state, and sleep awakenings. These additions allowed more complete behavioural profiles to be recorded however, the ethograms in the presented studies do not include a state of drowsiness, unlike Oliveira et al. (2022). Further additions could include behaviours linked to spontaneous collapse; however the next step will be to validate the existing ethogram using an objective measurement such as EEG or heart rate variability.

Similar to the way in which multiple grading systems exist for the purposes of grading lameness (Gómez Álvarez and Oosterlinck, 2023), different sleep ethograms may be appropriate under different experimental situations. However, clarity over terminology and definitions within an equine sleep ethogram is undoubtedly of critical importance, both to increase internal and external validity of video data analysis and in terms of analytical efficiency as observation on multiple/consecutive days across 24-hr involves large volumes of data. Typically, 24-hr can take 4+ h to review. Whilst technology has been used in animal behaviour research since the 1970s, increasing emphasis has been placed on the possibilities afforded by Artificial Intelligence (AI), for example to overcome the burden of video data analysis and improve monitoring of captive animal populations (Congdon et al. 2022). Thus, standardisation of terminology becomes increasingly important. Getting terms right for different measurements of sleep was a particular challenge when writing Paper 1, hence the inclusion of the glossary that appears within it. For example, we began by referring to the occurrence of sleep in bouts, however looking at human research we realised we were likely looking at sleep episodes borrowing from the work of Czeisler et al. (1980). The clarification of terminology was especially important to enable differentiation between sleep cycles and also for when we began to look at the duration of specific sleep states (sleep sequences). These terms linked through to our understanding of arousals that can also be described in a variety of ways e.g. micro-arousals when interrogating EEG output specifically (Halasz, 1998). Based on human research (Merica and Gaillard, 1986), we interpreted arousals as anything lasting less than three minutes in duration, after which the individual was considered to be in a state of full wakefulness and not just aroused from a state of sleep. Awakenings within sleep sequences longer than three minutes would are described as wakefulness rather than an arousal. Exploring the terminology associated with sleep research also highlighted there were a number of ways beyond the cumulative duration of sleep states in which equine sleep measurements could be investigated e.g., the frequency and duration of REM-NREM cycles.

The installation and maintenance of a high-quality CCTV system is an expensive consideration but enables easily accessible and clear data to be generated. There are many advantages of using cameras over live human observers such as:

- Enabling the observer to rewind and review behaviours to increase accuracy of results;
- The horse is not influenced by the presence of an observer;

- Use of infra-red offers a non-invasive method of nocturnal observation, and facilitates; increased accuracy for behavioural interpretation through high-quality imagery, and;
- More time efficient (e.g. multiple observers required for multiple horses, live overnight observation impractical).

However, video footage can still be affected by blind-spots that can hamper interpretation of behaviour. Introducing a category of ‘unknown’ when it is not possible to interpret the behavioural state reduces the likelihood of over or under-estimating the occurrence of behaviour. The published studies did not use the Observer software or Behavioural Observation Research Interactive Software (BORIS). These programmes are continuously updated, suggesting some limitations for the user. There is the additional expense of having to buy Observer software from Tracksys. Interobserver reliability tests go some way to address this, but human error is still an issue that requires smoothing of data before analysis, for example, where overlapping times or gaps in data are detected. This can usually be rectified by reviewing video footage, thus careful and accurate cataloguing of video files is required.

4.4 Recommendations and future research

Following critical review of the studies presented here for assessment, a number of recommendations can be made to future researchers in this field. The first, is to conduct observations over a 24-hr period on multiple days, to develop a representative average behavioural profile for each individual in the sample population. Power calculations are recommended to evaluate and justify the sample size (Eng, 2003) and to ensure statistical certainty around acceptance or rejection of hypotheses. The effect size should be considered where large variation within small cohorts is observed. To overcome limitations of convenience sampling, studies could be replicated at multiple sites but standardised routine/husbandry should be agreed, and confounding factors must be carefully considered. As a minimum, based on the research presented here, future studies should clearly state light intensity and exposure, the details around the light exposure routine, the season and relative temperatures, and bedding depth. But all of the confounding factors should be addressed e.g., excluding stereotypic horses. Repeated measure cross-over design, including wash-out periods for experimental studies, using continuous focal sampling must be considered gold standard. To further overcome the potential for large standard deviation and/or S.E.M., indicative of large individual variation, within-subject testing should be utilised within the study design. The ethogram must include wake and sleep states for each behaviour category, that are carefully described and include plates for ease of interpretation/ use by observers. Sleep quality as much as quantity should be included in data collection. Observer training is fundamental and inter-observer reliability tests must be reported to evidence consensus amongst observers, especially where multiple are employed. Ideally intra-observer tests should also be conducted where only one observer reviews video footage. Thus, the studies and the critical discussion around them presented within this critical commentary has helped to identify a framework upon which future research can be conducted to enhance reliability and validity of findings. It is important to recognise the novelty of some approaches but also the opportunity to apply these to a broader variety of animal species.

The results of these studies also present recommendations for horse care givers. To promote recumbent behaviour, and thus sleep, deep beds (equal to or greater than 10 cm in depth according to the findings of studies presented here) are recommended. Consideration should also be given to the intensity of and exposure to light, so as to promote a routine that does not limit normal circadian release of melatonin. Future research (see section 4.4.1) aims to elucidate further detail on this. Horse care givers should remain mindful that these are only two of a number of influential environmental factors and above all else they should be observant for signs that confirm the horse has laid down over night, for example bedding in mane/tail or stables stains on body/rug. Even these signs however do not confirm that the horse has slept, and thus excessive or unusual fatigue in the stable or during exercise should not be written off as ‘lazy behaviour’.

The body of work presented here is applied research and much of it is quasi-experimental. A key aim to this approach was to provide evidence-informed practice that could be adopted within the wider equine industry. To achieve this, knowledge, research, and novel measurements of equine sleep have been shared with a variety of audiences (appendix 2). These have included:

- The lay horse owner via a webinar for drdavemarlin.com, in consultation with the yard manager at Hartpury Equestrian Centre, and through a number of lay press articles;
- Early career researchers via webinars for the Animal Welfare Slack Workspace for the International Society of Applied Ethology;
- Established researchers via presentations at the Surrey Sleep Research Centre and the 52nd congress of the International Society for Applied Ethology;
- Students via the Equine Performance Conference hosted by Bishop Burton, alongside my day to day teaching and project supervision.

Anecdotally, graduates that I have taught or supervised have described how yard owners adopted a deeper bed philosophy, based upon the knowledge we had created and shared. To further disseminate the findings from the studies presented here, a closed Facebook group page was set up (https://www.facebook.com/groups/753699465073014). The aim of this group is to build a community of academics and students with the same research interests, to facilitate sharing of research knowledge and experience, and publicise findings of research to wider audiences.

4.4.1 Areas for future research

A number of areas for future research exist but critically, behavioural observations require validation against EEG data. Being able to more accurately determine a behavioural state against brain wave activity will increase the validity of the ethogram and help to standardise terminology and categories used within it. As part of a bid writing team, in collaboration with Aberystwyth University and members of both the Sleep Research Centre and Department of Computer Science at the University of Surrey, we propose a validation exercise, cross-referencing EEG and behavioural data, that can then be used to train artificial intelligence to interpret video and/or EEG data, with the aim of increasing both accuracy and efficiency of future equine sleep research. Such measurements can then be used to determine the impact of sleep disruptions on equine welfare and performance, and offer more reliable and valid measurements with which to further explore environmental factors. More accurate sleep metrics are required with which to study the resilient and opportunistic nature of equine sleep. Novel metrics of sleep quality also require validation that can be used to investigate compensatory mechanisms employed by horses to achieve optimal sleep over time. To quantify sleep as poor, outcome measures need to be developed and tested for horses such as excessive daytime sleepiness and rest activity cycles (e.g., Hajek et al. 1989). Whilst the use of EEG in horses has improved (e.g. it is now ambulatory - Williams et al. 2008), the technology requires further development due to the limitations around application and data output (see discussion in Chapter Two). Thus, currently, a number of opportunities exist to enhance the methods used to measure equine sleep that should aim to provide agreement over (behavioural) sleep state definitions and associated terminology, ensuring accurate interpretation for future use in sleep research and potentially for practical application in industry. This is important as accessibility to EEG equipment is itself a barrier to the furtherment of knowledge in the field of animal sleep research. An ethogram offers an accessible, non-invasive method with which to record and quantify the occurrence of sleep behaviour that could be utilised by the lay horse owner. However, expanding the types of measurement we use to quantify the occurrence of sleep in horses is also important. Duration measurements seem rudimentary, as though there is a perfect amount of sleep horses need to achieve on a nightly basis. Measurements achieved thus far reflect the domestic horse population, which perhaps shouldn’t be a baseline or what is thought of as normal. This change in approach to measurements could be applicable across animal species, and there is an opening to apply the novel measurements of sleep quality to mammalian species beyond the horse, providing insight into sleep quality for other species as suggested in Paper 1.

The impact of lighting on circadian control of equine sleep deserves further investigation. In collaboration with University College Dublin and the Royal Agricultural University, I was principle investigator on a recent project comparing the influence of a standardised LED (red/white) light system to traditional fluorescent lighting system on equine sleep behaviour and also circadian clock gene expression. We are currently in the final stages of data curation before data analysis is scheduled to begin. The study design followed the recommendations made within this section to enhance the validity and reliability of results so we may increase our knowledge of the influence of lighting on circadian control of equine sleep. The study could help to inform current stable lighting systems and could also be adapted and replicated in other domestic animal species. Linked to circadian control is the influence of core body temperature. Equine husbandry regularly includes clipping and rugging horses that may influence core body temperature and thus latency to sleep onset and sleep profiles. Data from a preliminary study conducted at Hartpury (Long and Greening, 2023, Appendix 2) suggest that the under-rug temperature of horses is associated with different nocturnal behavioural profiles. Future studies may wish to use test the validity of under-rug temperatures and rectal temperature as inferred core body temperature metrics to determine whether the sleep profiles are affected by different rugging scenarios.

Future studies may choose to include measurements of stress including glucocorticoids, facial action units (e.g. equine FACs, Wathan et al. 2015) and thermal cameras looking at eye temperature (Kim and Cho, 2021). These could be used for example to investigate the bidirectional relationship between sleep and stress (Paper 1). For example, does sleep influence performance after training with regard memory consolidation but also, how might novel stress-inducing scenarios influence sleep for example, training reinforcement schedules where the horse experiences concurrent and opposing tactile forces or relentless pressure. The link between early life social experiences, stress and the development of stereotypies also require investigation, in terms of how the horse copes within the stable. For example, stereotypic horses spend less time resting (McGreevy et al. 2001), and consideration should be given to how these horses self-limit and compensate for reduced.The opportunity to use micro-expressions and facial action units, alongside blink rate, might also be useful to distinguish between states of drowsiness and light sleep. Deep learning and facial units have been used to detect fatigue in humans whilst driving (Sikander et al. 2020). This would involve careful placement of cameras to enable capture of this behavioural data, as cameras used in sleep studies tend to be erected high above the stable to enable a field of vision encompassing the whole of the stable.

CHAPTER FIVE

Conclusions

Fundamentally, the sleep state is a behaviour that occurs within all mammalian species (Cirelli and Tononi, 2008). Species-specific profiles of sleep behaviour indicate how animals have evolved differently and respond differently to the homeostatic pressure to sleep. Individual differences within each species also indicate how vulnerable sleep is to both homeostatic and environmental pressures. The functional importance of sleep is explained by a number of consequences associated with sleep loss. However, for animals beyond human and rodent models, little is known about how reduced sleep impacts upon welfare and wellbeing.

Using published data pertaining to stabled horses, equine sleep appears to follow the characteristics of other large ungulate prey species in the domestic environment, being polyphasic in nature and taking up a relatively small proportion (roughly 17%) of the 24-hr time budget. Results from this collection of studies demonstrate how equine sleep is susceptible to changes in the domestic environment, highlighting for example how bedding depth and lighting can be manipulated to facilitate equine sleep (measuring both quantity and quality). These are easy changes that can be made to enhance equine welfare. However, a number of additional variables require consideration, for example the presence (or not) of companions and stable design relative to social interaction/the occurrence of sleep, to ensure robust and accurate data especially in quasi-experimental studies. This provides a wealth of opportunities for future studies alongside avenues for additional investigation such as the relationship between training of performance horses and their sleep profiles, clinical disease such as PPID and obesity, the influence of the sensory environment on the occurrence of sleep, and the influence of clipping/ rugging on core body temperature linked to the onset of sleep.

The field of equine sleep research currently however faces a number of challenges such as limited access to resources, including funding for research and accessibility of gold standard equipment. There is growing interest in the area of equine sleep but improvements to methods of measuring equine sleep are required. These are identified within the critical commentary with suggested recommendations to enhance the quality of future equine sleep behaviour research. The use of behavioural data is justified as a non-invasive, accessible method with which to measure equine sleep, but future work must now also explore opportunities to cross-reference behavioural data alongside digital technology, such as electroencephalography, to enhance the reliability and accuracy of results to enhance their application within equine welfare assessments and measurements. This equipment could also provide rich information about the transition into sleep compared to the state of drowsiness and thresholds of wakefulness. This and exploration of the occurrence of light sleep could also yield important data on the compensatory mechanisms that can be employed to make up for sleep loss, considering the different roles of horses in society especially those that travel.

Observing horses in their stables across 24-hr+ has enabled us to develop a representative average sleep profile. However, information is required on horses that have greater free choice than stabled horses, including those at pasture and those in the free-living environment. Such data can be used to establish a normal sleep behaviour profile. An observer watching 24-hr behavioural video footage cannot help but to develop an appreciation of the nature of the horse in the stable, in terms of the limited choices it is provided with and social interaction it experiences. Such footage is valuable educational material to help motivate changes within management practices/systems to better cater for the horse’s telos within the stable.

Although currently we can describe horses as resilient and adaptable in the context of achieving sleep, standardised and reliable methods with which to research sleep are required to better understand sleep occurrence, adaptive/ compensatory mechanisms, and the impact of sleep and disrupted sleep on emotional/ cognitive/ performance outcomes. The aim of increasingly accurate methods of measuring sleep should be to advance our understanding of equine sleep (using a variety of measurements including quantity and quality) to address a relatively unknown element of equine welfare.

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7.0 APPENDICES

Appendix 1. The collection of published work

This appendix presents the published output of research projects conducted between 2013 and 2023, stating the separate and distinctive nature of my contributions to each project using CRediT taxonomy, and including proposals for future contributions. These publications help to demonstrate how I meet the requirements of the Doctoral Descriptor (Table 1).

Paper 1. A review of equine sleep: implications for equine welfare

1.1.1 Published as

Greening, L. and McBride, S, D. (2022) A review of equine sleep; implications for equine welfare. *Frontiers in Veterinary Science: Animal Behaviour & Welfare*. 9, p.916737.

1.1.2 Objectives of the study

- Review existing research in the field of equine sleep for comparison against other species-specific sleep research to highlight key features of equine sleep.

- Highlight areas for future research, including the proposal of novel measurements.
- Explore the link between sleep and equine welfare.

1.1.3 Why this journal

Frontiers in Veterinary Science is a global, peer-reviewed, Open Access journal with an impact factor of 3.471. It describes itself as a networked and collaborative journal that takes a 21st century approach to communicating progress and innovation to both the specialist and to the wider audience of readers in the field of veterinary science. The animal and behaviour specialty section looks for submissions that address any, or all, of the three philosophical approaches to animal welfare; "naturalness", affective states, and basic health and functioning. It especially aims to contribute to improvements in animal welfare through the identification of potential risks to welfare, and their subsequent reduction or removal, through optimal management and housing practices. The aims and content of the review paper met these eligibility criteria. Impact statistics from Frontiers report that, from publication in August 2022, the article has been viewed 4,930 times (83% more views than all Frontiers articles) and has been downloaded 1,064 times (77% more downloads than all Frontiers articles). At the time of writing, the article has been cited once. The journal did indeed provide an excellent platform from which to share the review and, following publication of the review, I was invited to provide talks on the topic of equine sleep by the University of Edinburgh and the University of Surrey. I have also delivered webinars for the lay audience for Dr David Marlin.

1.1.4 Author contributions

	Linda Greening	Sebastian McBride
Conceptualization	50%	50%
Methodology	50%	50%
Formal analysis	50%	50%
Resources	50%	50%
Writing the paper	50%	50%
Review/editing	75%	25%
Visualization	50%	50%
Supervision	0%	100%
Project administration	50%	50%

1.1.5 Future contributions

Within the review a number of areas were highlighted as important in terms of future research including the need to determine what 'normal' equine sleep is, describing the duration and frequency of NREM/ REM cycles, or sequences of wakefulness, which would yield novel information providing a deeper understanding of equine sleep quantity and quality. I have since published a paper reporting on a novel measurement of sleep quality in a non-verbal animal species and have championed the inclusion of sleep interruption measurements in current projects that I am involved in.

The review also highlights the opportunity to improve the accuracy of sleep quantification in animals through mobile wireless EEG and polysomnography (PSG) equipment. I am currently a member of a collaborative bid-writing team, seeking funds from the Morris Animal Foundation for a project to utilise EEG equipment from the University of Surrey, and to explore how video analysis by artificial intelligence could replace human observations.

1.2 The published article in full

Abstract

Sleep is a significant biological requirement for all living mammals due to its restorative properties and its cognitive role in memory consolidation. Sleep is ubiquitous amongst all mammals but sleep profiles differ between species dependent upon a range of biological and environmental factors. Given the functional importance of sleep, it is important to understand these differences in order to ensure good physical and psychological wellbeing for domesticated animals. This review focuses specifically on the domestic horse and aims to consolidate current information on equine sleep, in relation to other species, in order to (a) identify both quantitatively and qualitatively what constitutes normal sleep in the horse, (b) identify optimal methods to measure equine sleep (logistically and in terms of accuracy), (c) determine whether changes in equine sleep quantity and quality reflect changes in the animal's welfare, and (d) recognize the primary factors that affect the quantity and quality of equine sleep. The review then discusses gaps in current knowledge and uses this information to identify and set the direction of future equine sleep research with the ultimate aim of improving equine performance and welfare. The conclusions from this review are also contextualized within the current discussions around the "social license" of horse use from a welfare perspective.

Keywords: equine, horse, behaviour, sleep cycle, sleep quality, sleep quantity, sleep deprivation, welfare

Introduction

The primary aim of this review is to create a greater understanding of equine sleep and to discuss and identify its role in equine welfare. Although a fundamental process in all mammals, sleep is not commonly considered as a factor that affects animal welfare (1). For example, in many of the animal welfare frameworks and guidelines, there are specific references to factors such as sufficient air and light, food and water, adequate spaces for movement and contact with conspecifics, but not to creating environments that facilitate maximum levels of species-specific sleep. In addition, whilst some standards have been written to ensure the provision of species-appropriate spaces to rest (e.g., Department for the Environment, Food and Rural Affairs, UK), very little information exists about how to implement this in practice. Furthermore, very little information exists that describes species-appropriate spaces to promote rest in the domestic environment. In this respect, more consideration needs to be given to understanding species-specific sleep requirements in a domestic setting and how best to accommodate these in order to help optimize animal welfare.

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

Definition, sleep stages and variation of animal sleep

Definition and Different Sleep Stages

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

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

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

Micro-Arousals, Wake Sequences and Wakefulness

Sleep stages are an ever-changing dynamic process due in part to the cyclic nature of sleep and the occurrence of arousing stimuli in the internal and external environment. Stages of sleep are, thus, often interrupted by either micro-arousals or slightly longer wake sequences and can be broken completely into a full state of wakefulness. During sleep, the brain continues to interpret information such that arousal eliciting factors work against sleep promoting forces. When there is higher pressure to sleep during the descending loop of the sleep cycle (N1–N4), phasic changes from sensory input often don't disrupt sleep and can in fact result in deepening of SWS (15). During the ascending loop of the cycle (N4 to REM), however, when there is lower sleep pressure, sensory input has a more pronounced modifying and disrupting influence on sleep. These phasic changes are referred to as micro-arousals (MA), defined as momentary adaptations to vigilance levels in response to internal and external sensory input. Within the EEG profile, MA are seconds in duration and are associated with the emergence of K-complexes (high and low voltage waveform) (15). According to the American Sleep Disorder Association, a micro-arousal should be scored when there is an increase in EEG frequency for 3 s or more but that K- complexes should not be scored as arousals unless accompanied by increased EEG frequencies (16).

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

The definition of full wakefulness from sleep relates to the probability of re-entering sleep within a specified time period. There are a number of specific neurophysiological events that occur on entering full wakefulness that are largely driven by the activation of efferent hypocretin neurons from the lateral hypothalamus (18). One of the main differences between sleep and wakefulness is increased sympathetic tone and decreased parasympathetic tone that maintains most organ systems in a state of action or readiness. In humans, spontaneous awakenings lasting longer than 3 mins are generally acknowledged as a state of wakefulness that modifies the sleep cycle (19).

Ecological and Biological Factors Affecting Mammalian Sleep

A comparison of average total sleep time between polyphasic mammalian species reveals large-scale differences and several factors have been proposed to explain this (Table 1). One of the primary factors is body mass which negatively correlates with total sleep time (22, 23). This is considered to reflect the risk of predation for larger prey species which, due to their size, are required to sleep in exposed locations (24), and thus tend to not enter into prolonged periods of deeper stages of NREM sleep. The severity of predation and safety of sleeping place are often scored from one to five using a sleep exposure index; a score of one is given to a sleep site that is well-protected with minimum predation, a score of five is a sleep site of high predator risk/exposure (20, 22) (Table 1). Mammalian prey species sleeping in riskier locations are commonly observed to engage in lower proportions of REM sleep, as this sleep stage can only be attained in the recumbent position (20).

Table 1. Comparison between sleep profiles and biological characteristics of various bi-hemispheric species, ordered by high to low sleep exposure indices (3, 20, 21).

Species	NREM (h/day)	REM (h/day)	Total sleep (h/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 (*Equus caballus*)	2.98	0.67	3.85	534.0	260,000	0.21%	65,000.0	337.0	5
Cow (*Bos taurus*)	3.2	0.8	4.0	460.0	272,000	0.17%	46,240.0	280.7	5
Sheep (*Ovis aries*)	3.3	0.6	3.8	100.0	30,000	0.33%	10,200.0	146.3	5
Goat (*Capra aegagrus*)	4.7	0.7	3.8	115.0	29,000	0.4%	6,840.0	163.9	5
Pig (*Sus scrofa*)	6.4	1.9	8.4	180.0	75,000	0.24%	8,250.0	117.0	4
Dog (*Canis familiaris*)	7.1	1.6	10.7	70.0	14,000	0.52%	–	62.0	2
Cat (*Felis silvestris*)	10.0	3.2	13.2	28.4	3,260	0.87%	2,314.6	63.9	1.5
House mouse (*Mus*	11.9	1.3	12.8	0.4	21	1.90%	69.7	21.2	1.33
Human (*Homo sapiens*)	6.1	1.9	8.0	1,320.0	62,000	2.13%	14,700.0	280.1	1

Sleep exposure index concerns a measure of predation risk based on vulnerability associated with sleep site. The index ranks relative exposure of a given species at its typical sleep quarters in the wild, where 1 = low risk e.g., caves/burrows and 7 = high risk, e.g., open water (22).

Another factor influencing sleep time is the degree of species encephalization which positively correlates with total REM sleep and supports the role of REM in memory consolidation (22). However, this is a complex relationship due to additional influencing factors including whether the species bears precocial or altricial young (21). For example, precocial species that experience longer gestation periods have high levels of brain maturity at birth so that offspring can adapt quickly to the external world (25). This would suggest that precocial species would have higher levels of REM sleep, however, because these animals tend to live in exposed environments of high predation risk, the total level of REM sleep tends to be lower (23). In contrast, neonates of less developed altricial species display larger amounts of REM sleep (7) potentially as the result of being maintained in a protected safer environment (21). Research has also shown a negative correlation between total sleep time and basal metabolic rate (BMR) with the hypothesis being that species with greater energy expenditure relative to their size are required to make a trade-off between sleep and foraging, with foraging superseding sleep (7, 26). Additionally, higher BMR is linked to higher levels of restorative neurophysiological processes thus potentially reducing the need for long periods of restorative sleep (22).

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

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

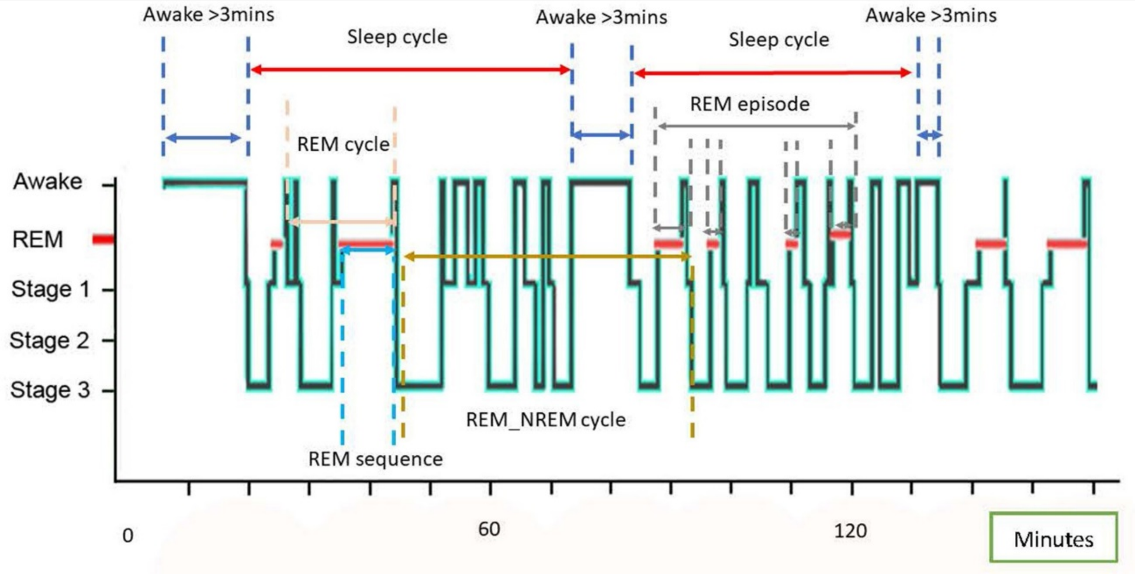
Comparison of Human and Equine Sleep Stages

In the awake state, human EEG patterns present as two types of waves, beta and alpha. Beta waves are associated with full wakefulness and have the highest frequency (13–30 Hz) and lowest amplitude (10–30 µV) (31). Beta wave forms have few rhythmic components and are thus more dyssynchronous than other wave forms (32). Beta wave forms have been measured in the horse during wakefulness and active states (33). During more inactive states of reported relaxation in humans, alpha brain waves predominate (34). These brain waves are slower (8– 13 Hz) with increased amplitude and are more synchronous in nature (35) with occasional bursts of high frequency (beta) wave forms associated with periodic alertness (34). Alpha waves have been measured in the horse (33, 36) and are associated with the behavioural markers of weight bearing on 2 fore and 1 hind limb with the head above withers (36). The transition into the sleep state is associated with a further reduction in brain wave frequency to a theta wave state (3–7 Hz) but with occasional presentation of alpha waves (8–13 Hz) (37). This is referred to as the N1 stage of NREM sleep and is often preceded by a state of drowsiness, particularly in animals (38). Full sleep onset is typically considered to occur at the N2 stage of NREM, here the EEG profile is similar to N1 (theta waves, 3–7 Hz) but now also contains sleep spindles and/or K complexes with at least 1 sleep spindle or K-complex occurring per 30 s on a N1 background (10). In humans, the N2 sleep stage tends to predominate during periods of sleep across all ages (39) and this sleep stage is routinely observed in the horse (36, 39). The next two stages of NREM sleep (N3–N4) are collectively referred to as SWS and are characterized by slow wave oscillations (0.5–2.0 Hz) of high amplitude (75 mv), referred to as delta waves. The N3 stage of SWS is defined as having between 20 and 50% delta waves whereas the N4 stage contains above 50% (40). More recently within the human literature the N3 and N4 stages of sleep have been merged (41). N3 but not N4 NREM sleep has been explicitly reported in horses and is commonly referred to as SWS (36, 42).

REM sleep is often referred to as “paradoxical” sleep because of the mixed frequency (3–30 Hz), low amplitude (10–30 uV) nature of brain waves associated with this sleep stage that is often also observed during wakefulness (40). REM sleep is associated with tonic suppression of skeletal muscle tone and reflexes through inhibition of the brain stem and spinal motor neurons (43) but with episodic bursts of rapid eye movements and muscle twitches arising from ponto-geniculo-occipital brain waves (37). REM sleep has been observed in the horse and is associated predominantly with lateral recumbency (reflecting full muscle atonia) (42) and also sternal recumbency with the head propped on the floor (36). In some instances, short episodes of REM sleep occur in the horse whilst standing accompanied with complete loss of tone in the neck muscles, head dropping to just above the floor and the horse buckling forelimbs with the muzzle hitting the floor (36). REM sleep in the horse is also associated with rapid eye movements and rhythmic ear twitching (36). In order to characterize and compare sleep profiles within and between species, standard descriptive terminology of the different sleep states in accordance with previous definitions is presented in Table 2, alongside some additional terminology that may be particular to the equine sleep profile.

Table 2. A glossary of terms and standard definitions of sleep states (19, 44, 45) with visualization of specific terms using an example equine hypnogram.

Term	Definition
Sleep onset	An episode of sleep lasting >1 minute and containing at least 1 min of sleep other than N1 NREM (19).
Sleep cycle	The interval from sleep onset (see definition above) to the start of a period of wakefulness that is greater than 3 mins (19), containing sequences of REM, NREM and wakefulness.
Epoch	A short interval of arbitrarily defined length (usually 20–60 s) of sleep stage normally determined from a polygraphic sleep recording (44).
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 (46)
Sequence	A consecutive series of epochs in the same sleep stage (44) 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 (44) e.g., REM episode is a series of consecutive sequences of REM sleep which are separated by less than 15 mins of NREM sleep or 3 mins 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 (45).
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 rhythms.	A rhythmically occurring natural phenomenon which acts as a cue in the regulation of the body’s circadian



Comparisons of the Human and Equine Sleep Cycle

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

A normal pattern of human sleep is often difficult to characterize due to individual sleep duration variability, compounded by factors such as duration of prior waking, staying up late, and waking by alarm (37). In addition, internal (e.g., drugs) and external (e.g., sound) stimuli will also influence the dynamic process of sleep (15). Sleep architecture also changes considerably with age. Twelve month old infants typically sleep for 14–15 h per day (52), which drops to 11 h by age 5, whilst adolescents require 9–10 h of sleep each night (53). Differences in the profile of sleep stages within a sleep episode are also apparent between young adults and the elderly (Figure 2). This is especially true when comparing the increasing number of brief arousals and wake sequence as a function of age (49, 54). In total, the amount of time awake after initial sleep onset (WASO) increases with increasing age (Figures 1, 2) along with a decrease in duration of REM and SWS sleep. Since arousal thresholds are higher during SWS and this sleep stage decreases with age, older adults become prone to experience more frequent awakening during a sleep episode (53).

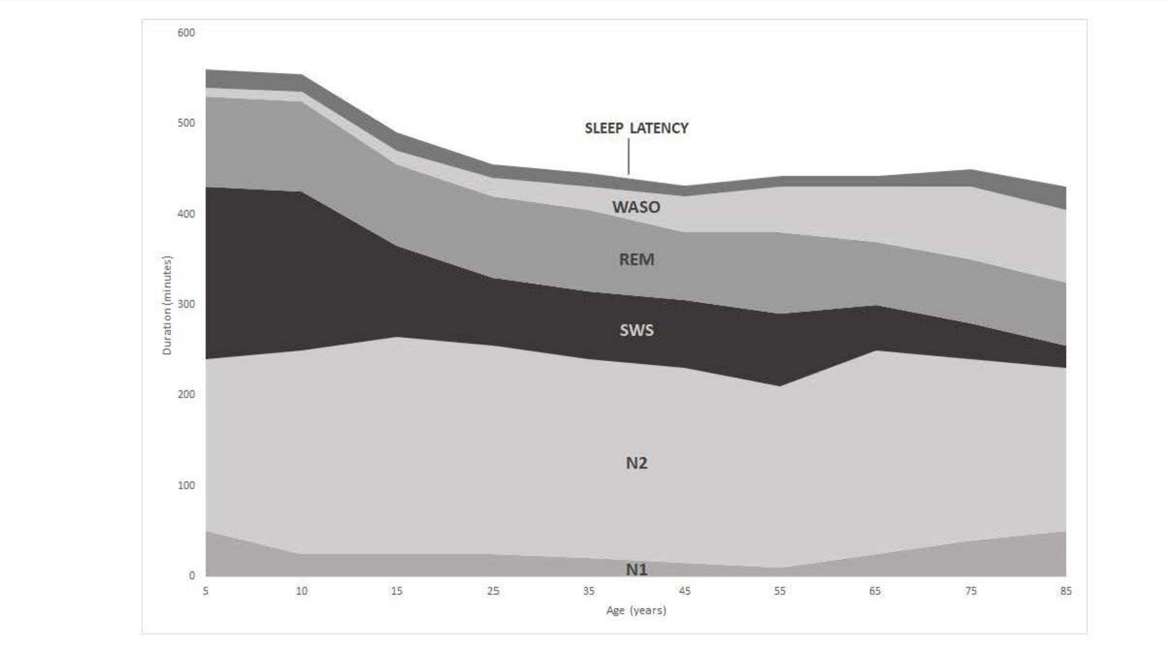


Figure 1. Changes in duration of sleep stages with increasing age [adapted from Ohayon et al. 2004]

Characteristics of the Equine Sleep Cycle in Comparison with the Human Sleep Cycle

Most mammalian species, including the horse, are polyphasic sleepers engaging in multiple sleep episodes across 24 h (7). This is considered to be an adaptation for increased vigilance (and thus survival) but may also indicate limited ability to sustain wakefulness (55). Table 3 presents data on all reported studies of equine sleep to date. This table sets out a number of sleep parameters to help define the characteristics of equine sleep and the equine sleep cycle, and allow for comparison with the human equivalent sleep profile.

Total Sleep Time

Studies of equine sleep describe on average 230.72 min (82.87) total sleep time (TST). This is in comparison to an average TST of 429.63 min (25.6) observed for humans (66, 68). Studies using EEG or ECoG reported mean total equine sleep duration ranging from 172 to 262 mins. Meanwhile, studies using behavioural observations record total equine sleep as between 65 and 382 min, depending on whether standing sleep was included and how total sleep time was defined. Differences in age of horses between studies (from 6 months to 20 years), use of sampling techniques, and total sleep observation time (e.g., 12 vs. 24 h) are also likely to cause variation within reported TST between studies. This latter point identifies the importance of using standardized methodology when taking a behavioural approach to quantifying animal sleep (discussed in more detail in Section Enhancing the Behavioural Measurement of Equine Sleep Quantity).

Total REM and Total NREM

For horses, NREM sleep consistently accounts for the largest proportion of total sleep time (77.50%) (mean 178.74 ±87.53 min) compared to REM sleep (17.50% of total sleep time) (40.27 ±24.15 min). Although the average total NREM and REM sleep duration is greater in humans (352.86 and 72.92 min respectively), the ratio of NREM to REM for human sleep (82 and 17%) is similar to the horse (66, 68), with a larger proportion of total sleep time devoted to NREM sleep for both species.

Table 3. Reported measurements and characteristics of the equine and human sleep profile.

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.)	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) (56)	7	Adult horses	7hrs at night	210.0min 50% TOT	40.0 min 65% TST 9.52% TOT	30.0 min 5%TST 7.14% TOT	NM	NM	NM	NM	NM	NM
Greening et al., (2021) (57)	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) (58)	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) (59)	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
(Dallaire and Ruckebusch, 1974a) (60)	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) (61)	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) (62)	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) (42)	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) (63)	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) (64)	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) (65)	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) (27)	3	Adult horses	10hrs (over-night)	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
Human – children (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
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) (68)	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**

(

In total, NREM and REM sleep constitute 95% of the total sleep time for the horse compared to 99% for the human which may be indicative of greater levels of wakefulness in the horse during sleep cycles.

Sleep Cycle Duration and REM and NREM Components of the Sleep Cycle (Sequences and Episodes)

The average number of sleep cycles for human sleep has been recorded at 4.23 with a range of 2–6 cycles per night that last for an average of 119.70 mins, ranging from 80.7 to 199.1 mins (66). The horse also engages in 2–6 cycles (averaging 4.1 cycles) but these are shorter on average than in humans (40.74 mins). Many equine studies only measure sleep at night and thus additional sleep cycles, that are known to occur during the day for the horse, will not have been taken into account. In this respect, the average value of 4.1 cycles pertains only to the nocturnal sleep profile.

In the horse, the average duration of NREM sequence within the total sleep time is 6.02 min (ranging from 3.37 to 9 min) with the average number of sequences being 31.96 (ranging from 18 to 52). This is substantially different to humans where the average duration of NREM sequence is 67.97 mins (ranging from 62 to 77.5 min) within the total sleep time. The number of these prolonged NREM sequences in humans during the total sleep time is therefore also substantially lower, with one sequence per sleep cycle and an average of 4 sleep cycles per night (66). This is reflected in recorded durations of NREM episodes which are substantially greater in humans (101.36 min) compared to the horse (17.14 min) (42, 66).

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

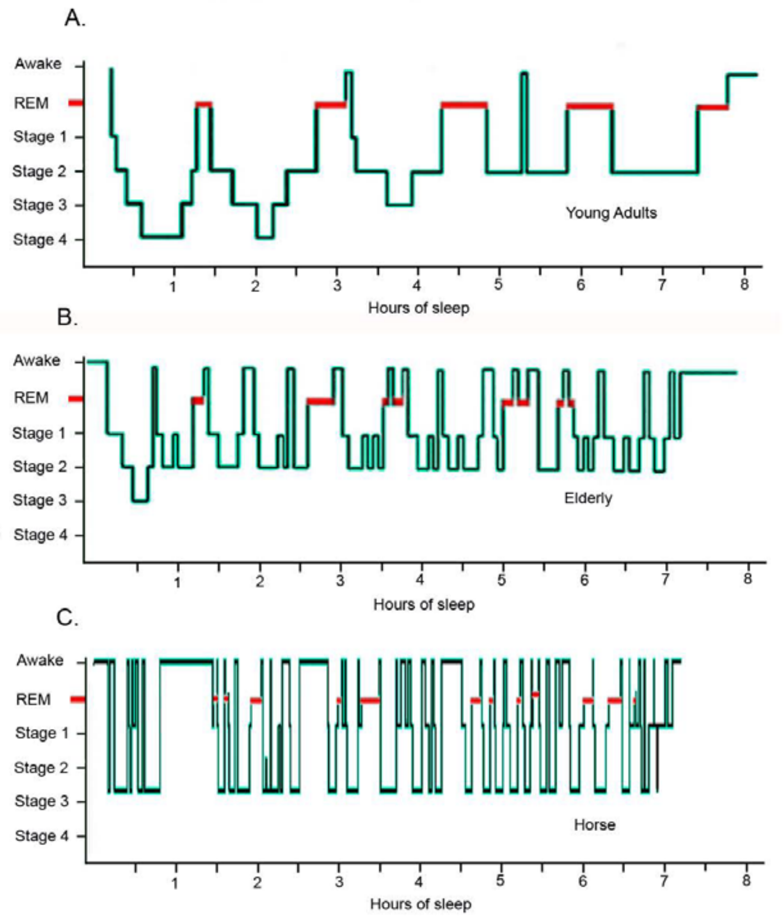


Figure 2. Hypnogram comparing sleep architecture of a typical young human adult, an elderly human adult and an adult horse over an eight-hour sleep episode: (A) the young adult experiences 5REM sequences and 2 short sequences of wakefulness; (B) the elderly individual experiences 8 disturbed REM sequences and multiple sequences of wakefulness accompanied by a distinct lack of stage 4 sleep; (C) the horse experiences 13 disturbed REM sequences and multiple sequences of wakefulness accompanied by a distinct lack of stage 4 sleep [adapted from (42, 51)].

Wake Sequences (Duration and Number)

The mean number of wake sequences per sleep cycle is 0.25 for children, 0.77 for adults, 0.9 for elderly adults and 6.89 for the horse (Table 3). The mean duration of wake sequences for children is 1.64 vs. 2.75 mins for adults, 11.56 mins for elderly adults and 0.96 mins for the horse. In this respect, elderly humans are experiencing wake sequences greater than 3 mins which are thus more likely to be complete breaks in the sleep cycle with a new sleep cycle restarting after the wakefulness has occurred. Thus, whilst the number of wake sequences within a sleep cycle for the elderly adult has greater similarities to the horse compared to younger humans, the nature of the wake sequence appears to be quite different (Figure 2). The increased number of long duration wake sequences in the elderly human is considered to reflect non-functional age-related changes in the brain (70), whereas the large number of short duration wake sequences in the horse is considered to serve a much more functional role in maintaining vigilance levels against predators (51).

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

Enhancing the Behavioural Measurement of Sleep

As previously discussed (Section Definition, Sleep Stages and Variation of Animal Sleep) EEG profiles, often in combination with polysomnography (PSG), give an accurate quantification of specific sleep states in a range of species including the horse (3), however, equipment is needs to be specialized and the use of surface electrodes often produces data loss thus producing incomplete data sets (71). The behavioural quantification of sleep has historically provided a viable alternative to EEG measurement (3) and thus, there is an argument for developing this approach for the measurement of equine sleep when EEG equipment is not available. One of the primary problems with this approach, however, is that the horse is capable of achieving various stages of sleep in multiple body positions (Table 4). For example, NREM N1–N3 sleep stages are differentiated only by the position of the eye lid and the position of the poll in relation to the withers when the horse adopts these sleep states whilst standing (36). Although NREM sleep tends to occur in standing positions, it can also occur when the horse is recumbent making the differentiation between NREM and REM sleep states more difficult. REM sleep, due to muscle atonia, can only occur in recumbent states (lateral and sternal) with the muzzle being placed on the floor in the sternal position (56). Some horses (although rare) can enter a REM sleep state in a standing position but only momentarily before muscle atonia occurs and the horse collapses bringing it out of sleep (36). Individual horses thus appear to have different strategies of sleep with different proportions of sleep states occurring in different behavioural positions. For example, in an EEG study of seven horses (42), the quantity of total sleep in the standing position ranged from 26.4% in one horse to 65.7% in another, in sternal recumbency it ranged from 21 to 59.7% and in lateral recumbency from 1.8 to 13.9%. By comparing EEG data with behavioural data, it may be possible to derive better estimates of sleep state from the behavioural assessment of the animal. For example, by plotting the duration of the EEG sleep state against the duration of associated behavioural state, the linear regression equation that is derived from this plot can be used to more accurately estimate the sleep state from the behaviour of the animal. To illustrate this, **Figure 3** presents the linear regression of the average duration of EEG sleep states (Light sleep, N1; SWS N2–N3; REM) for 7 horses (over 4 nights) against the average duration of sleep behavioural states (standing sleep; sternal recumbent sleep; lateral recumbent sleep) [data taken from Kalus (42)]. From **Figure 3A**, it can be seen that both light sleep (NREM N1) and SWS sleep (NREM N2–N3) can occur whilst the horse is in standing sleep with predominantly more time spent in the latter sleep state compared to the former. The linear equations give the estimation of the time spent in each sleep state when the animal is in standing sleep (Light sleep [NREM N1] 0.25 Total time spent in standing sleep – 3.13; SWS[NREM N2-N3] = 0.75xTotal time spent in standing sleep + 3.13). Interestingly, the variation within this estimation (as indicated by the R^2 value) can be reduced by taking into consideration other behavioural features during the sleep period. For example, the number of wake sequences (periods of wakefulness during sleep cycles that are less than 3 minutes) that occur within the total period of sleep increases the accuracy of the estimation. Taking the Kalus (2014) data for example (42), including the wake sequence data into the regression analysis marginally increases the R^2 value for Light sleep (NREM N1) from 0.19 to 0.34 and for SWS sleep (NREM N2-N3) from 0.68 to 0.74.

The two other primary behavioural sleep states within which sleep can occur are sternal and lateral recumbency. For sternal recumbency, all three sleep states are present as can be seen in Figure 3b. SWS (NREM N2-N3) predominates (SWS [NREM N2-N3] = 0.66xTotal time spent in sternal recumbency – 9.85) followed by REM sleep (REM= 0.12xTotal time spent in sternal recumbency +14.97) and then light sleep (NREM N1) (Light sleep [NREM N1] =0.22xTotal time spent in sternal recumbency – 5.11). Again, adding in additional behaviours into the estimation analysis can increase the R^2 value and the accuracy of the estimation. In this instance, the inclusion of the total number of wake sequences into the regression analysis marginally reduces the variation of estimation from R^2 value for Light sleep (NREM N1) from 0.73 to 0.74, from 0.90 to 0.91 for SWS sleep (NREM N2-N3) and from 0.23 to 0.24 for REM sleep. For lateral recumbency, again all three states can occur as illustrated in Figure 3c, but with a more equal distribution across the three states (Light sleep [NREM N1] =0.36xTotal time spent in lateral recumbency – 1.04; SWS [NREM N2-N3] = 0.28xTotal time spent in lateral recumbency +1.24; REM= 0.36xTotal time spent in lateral recumbency -0.36). Again, inclusion of the total number of wake sequences into the regression analysis marginally reduces the variation of estimation from R^2 value for Light sleep (NREM N1) from 0.55 to 0.57, from 0.35 to 0.36 for SWS sleep (NREM N2-N3) and from 0.50 to 0.51 for REM sleep. Further EEG studies that monitor in close detail the changes in behaviour of the horse as it transitions between the three primary sleep states has the potential to increase the accuracy of behavioural sleep analysis through this multiple regression approach.

Table 4. Behavioural markers of different EEG sleep states in the horse. Ticks (✓) indicate behavioural states required to be in the sleep state. Crosses (x) indicate behavioural markers that cannot occur during the sleep state. Dashes (~) indicate behavioural states that may or may not occur during the sleep state (36, 59, 72, 73) (Williams, Aleman et al. 2008; Dallaire and Ruckebusch 1974, Hale and Huggins 1980, Dallaire 1986,).

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	~	✓	✓	~	~	~	~	~	x
NREM N3 (SWS)	~	✓	✓	~	✓	~	~	~	x
REM	x	✓	✓	~	x	~	~	~	~

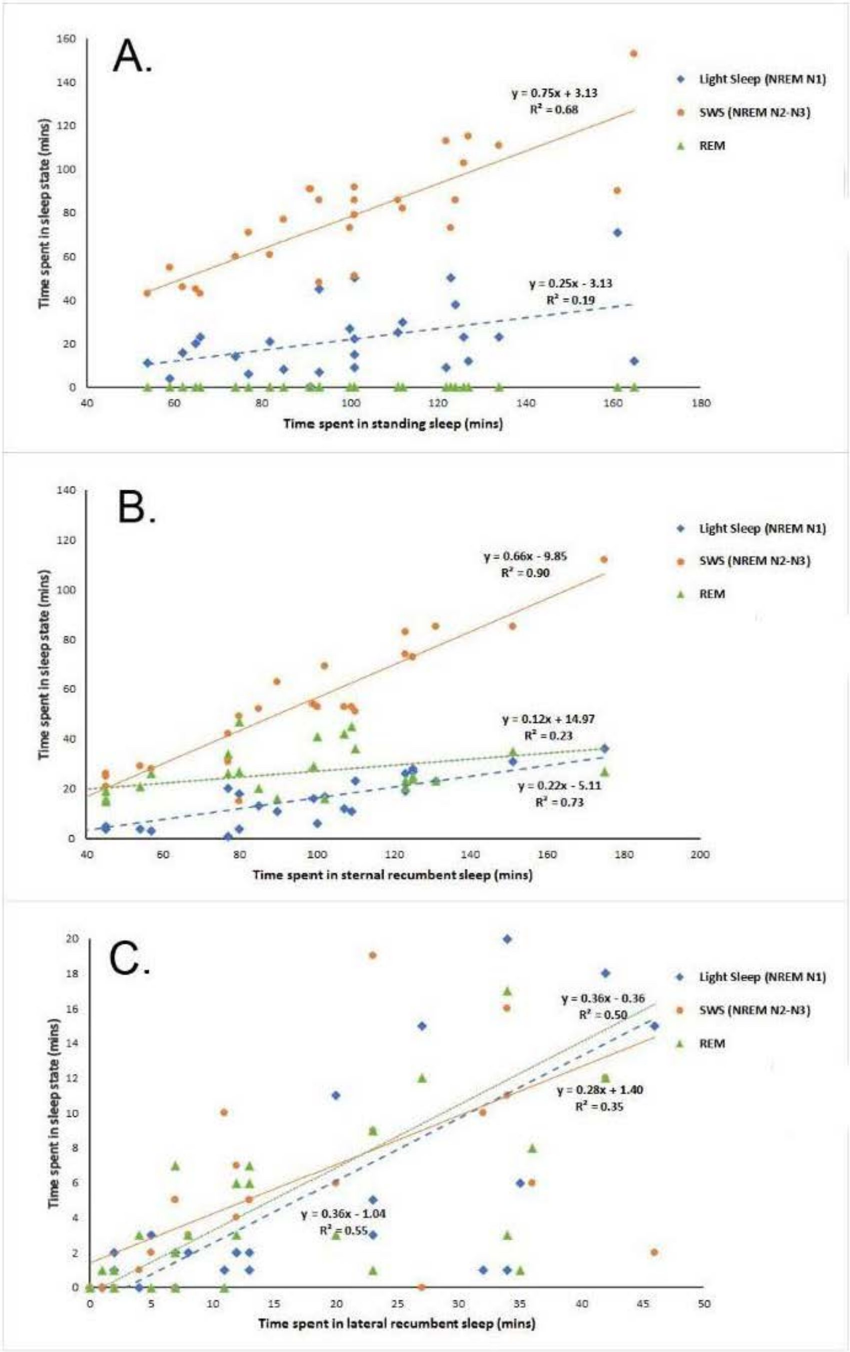


Figure 3. Linear regression analysis of average duration EEG sleep states against average duration behavioral states for 7 horses over 4 nights. Data taken from Kalus (42) [(A) standing, (B) sternal recumbency, (C) lateral recumbency].

Measuring sleep quality

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

General concepts of sleep quality versus quantity

Although the average (and thus potentially optimal) quantity of total sleep time for a range of animal species is well documented (3), sleep quality is an uncommon measurement within animal sleep research and is therefore very poorly defined for the majority of mammalian species. In humans, the subjective experience of sleep quality has been quantified using sleep continuity measures such as reduced latency to sleep onset, the number of awakenings, and duration of wakefulness after sleep onset (76), correlating with a reduction in total sleep time. Poor human sleep quality is also associated with patterns of sleep fragmentation or interruptions described as sleep that is punctuated by repeated periods of waking throughout the night (77). These can involve transient arousals (transition to brain alpha activity [2 seconds or more]) not associated with a change of sleep stage and body movements lasting 0.5 seconds or longer (37) or wake sequences (<3 minutes) (19). Reduction in both sleep quality and quantity produce a state of sleep deprivation and subsequent sleep debt (78). Sleep deprivation is defined as either a complete lack of sleep or a shorter than optimal sleep time (75), for example quantified for humans as less than six hours of sleep per night (79). A distinction is made between acute and chronic sleep deprivation based on the number of days the individual experiences less than the optimal sleep time. For example, in humans acute sleep deprivation has been defined as three consecutive nights of restricted or no sleep (80) whereas chronic deprivation has been described as persisting over longer periods of time e.g. fourteen consecutive nights of restricted sleep (81). In animals, the former has been associated with reduced energy whereas the latter has been associated with generalized inflammatory and stress responses in the brain (82) leading to the death of the animal (83).

Due to an accumulation of hours of lost sleep relative to the daily sleep requirement at an individual level, there is a need for recuperative or recovery sleep (81, 84). For example, human individuals maintained on a sleep wake pattern that induced a reduction in total sleep time were described as sleepier and less alert (78). The effects were reversed via extended sleep following the sleep reduction, and the individuals were described as having ‘repaid the sleep debt’ (78). The sleep debt can also be repaid by higher intensity sleep in the form of deeper slow wave sleep, where EEG slow wave activity (SWA) observed during NREM sleep is considered to represent a parameter of sleep intensity (9). In this context, slow wave sleep has also been described as a function of the duration of prior wakefulness (85) where it occurs closer to the point of onset of sleep during the sleep cycle (86), therefore providing an efficient mechanism with which to recover the sleep debt if required.

After sleep deprivation, increased levels of SWA during NREM sleep are also associated with a decreased number of spontaneous awakenings and an increased threshold for induced awakening (85, 87), which are characteristic of deeper/more intense/higher quality sleep. REM sleep is less sensitive to sleep deprivation, however sustained deprivation of REM sleep results in elevated REM sleep that is not always immediate but lasts for several nights compared to SWS which tends to be elevated during the first recovery night (9). To summarise, whilst acute changes to sleep cause an immediate, short-lasting compensatory SWS response, only a severe deficit in REM sleep results in a rebound which is often delayed and prolonged (9).

Potential measures of sleep quality in the horse

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

Additional measures of the equine sleep profile that may also be useful in quantifying equine sleep quality is the total duration of NREM sleep and also the number of wake sequences (<3min) or micro-arousals within a sleep cycle. Previous work in humans has shown that an increased number of wake sequences negatively correlate with subjective sleep quality, whilst increased quantity of NREM sleep positively correlates with better motor function and accuracy (89). As indicative baseline values for horses the mean total duration of NREM sleep is 178.74 minutes and the mean number of wake sequences within a sleep cycle was 6.89 (Table 3).

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

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

SQI= 178.74/1.98 + 40.3/4.91 = 98.48

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

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

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

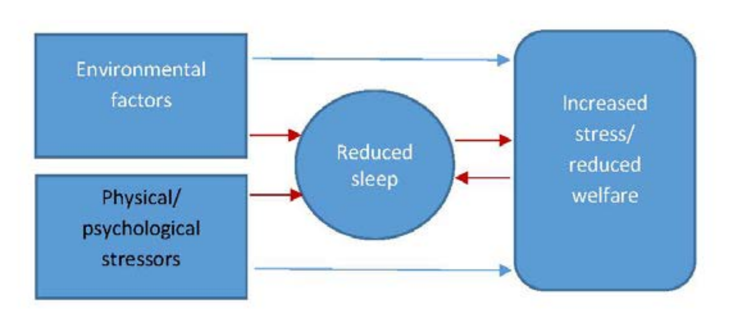


Figure 4. The interrelationship between factors of well-being affecting sleep and sleep affecting well-being (red arrows indicate the pathways to reduced sleep and the interplay between increased stress/reduced welfare and reduced sleep).

Using changes in sleep as a marker for poor welfare

It is often difficult to establish whether changes in sleep can be used as a marker for stress or whether changes in sleep are partially or wholly responsible for the animal being stressed. It is likely, as intimated in Figure 4, that changes in sleep are both a marker and cause of stress with the importance of the latter increasing over time as the quality of the animal's sleep progressively diminishes. For example in rodents, sleep has been used as a behavioural marker of stress alongside other standard biomarkers (adrenal weight, corticosterone) in response to cage size and social stress (90). It is difficult however to ascertain within this study whether it was social stress directly that was having the stress effect or whether the physiological response was due to the significant reduction in sleep duration and interruption. In this sense, reduced quality sleep may be a reasonable marker of stress in the first instance but ultimately becomes a compounding stressor in its own right over the longer term. When investigating the relationship between sleep and welfare, non-significant relationships have been reported between total time spent asleep, judgement bias, and behaviour-based measures of welfare for shelter dogs (91) potentially highlighting the need for more sensitive measurements of sleep beyond total sleep time. As previously discussed, measurements of sleep quality such as number of disturbances or micro-arousals might yield more valid results. Little research has been carried out assessing how welfare-reducing factors might manifest as changes in the sleep profile in the horse. However, horses performing stereotypy are reported to display different sleep profiles compared to non-stereotypy animals with significantly less time ($p<0.001$) spent in REM (2.2±1.7 versus 6.7±1.9) and N2-N3 SWS (13.8±8.2 versus 29.5±3.4) sleep states and significantly more time ($p<0.001$) spent in light sleep (N1) (22.6±4.5 versus 8.8±3.4) (194). Stereotypy is associated with current and/or historic states of reduced welfare (195) and the result of this study, given the welfare consequence of sleep deprivation, suggest that stereotypy horses may be suffering from compounded state of negative welfare.

Effect of sleep deprivation on animal behaviour and welfare

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

Table 5. The after effects of sleep deprivation in human and rodent models.

Impaired visual perception Human (100)	Human	100
Reduced capacity to engage in tasks requiring simple sustained concentration/attention	Human	101
Impaired decision making including more high-risk strategies and reduced concern for negative consequences of these	Human	102
Impaired memory consolidation	Rodent	103
Negative effects on vigilance and simple reaction time	Human	104
Increased daytime sleep propensity/ micro-episodes of sleep leading to lower capabilities and efficiency of task performance and to increased number of errors	Human	68
Poor memorization and schematic thinking which yields wrong decisions	Human	105
Emotional disturbances such as deteriorated interpersonal responses and increased aggressiveness	Human	For review see Fairholme and Manber 107
Changes to pain perception, specifically hyperalgesia	Rodent	For review see Lautenbacher et al 98

Factors reducing sleep

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

Stressors

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

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

Beyond comfort, perceived safety within the environment also influences sleep. For example, human sleep is sensitive to a novel environment and stimuli, described by the ‘first night effect’ (FNE) and this is also observed in dogs (127). However, humans have the capacity in most instances to modify the stress-inducing factors within the environment which is often in stark contrast to domesticated mammalian species that lack a level of control over factors within their environment. For example, moving horses from a period of turnout to overnight stabling has been shown to affect their daytime behavioural profile (128) whilst nocturnal recumbency significantly increased six weeks after horses were brought into an overnight stabling management regime from a period of overnight turnout (129). Seminal EEG data states that horses require a period of acclimatisation to novel environments (40), observed as greater levels of vigilance displayed when horses are initially stabled after a period of turnout (128, 129). It seems that stabling in isolation removes the aspect of shared safety through group vigilance during turnout, whilst presenting a wealth of novel (auditory and other) sensory stimuli.

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

Environment

Light is one of the most important environmental factors affecting sleep across a range of species. The sleep-wake cycle is driven by a central clock, the superchiasmatic nucleus (SCN), and in most mammals, by changing concentrations of melatonin due to light exposure (137). Photoentrainment of sleep to circadian rhythms is usually mediated by photoreceptors that detect changes in the quantity and quality of light over the 24 h dawn/dusk cycle (138). The sleep-wake cycle is vulnerable to changes in the timing of circadian rhythms (phase shifting) (139) via exposure to bright light at specific points during the light-dark cycle, even during sleep. Because of the increased use of artificial light within society, humans tend to spend less time in the dark which has been described as influential in the shift from biphasic to monophasic sleep patterns in humans (140). This includes exposure to artificial light at night (ALAN), which could be considered an environmental stressor due to the fact that it has been shown to disrupt the biological clock via suppression of melatonin (141). Prolonged exposure to ALAN induces adverse effects on mood and productivity (142), and in laboratory rodents has been associated with reduced anxiety-related behaviour including more time spent in the open (143). The latter is considered a maladaptive response specifically for urban dwelling prey species (143). Changes in exposure to light and associated phase shifts (waking earlier or later) are important to humans, enabling them to adjust to travel across time zones or facilitating adaptation to night shift work or early awakening (144). However, sleep disruption can be a biproduct of these phase shifts, for example in the form of 'jet lag' (145). Domesticated horses are often housed in situations that include artificial light, although the way in which this acts as a zeitgeber for equine sleep is little understood. A recent study specifically examined the effect of overnight light on sleep behaviour in horses and reported a significant reduction in sternal recumbency linked with the REM sleep state (56). Interestingly breeding mares are routinely exposed to artificial light to manipulate the breeding cycle in the northern hemisphere horse racing industry (146) but very little is known about the impact of this procedure on quality of sleep for those animals. The use of red light at night has been advocated for use within equine husbandry as a means to minimize circadian disruption (147). Competition horses are regularly travelled internationally although little is known about the effects of changing time zones and rates of adaptation relative to sleep and performance.

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

Other non-photic entrainment factors/ zeitgebers for sleep include temperature and humidity. Sleep and rest in many mammalian species are associated with a reduction in core body temperature (CBT) (163), a thermoregulatory process whereby heat is redistributed from the core to the outer layer of the body. The CBT rhythm is suggested to be able to entrain peripheral pacemakers around the body and can affect normal sleep patterns. For example, increasing distal skin blood temperature via exercise (164) is considered to be one of the factors that can reduce the latency of sleep onset (163). Sleep is also highly susceptible to environmental heat, as demonstrated in rats (165), and in cows heat stress is known to reduce lying time that can subsequently impact on sleep levels (166). Meanwhile, recent work by Yadhapalli et al (167) suggests that sleep-promoting circadian clock neurons are inhibited by heating and excited by cooling in Drosophila melanogaster and evidence that these neurons are continuously integrating temperature changes to coordinate the timing of sleep and activity. Horses tend to sleep less during higher (57) and longer during lower (168) ambient temperature when theoretically core heat redistribution is harder and easier respectively. However, this also tends to coincide with changes in daylight season and thus it is difficult to identify the predominating sleep affecting factor. According to Duncan (1985) (169), free-living Camargue horses adopt recumbent positions more so in spring with a higher prevalence of standing alert and walking during the summer although these behavioural patterns are also thought to be driven by availability of forage (specifically crude protein) and the presence of biting flies. During the autumn and winter months these horses also increased the proportion of time spent resting whilst standing but with decreased time spent in a recumbent posture, correlating with low ambient temperatures and increased rain (169). All in all, equine sleep-related behavioural patterns appear closely associated with environmental seasonal fluctuations with temperature being an important factor in this respect. However, limited research exists specifically on the direct effects of ambient temperature on equine sleep and CBT, which is of particular interest since many horses have their coats clipped, are fitted with rugs and experience different climates due to international travel.

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

The level of stimulation from the environment may also influence sleep due to varying levels of arousal and alertness. In humans, an example of a hyper-stimulating environment would be an Intensive Care Unit (ICU), with evidence of very poor sleep occurring during stays in ICU due to noise, critical illness itself, and treatment events throughout the day and night (182). Noise is generally accepted as unwanted audible acoustic phenomenon (183). The human auditory system continues to scan, evaluate and react to environmental sounds even whilst asleep where more meaningful noise events are more likely to cause arousals from sleep than less meaningful events (184). The depth of the sleep phase, background noise level and individual characteristics affecting sensitivity to noise are known to determine whether or not noise will disturb sleep (185, 186, 187). Other factors include the type of noise (e.g., continuous, intermittent, impulsive), noise intensity, noise frequency, noise spectrum, and noise interval (e.g., duration, regularity, expected) (183). It is currently unclear how many additional noise-induced awakenings are acceptable/ without consequences for sleep recuperation and health, especially given the large inter-individual differences in susceptibility to noise. Prey species typically tend to remain vigilant for the rest of the night, even after initial adaptation to the nocturnal environment, following one awakening elicited by a spontaneous/ startling stimulus (3). However, continuous auditory stimulation provided overnight (e.g., music) can have a masking and relaxing effect in animals (61, 188). For example, in horses, overnight music appeared to facilitate increased displays of biologically significant behaviours including lateral recumbency and the behavioural benefits continued beyond the enrichment period (61).

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

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

Discussion and Future Directions

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

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

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

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

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Paper 2: Investigating duration of nocturnal ingestive and sleep behaviours of horses bedded on straw versus shavings

2.1.1 Published as

Greening, L., Shenton, V; Wilcockson, K; Swanson, J. (2013) Investigating duration of nocturnal ingestive and sleep behaviours of horses bedded on straw versus shavings. *Journal of Veterinary Behaviour; clinical applications and research*. 8 (2), pp82-86.

2.1.2 Objectives of the study

- Determining whether differences in sleep quantity could be detected in response to:
    - Different bedding substrates

2.1.3 Why this journal

The study was presented as a poster and the abstract was published in the proceedings of the 7th International Conference for Equitation Science following which, presenters were invited to submit a full article for a special issue within the Journal of Veterinary Behaviour. The journal is the official journal of the Australian Veterinary Behaviour Interest Group, the British Veterinary Behaviour Association, the Association Veterinaire Suisse pour la Medecine Comportementale, The American Veterinary Society of Animal Behavior, and the American College of Veterinary Behaviorists, and accepts submissions covering topics such as normal signalling or social behaviours, welfare and/or housing issues, and applied behavioural issues (e.g. working dogs) that may have implications for clinical interest or assessment. It currently has an impact factor of 2.172. PlumX Metrics report that the article has been cited 21 times with 109 captures, and 101 readers via Mendeley.

2.1.4 Author contributions

	Linda Greening	Victoria Shenton & Kate Wilcockson	James Swanson
Conceptualisation	20%	80%	0%
Methodology	50%	50%	0%
Formal analysis	70%	20%	10%
Resources	0%	100%	0%
Data curation	0%	100%	0%
Writing the original draft	75%	0%	25%
Editing the original draft	100%	0%	0%
Visualisation	20%	70%	10%
Supervision	50%	0%	50%
Project administration	25%	50%	25%

2.1.5 Future contributions

This study provided an entrance into the field of equine sleep research, offering an overwhelming opportunity to upskill and increase my knowledge that contributed to the publications that follow. Based on the fact that Paper 4 highlights how depth of the bed is a significant factor coupled with a pilot study run with shavings that shows depth rather than the substrate is more likely the influential factor linked to equine sleep behaviour, and the associations being drawn between straw bedding and equine asthma, future and further research looking at bedding substrate is unlikely.

2.2 The published article in full

Abstract

Horses are stabled overnight for a number of practical reasons; however, there is little research quantifying nocturnal equine behavioural patterns or the extent to which different environments influence nocturnal behaviour. The aim of this study was to establish whether differences in duration of sleep and ingestive behaviours were apparent for horses bedded on straw (group 1) or shavings (group 2). Ten geldings of mixed breed (mean age: 7.3 ±3.53 years) bedded on either shavings (n = 5) or straw (n = 5) were observed between 7 PM and 7 AM. Duration of behaviours according to a predefined ethogram was recorded in minutes using a video recorder and continuous focal sampling. Mann–Whitney U tests were used to identify whether any significant differences in duration of ingestion and sleep behaviours occurred for horses bedded on straw compared with shavings. Of the total observation period, group 1 spent, on average, 29.3% of their time budget engaged in recumbent behaviours, compared with 12.2% for group 2. However, no significant differences in duration were established between horses bedded on straw or shavings for standing sleep, sternal recumbency, and lateral recumbency behaviours ($P > 0.05$). Ingestive behaviours occupied approximately one-third of the time budget, with no significant difference ($P > 0.05$) observed between groups. On average, group 1 spent a longer proportion of the observation period ingesting bedding (8.1%) compared with group 2 (1%). Duration of bedding ingestion appeared to peak between 1 AM and 7 AM for both groups. Although not quantified, general observations revealed horses were motivated to alternate between eating hay and bedding in both groups, owing to the prevalence of bedding ingestion. The results indicate that straw bedding facilitates the display of ingestive and sleep behaviours, whereas horses bedded on shavings spent a greater proportion of their nocturnal time budget engaged in ‘‘other’’ behaviours. Further research is required to investigate the extent to which different types of bedding material enrich the environment of horses that are stabled overnight.

Keywords: equine; nocturnal; recumbent; ingestion; behaviour; bedding

Introduction

The domestic environment within which the horse is kept and managed can present challenges to instinctive and innate behavioural patterns. Research indicates that within this environment, stabling and associated practices are often the most challenging aspects that the horse is expected to cope with (e.g., McGreevy et al., 1995; Henderson & Waran, 2001; Piccione et al., 2008). Intense stabling practices, involving long periods of confinement with little access to (free) exercise, have been associated with increased restlessness and aggression (Werhahn et al., 2011). Extended periods of confinement can also be associated with increased risk of abnormal behaviour development (McGreevy et al., 1995). Barriers within traditional and conventional stabling systems therefore appear to reduce the opportunity for the horse to display normal behaviour and increase the likelihood of abnormal behaviour display (Cooper and Albentosa, 2005; Rose-Meirhöfer et al., 2010). However, the role of the horse in modern society seems to necessitate stabling (Henderson, 2007), and as a result, research exists to investigate methods to enrich the stable environment using feed-balls (Henderson and Waran, 2001), increased opportunities to forage (Thorne et al., 2005), and increased visual horizons, such as mirrors (Cooper et al., 2000; Mills and Davenport, 2002). The use of bedding in the stable is a traditional practice that is receiving increased attention as a stimulus that is reported to exert variable positive and negative influences (Werhahn et al., 2010). However, previous studies investigating bedding report contradictory evidence, possibly owing to noncomparable sample populations or aspects of the study design. For example, some studies included female populations exclusively (Houpt et al., 1986; Werhahn et al., 2010), used relatively small stalls (Pedersen et al., 2004), and used crossover designs using different bedding materials (Werhahn et al., 2010). During preference tests, horses have been observed to choose straw bedding when given the choice (Mills et al., 2000), but some have also demonstrated no significant preference (Hunter and Houpt, 1989). Some of the negative conclusions from bedding research suggest that bedding material has little significant influence on behaviour (Thompson, 1995) and is linked with colic (Greet and Rossdale, 1987). More recently, research has shown that straw bedding encourages more bedding-directed behaviours, less standing behaviour, and longer duration recumbent behaviour (Pedersen et al., 2004; Werhahn et al., 2010). The current study therefore aimed to establish whether significant differences existed for a sample population of mixed-breed/age geldings, bedded on either straw or shavings, looking at the duration of nocturnal ingestive and sleep behaviours.

Methods and materials

Materials

Ten geldings of mixed breed and age (average age: 7.3 +3.53 years; range: 4-13 years) were allocated to either group 1 (straw; n = 5) or group 2 (shavings; n = 5) based on the bedding material that appeared in their stable. Each horse had been bedded on the material for at least 5 months previously. Each stable measured 12 feet by 12 feet, the design of which prevented physical contact between neighbouring horses owing to solid walls separating each horse; they were able to see each other from the stable door. No deep litter systems or rubber matting was present in any of the stables. The bedding that appeared in the stable was either wheat straw or pinewood shavings that were not entirely new and varied in depth according to owner preference (minimum: 10 cm). Each horse was provided with access to pasture for between 6 and 10 hours during the day and given its normal feed and/or individual ration of hay at 6.30 PM. All horses were individually stabled on the same yard and underwent light-to-medium work but were privately owned and therefore subject to different daily regimens.

Experimental design

Each horse was filmed once for a period of 12 hours between 7 PM and 7 AM. The light was left on during the study, and horses were habituated to this for a period of 2 nights before data collection. A Sony Handycam DCR-SX15E (Sony, Tokyo, Japan) with a NP-FV100 battery was secured in the stable rafters in the top corner above the stable door and set on a wide-angle lens so that the entire stable was visible to facilitate data collection. Duration of ingestion and recumbent behaviours according to a predetermined ethogram (Table 1) were recorded using continuous focal sampling. Sleep was recorded where the behaviour lasted for longer than 1 minute. Ethical approval was granted by Hartpury College.

Table 1. Ethogram of ingestive and sleeping/recumbent behaviours (Adapted from Dierindonck et al., 1996; Winskill et al., 1996; Flannigan & Stookey, 2002; Souris, 2007)

Category	Description of activities
Ingest hay	Masticating, prehending or swallowing hay
Ingest concentrates	Masticating, prehending or swallowing concentrates
Ingest bedding substrate	Masticating, prehending or swallowing bedding substrate
Sleep whilst standing	Standing immobile, no or limited ear movement, relaxed tail, limited leg movement, eyes closed or half shut
Sternal recumbency	Recumbent, with sternum in contact with the ground, legs folded beneath the body, no or limited ear movement

Lateral recumbency	Recumbent, either lateral thoracic area parallel to and in contact with the ground, head immobile and in contact with the ground, legs extended

Statistical analysis

To establish whether differences in frequency or duration of ingestive and recumbent behaviours existed for horses bedded on straw or shavings, Mann–Whitney U tests were used (n1 = n2 = 5). Significance levels were set at $P = 0.05$. Total mean duration of individual behaviours was calculated from the sum of the total time spent engaging in that behaviour by each horse, divided by the size of the sample population in each group. Mean duration data were also used to calculate the proportion of the total observation period (720 minutes) that each behaviour was displayed for.

Results

Sleep behaviour

No significant differences between group 1 or 2 were observed for average total duration sleep standing ($z = 20.104$; $P > 0.05$), sternal recumbency ($z = 0.301$; $P > 0.05$), and lateral recumbency ($z = 1.985$; $P > 0.05$) (Table 2). During the total observation period, horses bedded on straw spent, on average, 29.3% of their time budget engaged in recumbent behaviours, compared with 12.2% for those bedded on shavings. Overall, horses bedded on straw spent, on average, 56.9% engaged in sleep behaviours compared with 49.2% for horses bedded on shavings.

Table 2. Average duration (minutes) of sleep and sternal behaviours, also showing total duration as a proportion of the total observation period (%)

	Sleep standing		Sternal recumbency		Lateral recumbency	
	Shavings	Straw	Shavings	Straw	Shavings	Straw
Median duration (minutes)	263	273	113	148	6	45
Mean duration (minutes)	266	199	83.40	162.20	5	49
Proportion of total observation period (%)	36.9	27.6	11.6	22.5	0.7	6.8

Ingestion behaviours

Only one horse bedded on shavings was observed not to display any type of bedding ingestion behaviour. No significant differences were observed for median total duration of hay ingestion ($z = 20.940$; $P > 0.05$) or bedding ingestion ($z = 1.776$; $P > 0.05$) (Table 3). Of the total observation period, horses bedded on straw spent, on average, 36.2% of their time engaged in ingestion behaviours, compared with 33.1% displayed by horses bedded on shavings. General observations recorded peak duration of hay ingestion occurring between 7 PM and 10 PM, followed by a general decrease until 4 AM and 7 AM. Around this time, bedding ingestion was seen to increase. Additionally, horses with hay nets were observed to have some hay left in the morning, which may be explained in some cases by the prevalence of bedding ingestion.

Table 3. Average duration (minutes) of ingestion behaviours, also showing total duration as a proportion of the total observation period (%)

	Ingestion of hay		Ingestion of bedding	
	Shavings	Straw	Shavings	Straw
Median duration (minutes)	248	228	8	17
Mean duration (minutes)	231.20	202	7.20	58.20
Proportion of total observation period (%)	32.1	28.1	1	8.1

Nocturnal time budget

Distinct differences were observed in nocturnal time budgets for lateral recumbency and ingesting bedding behaviours, when comparing the shavings and straw (Figure 1) bedding groups. Overall, the average proportion of time spent on other activities was greater for horses bedded on shavings (17.7%) compared with straw (7%). This equates to 127.44 minutes and 50.40 minutes, respectively, where horses were not engaged in sleep, recumbent, or ingestive behaviours.

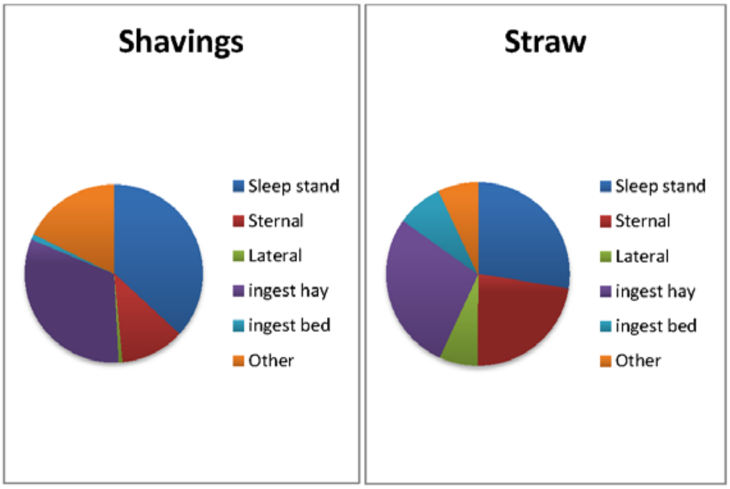

Figure 1. Time budgets of average total duration of nocturnal behaviour shown as a proportion for the entire observation period, enabling comparison between for horses bedded on shavings and straw.

Discussion

The results of the current study, although nonsignificant, reinforce previous research findings that straw bedding encourages increased bedding ingestion and recumbent behaviours and reduced standing-related behaviours (Pedersen et al., 2004; Werhahn et al., 2010). No standardization of bed size or condition was made, thus representing ‘‘normal’’ bedding conditions observed in the general population. Motivation to engage in bedding ingestion behaviours may have been influenced by bedding condition, where horses have been observed to display anti-parasite strategies when grazing at pasture (Fleurance et al., 2007). Further exploration of this theory in the stable may be useful to establish whether bedding condition influences nocturnal behaviour.

On average, the horses bedded on straw were observed to ingest their bed for longer than those bedded on shavings, although it is important to note that the latter still engaged in bedding ingestion behaviours to some extent. Research has shown that horses provided with multiple forage opportunities were observed to perform foraging behaviour more frequently and for longer (Thorne et al., 2005). In the current study, however, there was little difference in total average duration of ingestion behaviour between shavings and straw beds, suggesting that although straw offers variation in palatability, it does not significantly influence the amount of time that horses spend eating in the stable overnight. Although not quantified, general observations noted that horses moved between ingestion of hay to bedding ingestion, supporting the idea that some motivation exists to introduce variation into the diet. However, the ethogram used in the current study incorporated prehension of bedding under the heading ‘‘bedding ingestion,’’ which may have included hay that was mixed in with the bed, although the observer attempted to discern between hay and bedding ingestion. Interestingly, when the researcher asked the owners about each horse before the start of data collection and whether their horse had colicked in the past 5 months, all owners replied no. Although straw ingestion has been linked with colic in the past (Greet and Rossdale, 1987), the anecdotal reports from owners in the current study do not support this.

Some horses engaged in bedding ingestion more than others, suggesting different individual motivation to do so. Increasing access to different types of forage within the stable has also been shown to decrease straw bed forage behaviour (Thorne et al., 2005), which may offer a practical solution for owners of horses that have experienced colic associated with the use of a straw bed. Straw beds have been reported to facilitate more sternal recumbency (Werhahn et al., 2010) and lateral recumbency (Pedersen et al., 2004). In the current study, a straw bed appeared to encourage horses to spend a greater proportion of the observation period engaged in both lateral and sternal recumbency compared with horses bedded on shavings, suggesting that straw facilitates sleep, and more importantly paradoxical sleep, highlighting the potential for improved welfare. However, it is recognized that the exact amount of exercise each horse undertook was not standardized, which may have influenced the amount of rest that individual horses were motivated to undertake during the study (Caanitz et al., 1991). Similarly, the study could afford to be conducted over a longer period to gain more data for each individual, and the size of the horse relative to the dimensions of the stable should also be considered in future, as horses have been found to engage in more recumbent behaviour in large boxes (Raabymagle & Ladewig, 2006).

Overall, the average proportion of time spent on other activities was greater for horses bedded on shavings (17.7%) compared with straw (7%). Further research may help to verify the following suppositions: that this time may have been spent in standing alert, moving, rolling, engaging in stereotypic behaviour, or defecation. Generally, it is accepted that environmental enrichment aims to improve the biological functioning of an animal by making adjustments to the artificial environment, although measuring the success of such improvements quantitatively has proven to be challenging (Newbury, 1995). Where it is possible to account for biologically relevant behaviours within time budgets, it could be suggested that a straw bed encourages the display of more beneficial or functional behaviours from horses compared with shavings, although further research would be required to account specifically for the type and prevalence of these.

Conclusion

No statistically significant differences were observed for time spent engaged in ingestion or sleep behaviours when horses were bedded on shavings compared with straw. The proportion of time not spent engaged in sleep- or ingestion-related behaviours was greater for horses bedded on shavings, suggesting straw enables more functional behaviour to be displayed in the stable overnight. Although no episodes of colic were recorded during or before the study, owners are recommended to approach the requirements of individual horses with care. Future research should focus on quantifying the occurrence of other nocturnal behaviours, whereas a greater understanding of individual horse bedding preferences, including factors affecting motivation behind bedding-directed and recumbent behaviours, is still required.

Acknowledgments

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Paper 3. A Preliminary Study Investigating the Influence of Auditory Stimulation on the Occurrence of Nocturnal Equine Sleep-Related Behaviour in Stabled Horses

3.1.1 Published as

Hartman, N. & Greening, L. (2019) A preliminary study investigating the influence of auditory stimulation on the occurrence of nocturnal equine sleep related behaviour in stabled horses. *Journal of Equine Veterinary Science*. 82, p. 102782, https://doi.org/10.1016/j.jevs.2019.07.003.

3.1.2 Objectives of the study

- Determining whether differences in sleep quantity could be detected in response to:
    - The introduction of music to the nocturnal environment

3.1.3 Why this journal

The journal is an international publication designed for the practicing equine veterinarian, equine researcher, and other equine health care specialist. It is the official publication of the Equine Science Society and the official journal of the International Symposium on Equine Reproduction, and currently has an impact factor of 1.386. According to PlumX Metrics, the article has been cited six times, with 51 captures/readers and some presence on social media to date.

3.1.4 Author contributions

	Linda Greening	Naomi Hartman
Conceptualisation	50%	50%
Methodology	50%	50%
Formal analysis	75%	25%
Resources	75%	25%
Data curation	20%	80%
Writing the original draft	100%	0%
Editing the original draft	100%	0%
Visualisation	50%	50%
Supervision	100%	0%
Project administration	30%	70%

3.1.5 Future contributions

The research idea was developed from a previous study looking at behaviour of horses when different types of music were played, which was presented at the 8th International Society for Equitation Science conference. The idea that music could be used to reduce the novel auditory stimuli that horses experience overnight warrants further research, especially for horses exposed to novel environments such as clinical or competition settings. Sleep quality could also be included to understand whether music disturbs sleep beyond the changes in duration of behavioural states.

3.2 The published article in full

Abstract

The physical environment is known to influence nocturnal behavioural time budgets of the stabled horse, but less evidence exists to suggest how this might be affected by including additional sensory stimuli. This study aimed to establish the impact of novel auditory stimuli on the frequency of equine sleep-related behaviour. Seven horses stabled for 24 hours per day on the same yard receiving the same daily management routine were observed from 2030 to 0630 over nine nights. Frequency of nocturnal behaviour was recorded using focal intermittent sampling against a predetermined ethogram and an infrared CCTV camera system. Data were recorded under the following conditions: without music for two nights (phase A1), exposure to music for five nights (Beethoven's ninth Symphony) played at an average of 62.3 decibels (phases B1 [nights 3-4] and B2 [nights 6-7]), and two further non-consecutive nights (phase A2) when music was no longer played. A general linear model was used to determine differences in the frequency of parametric behavioural data with a significantly higher occurrence of “ingestion” (F [3,18] = 7.910, P = .001) during phases in B compared with A, and a significant decrease in the occurrence of “other” behaviour (F [3,18] = 10.25, P = .000) comparing phase A1 with all other phases. The Wilcoxon signed rank test highlighted significant differences in the frequency of “lateral recumbency” between specific phases (P < .05). The addition of music appears to have a significant effect on the equine nocturnal time budget that might be beneficial from an equine sleep perspective.

Keywords: Equine; Nocturnal; Sleep-related behaviour; Music; Enrichment

Introduction

Little is understood about the occurrence and specific functionality of equine sleep behaviour although undoubtedly from an evolutionary perspective it is critical to the normal functioning of the species [1]. For example, the horse is able to engage in some stages of the sleep cycle while standing and is able to survive on relatively little sleep in comparison to other mammalian species [2], all of which appears to be an adaptation to the potentially vulnerable state involving lateral recumbency (LR) as a prey species. Facilitating the occurrence of LR in free-living horses is a group behaviour that divides the occurrence of sentry behaviour between group members [3]. Lateral recumbency is critically important as the only position in which rapid eye movement (REM) sleep can occur for a prolonged period because of the complete muscle atonia associated with this sleep stage. Nonrapid eye movement (NREM) sleep is recognized as the only stage from which animals can progress into REM sleep and is typically observed in sternally recumbent horses [4]. Although both NREM and REM sleep have been observed in horses while standing [5], horses will invariably adopt a recumbent position if permitted by the environment because of the gradual loss of muscular tone. The various stages of sleep have been quantified using electromyography, electrooculography, electroencephalography, and behavioural indicators [6]. Behaviour remains a valid method of detecting sleep, especially in species that do not lend themselves to traditional methods of sleep detection [7], with familiar descriptions of equine sleep/sleep-related behaviour appearing within literature [5], [8], [9].

In general, little is known about the quality of equine sleep behaviour relative to the ratios of standing rest and different forms of recumbency, where the occurrence of LR in the domestic environment is variable [8]. For example, previous research has shown that the average duration of LR is reportedly less for horses bedded on wood shavings compared with straw [10], [11], [12]. Size of the stable and bedding depth [9], [13] are also implicated as influential factors relative to nocturnal equine recumbent behaviour. In addition, horses have been described as “bad sleepers” because of the amount of time taken to stabilize the overnight behavioural profile after a change to the nocturnal environment [1], [14]. Enrichment techniques are often used in the domestic environment to address hypo-stimulating/hyper-stimulating environments and encourage the display of biologically significant behaviours [15] such as rest/sleep or ingestion, while reducing the likelihood of abnormal behavioural development or display [16]. Research using auditory stimulation as enrichment, specifically classical music, reports positive changes in domestic canine species [17], while equine research reports that classical music was associated with the reduced occurrence of alert state behaviours [18]. Music has been suggested to mask the occurrence of trivial novel auditory stimuli in the environment [19], offering an explanation for the reduced alertness and increased occurrence of biologically relevant behaviours such as ingestion in an equine auditory enrichment study. Meanwhile, genres other than classical music have been associated with reduced psychophysiological stress and positive emotional states for race horses [20], [21]. Investigations into the impact of music on behaviour have thus far been conducted during the stimulus-rich daytime. The aim of the present study therefore was to determine whether auditory stimulation may act as enrichment within the nocturnal environment indicated by changes to the equine nocturnal behavioural patterns with specific reference to recumbency.

Methods and Materials

Subjects

Seven horses (5 geldings, 2 mares; native mixed breeds; age range, 6–16 years; height range 14.0 hh-15.3 hh, none displaying stereotypic behaviours) used within riding lessons for College and

University students at Hartpury Equestrian Centre were observed while in their usual stable (3.6 m × 3.6 m). The floor surface of the stable was half exposed concrete and half concrete covered by rubber matting with a shavings bed (approx. 5 cm thickness with banks approx. 30 cm high), which were cleaned as required throughout the day. Stables were located within a barn and organized into two rows that faced each other, separated by a walkway through the middle. Stable half doors opened into the walkway and with metal bars enclosing the top half of the front of the stable. Horses were prevented from sensory communication with neighbours as the three remaining walls of the stable were solid. All observations took place between the 30th January and the 20th February, 2018 (mean regional temperature approx. 4.5°C), and horses were rugged individually and in accordance with this. Horses were maintained in their usual routine involving 24 hours stabling because of the time of year which limited grazing opportunities, with ridden exercise for two to three non-consecutive hours per day (using indoor and outdoor arenas). The daily routine involved provision of individualized forage rations at 07:00 and hard feeds at 08:00 repeated at 18:00, with ad lib access to forage and water throughout the day, a final forage ration at 19:30, and lights out at 20:00.

Methods

Similar to the study by Wells and Irwin (2008), this study used an ABA cross-over study design. Horses were observed first under control conditions with no music playing; phase A1 = two consecutive nights (N1 and N2), followed by the experimental condition with music playing; phase B = five consecutive nights (N3 to N7), and finally a control condition with no music playing; phase A2 = two non-consecutive nights (N8 and N9). Horse behaviours were observed between 20:30 and 06:30 using night vision cameras (Sony Super HAD Bullet CCTV Camera, 650 TV Line Infrared Night vision Hikvision 4 Channel H.263 960H USB DVR, and Spy Camera digital wireless CCTV infrared cameras Model: DIGIRC1003 with Spy Camera Portable LCD Model: DIG03SCR) mounted above the stable. Beethoven's ninth Symphony [18], [20] was played in its entirety and on loop, at an average of 62.3 decibels between 20:30 and 01:30 to comply with the wishes of the horse's caregivers, using an iPod and speaker (Apple iPod and Anker miniboom speaker). Frequency of behaviour was recorded using continuous intermittent focal sampling every 2 minutes and a predetermined ethogram (Table 1). Ethical approval was granted by Hartpury Ethics Committee.

Table 1. Ethogram of nocturnal behaviour observed (adapted from [8, 20])

Behavioural label	**Definition for the purpose of the study**
Ingestion (hay, bed, water)	Muzzle is lowered to ground/ within bucket; lips grasp hay/ bedding; masticating, prehending or swallowing food/ water

Lateral recumbency	Recumbent, either lateral thoracic area parallel to and in contact with the ground, head immobile and in contact with the ground, legs extended
Sternal recumbency	Recumbent, with sternum in contact with the ground, legs folded beneath the body, no or limited ear movement
Standing	Immobile displaying either of the following; no or limited ear movement, relaxed tail, eyes closed or half shut, head close to level with the withers or lower
Head over door	Head out of view of the camera i.e. over the stable door
Other	Any behaviour other than those listed above including stand alert, locomotion, excretion.

Data Analysis

Behavioural data are presented as frequency of occurrence (sampled every 2 minutes over 600 minutes per night of observation) and arithmetic means are presented with standard error of means. Parametric and nonparametric repeated measures statistical analyses were used to determine differences in behavioural frequency between phases. Phase B was divided into two subphases to observe specifically the initial versus longer term effects of music exposure for the purpose of analysis. Thus, difference in total frequency of behavioural display for each horse was compared between four phases; phase A1 = nights 1 and 2 (no music), phase B1 = nights 3 and 4 (first two nights with music), phase B2 = nights 6 and 7 (last two nights with music), phase A2 = nights 8 and 9 (no music). All behavioural frequency data were tested to meet the assumptions of parametric testing. For the behaviours that met parametric assumptions, a general linear model was used to determine whether the frequency of behaviours was equal across the four phases ($P < .05$). Differences in behavioural frequency between phases for LR were determined using the Wilcoxon signed rank test (significant at $P < .05$) as data were found to be nonparametric. All statistical tests were conducted using IBM SPSS, version 24.

Results

On average, across nine nights, horses were observed to engage in similar proportions of the following behaviours: recumbency (22%), head over door (23%), and standing (24%). Ingestion occupied the greatest proportion of the nocturnal time budget on average (29%). An anomalous horse was observed engaging in recumbent behaviours on average for 3.6% of the nocturnal time budget, with most of the time spent displaying head over the door behaviour (62%) followed by foraging behaviour (31%). On average, the frequency of behaviour was characteristically different between phases (Table 2). All of the horses in this study were observed to adopt a recumbent position during at least one phase of the study.

Table 2. Summary of mean (standard error of means) total frequency of behaviours for each phase.

	Phase A1	**Phase B1**	**Phase B2**	**Phase A2**
Lateral recumbency (REM)	14.3 (7.4)	22.3 (11.4) a	10.9 (6.2) a, b	20.0 (10.6) b
Sternal recumbency (NREM)	115.4 (±65.5)	119.7 (±70.0)	119.4 (±81.0)	124.1 (±70.2)
Ingestion	152.7 (10.0) a	188.6 (12.8) b,c	187.9 (15.0) b, c	144.7 (15.4) d
Standing (Non-REM sleep)	156.1 (±129.8)	132.6 (±85.3)	126.6 (±101.2)	165.1 (±100.3)
Head over door	137.9 (56.0)	130.6 (51.9)	136.9 (60.6)	131.0 (55.4)
Other	20.6 (±8.0) a	10.6 (±4.1) b	7.9 (±2.4) b	12.3 (±6.4) b

Means in a row with different letters differ significantly.

The frequency of LR was significantly higher in phase B1 compared with B2 (Z = -2.197, P = .028) and higher in phase A2 compared with B2 (Z = −1.997, P = .046). No significant differences were detected for sternal recumbency (F [3,18] = 0.093; P = .963) and standing behaviour (F [3,18] = 1.882; P = .169) between different phases. A significant change in the frequency of ingestion (F [3,18] = 7.910, P = .001), specifically between phase A1 and B1 (P = .038), A1 and B2 (P = .019), B1 and A2 (P = .02), and B2 and A2 (P = .004) was apparent. The occurrence of “other” behaviours was also significantly higher (F [3,18] = 10.25, P = .000) in phase A1 compared with B1 (P = .008), B2 (P = .007), and A2 (P = .017). Finally, no significant difference (F [3,18] = 0.157; P = .924) for head over door behaviour was detected between different phases.

Discussion

The introduction of music had a significant effect on the nocturnal time budget, resulting in significantly more ingestion and recumbent behaviour. These results are similar to nocturnal effects of auditory enrichment observed for behaviour of kennelled canines, where the use of classical music resulted in species-specific positive changes to behavioural repertoires during the day, namely increased resting/decreased barking [17], [23]. Increased heart rate variability and reduced heart rate have been documented previously for horses exposed to music in the stable [20], [21], suggesting a music might induce a relaxed state that encourages more rest behaviour. Increasing ingestion in the present study was complimented by a decrease in the occurrence of “other” behaviours, a pattern that was consistently observed while music was played. Classical music resulted in significant effects on the behaviour of zoo-housed Asian elephants, most notably a reduction in the occurrence of stereotypic behaviour [19]. The mechanism for the way in which music enriched the environment was discussed in terms of masking external auditory stimuli, but the authors also proposed a neurological effect. As a neophobic species [24], the benefits of auditory stimuli may lie with the way in which unidentifiable external stimuli are masked, thus reducing distractions for the solitary horse confined to its stable. The relaxing nature of music may also induce more positive emotions [20], [21] linked to the display of more biologically significant behaviours. To confirm, none of the horses in the present study had exhausted their evening forage ration before receiving their morning ration, with or without increased ingestion behaviour.

While the duration of music exposure was longer when compared with previous auditory enrichment studies, the novel effect of playing music every night needs further investigation to understand longer term responses to auditory enrichment. Changing the time at which music was played might impact on the nocturnal behavioural time budget, as horses tend to engage in recumbent behaviour/REM sleep after midnight [6], but music in this study ceased at 1.30 am. Increased vigilance/reduced LR in phase B2 could be linked to the occurrence of anomalous environmental occurrences that the auditory enrichment failed to mask or that occurred after the music ceased. Similarly, when considering average behavioural profiles, it is important to consider the impact of individuals within the sample population alongside factors that influence sleep-related behaviour beyond the addition of auditory enrichment. For example, the initial increase in LR in phase B1 was not maintained in B2, but an increase was observed again in phase A2, which could be linked to a sleep rebound effect rather than the effect of the music [25]. On average, the horse is recognized to engage in approximately 3 hours sleep over a 24-hour period with approximately 30–50 minutes of this devoted to REM sleep [4], and it is suggested that sleep debts may need to be repaid if the critical amount is not achieved [25]. The pattern of reduced LR followed by increased prevalence between phases could also be indicative of different exercise intensities during the daytime that were not accounted for during this study [26].

Conclusion

Horses stabled overnight face a hypo-stimulating environment that at times may result in an increased state of vigilance because of isolation and confinement and being less able to identify the source or cause of external environmental stimuli. Music is reported to provide both masking and relaxing effects, which might explain the results of this study where playing music at night significantly affected the nocturnal behaviour profile, specifically the display of more biologically significant behaviours, such as ingestion and LR. Overall, vigilant behaviour (‘head over door’ and ‘other’ behaviours) appeared to decrease in a trade-off with restful behaviours and ingestion suggesting music may have a calming influence. An understanding of the longer term effects of music on the equine nocturnal behavioural profile is required, along with supporting physiological data and investigations of equine sleep rebound and the impact of daytime exercise.

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Paper 4. The effect of altering routine husbandry factors on sleep duration and memory consolidation in the horse

4.1.1 Published as

Greening, L., Downing, J, T., Amiouny, D., Lekang, L. & McBride, S. (2021) The Effect of Altering Routine Husbandry Factors on Sleep Duration and Memory Consolidation in the Horse. *Applied Animal Behaviour Science*. 236, p.105229

4.1.2 Objectives of the study

Determining whether differences in sleep quantity could be detected in response to:

- Different bedding depth
- Different overnight lighting schedules

4.1.3 Why this journal

This journal publishes relevant information on the behaviour of domesticated and utilized animals, looking at the behaviour of farm, zoo and laboratory animals in relation to animal management and welfare. It currently has an impact factor of 2.569 and is the official journal of the International Society of Applied Ethology. The article received attention on social media with 131 interactions to date, plus six citations and 68 captures.

4.1.4 Author contributions

	Linda Greening	Josh Downing, Daniella Amiouny, and Line Lekang	Sebastian McBride
Conceptualisation	30%	30%	50%
Methodology	20%	25%	55%
Formal analysis	50%	25%	25%
Resources	0%	25%	75%
Data curation	10%	60%	30%
Writing the original draft	50%	0%	50%
Editing the original draft	75%	0%	25%
Visualisation	40%	0%	60%
Supervision	0%	0%	100%
Project administration	0%	70%	30%

4.1.5 Future contributions

The study highlighted how the ethogram could be used to measure sleep behaviours effectively. Following the discussion in chapter three, it would benefit from additional behaviours to further distinguish between wakefulness and sleep states within behaviours before being used to address the need to determine ‘normal’ sleep profiles for horses. The ethogram could also be a tool with which to determine the impact of changes to sleep behaviours. The cognitive task in the study was ineffective and it could be replaced with a more demanding test or with welfare indicators such as salivary cortisol.

Following this study, I was invited to join a group bidding for funding for money to look at the influence of a standardised lighting system on equine behaviour and clock gene expression. The group was awarded $10,000 to carry out the project, which ran from the middle of January to the middle of March 2023. Results from this study will offer greater detail on the influence of light on circadian control of sleep, including sleep quality metrics.

4.2 The published article in full

Abstract

Sleep is a critically important behaviour for all mammals due to its fundamental role within homeostatic/ circadian systems and memory consolidation. As a large and vigilant prey species that is highly sensitive to stimuli at night, the horse sleeps less than other mammalian species. For this reason, the domestic environment has the potential to greatly affect the duration and quality of equine sleep. This study aimed to determine the effect of environmental factors on equine sleep stages, and whether this would influence cognitive performance during a spatial memory task. Ten riding school horses (mixed breed/ height/ sex; average age 14.9 + 2.4 years) were randomly assigned to two groups (n = 5) within a five-week crossover repeated measures design experiment. Each group experienced a combination of one of two light conditions (lights on = Treatment; lights off = Control), and one of two bedding depth treatments (15 cm bed = control; 5 cm bed = treatment) for six days. Duration of sleep stage behaviours (standing Non-Rapid Eye Movement [NREM]), sternal NREM, sternal Rapid Eye Movement [REM] and lateral REM) were measured continuously using CCTV infrared cameras. For the spatial memory task, latency, number of correct responses, and differences between these parameters during training and testing days were measured. A repeated measures general linear model assessed the effects of treatment conditions on duration of sleep stage, and changes in sleep stage over time (bedding and light set as within-subject factors). Wilcoxon Signed-Rank and paired t-tests determined differences in memory task parameters between treatments. Comparing Treatment Bedding with Control Bedding conditions, horses spent on average significantly less time in lateral REM (0.34 ± 0.12 versus 0.46 ± 0.13 h; p = 0.032) and sternal NREM (0.64 ± 0.10 versus 0.80 ± 0.12 h; p = 0.007), and significantly more time in standing NREM (3.69 ± 0.76 versus 3.17 ± 0.77; p = 0.024). Only sternal REM was significantly affected during the Treatment Light condition compared to control conditions (0.53 ± 0.07 versus 0.67 ± 0.11; p = 0.031). Interactions between day and treatment were apparent for specific sleep stage behaviours indicative of acclimatisation. No significant effects ($p > 0.05$) of Treatment Light or Bedding conditions were detected for performance during the spatial memory test. Overall, horses exposed to sub-optimal conditions tended to display significantly less time in recumbent sleep stages (NREM and REM) and increased time in a standing NREM stage. The impact of reduced sleep on equine cognition requires further study.

Keywords: Equine Nocturnal Behaviour Sleep Bedding Light

Introduction

Sleep is one of the most critically important behaviours to all domestic animals due to its fundamental role within homeostatic and circadian systems (Toth and Bhargava, 2013). Both of the primary sleep stages (Non-Rapid Eye Movement [NREM] and Rapid Eye Movement [REM]) regulate a range of physiological processes including neuroendocrine modulation, restorative functions, and memory consolidation (Beccuti and Pannain, 2011; Mavanji et al., 2012; Toth and Bhargava, 2013). Thus, reduced sleep and states of sleep deprivation cause changes in a range of cognitive, emotional and physiological states such as cognitive impairment including reduced spatial memory (Guan et al., 2004), increased levels of anxiety and aggression, and depletion of glycogen stores along with changes in appetite (McEwen, 2006).

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

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

Other factors within the domestic environment can potentially affect horses' ability to adopt a recumbent position for the purpose of sleep. For example, the average duration of laterally recumbent behaviour is reportedly higher for straw bedding (44.0 min) compared to shavings and/or straw pellets (21.6 min) (Pedersen et al., 2004; Greening et al., 2013; Werhahn et al., 2010) with bedding depth also being important in this respect (Pedersen et al., 2004; Werhahn et al., 2010; Modena and Greening, 2019). Although no research has been directly carried out on the effect of lighting conditions on equine sleep stages, light (between 3 and 10 lx) is known to affect melatonin production in the horse (Walsh et al., 2013). Thus, artificial lighting within the stable environment will undoubtedly have an effect on the animal's ability to enter into stages of sleep. Traditional practice is that artificial lights are turned off within the stable environment overnight, however, in some instances this may not always be the case. In addition, late-night checks on horses involving lights being turned on could affect melatonin cycles and subsequently, sleep patterns. The aim of this study was to determine the effects of altering the environment (bedding depth and light) on the duration of different sleep stages (NREM and REM) in the stabled horse. In addition, to determine the functional and welfare consequences of potential sleep deprivation, the study also assessed the effects of bedding depth and light on performance within a spatial memory task.

Materials and methods

Animals

Ten school horses (6 geldings, 4 mares; mixed breeds; average age 14.9 +2.4 years; average height 163.5 ±7.4 cm, none displaying stereotypic behaviours) were observed in their usual 3.6m x 3.6m stable, experiencing the same feeding schedule (three forage rations presented in stables) were organised around the periphery of the barn so that study subjects were able to see stables and horses within the barn. Stable half doors were open, with metal bars enclosing the top half of three walls of the stable but not the back wall, facilitating some sensory communication with neighbours. Observations took place between November and December 2019 (mean regional air temperature 5.75◦c/ 42.85 days of sunshine/ sunset between 16:00 and 16:20, (Met Office, 2020) with individual placement of rugs on the horses. The study was given ethical approval from the Aberystwyth University Animal Welfare and Ethical Review Board (AWERB).

Experimental design

Horses were randomly assigned to two groups balanced for sex and age (Group A [n 5] or Group B [n 5]) and exposed to two treatment conditions (Light and Bedding) within a two factor, crossover repeated- measures experimental design (Table 1). Each treatment lasted for six days plus a one day wash-out period. Groups were staggered in the treatment sequence by seven days (Group B started seven days earlier and Group A finished seven days later) due to the logistics of applying treatment conditions within the same stable environment (Table 1). The disadvantage of this short temporal difference was considered to be outweighed by having all animals within the same experimental environment with similar work and husbandry routines. The Control Light condition involved the normal turning off (at 20:00) of fluorescent tube lighting (2 lx) overnight, whereas the Treatment Light condition maintained the fluorescent tube lighting on (180 lx) during this period. The fluorescent tube lighting was ‘warm white’ with primary spectral peaks of 490 nm, 550 nm and 625 nm. The Control Bedding condition was bedding at normal height of 15 cm and Treatment Bedding was maintained at the lower height of 5 cm. A yard stick marked at 5 cm and 15 cm was used to measure bedding depth from the same position every morning that enabled the addition or removal of bed as required.

Table 1. Details of treatments during the crossover experimental design for groups A and B.

Group	**Days 1-7**	**Days 8-14**	**Days 15-21**	**Days 22-28**	**Days 29-35**
A		Control Light; Control Bedding	Treatment Light; Control Bedding	Control Light; Treatment Bedding	Treatment Light; Treatment Bedding
B	Treatment Light; Treatment Bedding	Control Light; Treatment Bedding	Treatment Light; Control Bedding	Control Light; Control Bedding	

Sleep measurement

High-quality security infrared cameras (Reolink H.264 Digital Video Recorder and ANNKE model N28WEB) were used to record behaviour of all study subjects for 24 h across seven consecutive days during the five-week study period, according to a predetermined ethogram (Table 2). Cameras were mounted above the stable that enabled a field of vision spanning the entire floor of the stable and prevented study subjects from interfering with the cameras. Duration of behaviour was recorded (hours) using continuous focal behavioural sampling over a 24 h period for 6 days per experimental week for each horse, that was reviewed by three observers. Several training sessions were held to ensure accurate agreement on the four sleep stage behaviours and this was followed with an inter-observer reliability measurement using sample behaviour footage (R2 = 1).

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

Behavioural sleep state		**Definition**
Standing Sleep (NREM)		Subject is standing immobile, with the altanto-optical (poll) below the withers (with or without a resting hind limb)
Sternal Recumbency (NREM)		Subject is lying with the sternum in contact with the ground and legs folded beneath the body, the muzzle is not in contact with the ground
Sternal Recumbency (REM)		Subject is in sternal recumbency (as described left) but showing REM sleep signs, i.e. the muzzle is clearly resting on/in contact with the ground
Lateral Recumbency (REM)		Subject is lying on its side (either lateral thoracic area parallel to and in contact with the ground), legs extended, head is on the floor and immobile other than for ear/eye movement

Spatial memory task

Both groups were tested on the spatial memory task during two of the combined treatments periods 1) Control Light/Control Bedding and 2) Treatment Light/Treatment Bedding. The task required subjects to use distal spatial cues (signage, door apertures and view gallery) to locate a salient object (food within a bucket) by disregarding local cues (bucket colour), based on a standard Morris maze methodology used in rodents (Hamilton et al., 2007). The task was conducted in an indoor school (60m × 30m with a combi-ride waxed surface) containing a number of distal cues that study subjects were all familiar with (Fig. 1).

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

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

During the training session, each horse was first led around the inside of the arena to inspect and eat from all the buckets, which contained food (pre-training phase). The horse was then led around the outside of the arena for 5 min whilst food was placed in the designated bucket. The first trial started when the horse was released (lead rope unclipped from head collar) at a pre-designated random position and left to autonomously navigate the arena for 120 s. The lead rope was reattached to the head collar at the end of the 120 s period or once the horse had located the food. The horse was then led outside the arena to enable preparation for the next trial. The training phase continued until the horse reached a) learning criterion (four consecutive correct responses) or b) a maximum number of trials set at 18. A correct response was described as eating from the bucket containing food within 120 s. If horses did not respond for five consecutive trials, the training session was terminated and the horse returned to its stable. The testing session occurred on the day immediately following the training session and followed the same protocol, minus the pre-training phase. Trials were conducted until the horse reached a) the learning criterion or b) the maximum number of trials set at 6. Correct and incorrect responses and latency to locate food was recorded (seconds) for each trial giving the following measures of task performance: mean training latency (Md1), mean testing latency (Md2), mean difference of training-testing latency (Md1 - Md2), mean correct number of training responses (%d1), mean correct number of testing responses (%d2), and training-testing difference in correct responses (%d1- %d2). The latency for failed trials was recorded as 120 s. Data from horses that disengaged from the task (5 consecutive non-responses) were not included in the final data set. Even though the order of treatments was counterbalanced across the two groups of animals, in order to reduce the effects of learning due to repeat testing over the two treatment periods (and thus maximise treatment effects), a memory wash out session was conducted between the two treatment periods. Here, the salience of the previous location was diminished by leading all horses to baited buckets in all four locations (120 s per location) (Hamilton et al., 2007).

Data analysis

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

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

Results

Sleep

Mean duration

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

There was no significant effect (F(1,9) = 3.54, p = 0.09) of Treatment Bedding (4.43 ± 0.79 h) versus Control Bedding (3.98 ± 0.81 h) or Treatment Light (4.35 ± 0.76) versus Control Light (3.95 ± 0.84 h) (F(1,9) = 2.27, p = 0.17) on the total amount of NREM sleep. There was no significant effect (F(1,9) = 4.31, p = 0.07) of the Treatment Bedding (0.87 ± 0.11) versus Control Bedding (1.13 ± 0.12 h), nor for the Treatment Light (0.91 ± 0.11) versus Control Light (1.09 ± 0.11 h) (F(1,9) = 4.24, p = 0.07) on the total amount REM of sleep. However, horses spent significantly more time in standing NREM sleep for the Treatment Bedding (5 cm) compared with the Control Bedding (15 cm) (3.69 ± 0.76 versus 3.17 ± 0.77; F(1,9) = 7.303, p = 0.024;) (Fig. 2A.). Horses also spent significantly less time in sternal NREM sleep (0.64 ± 0.10 versus 0.80 ± 0.12 h; F(1,9) = 12.238, p = 0.007) (Fig. 2B), and significantly less time in lateral REM (0.34 ± 0.12 versus 0.46 ± 0.13 h; F(1,9) = 6.450, p = 0.032) during the Treatment Bedding week compared with the Control Bedding (Fig. 2D). Horses spent significantly less time in sternal REM during the Treatment Light compared to the Control Light (0.53 ± 0.07 versus 0.67 ± 0.11; F(1,9) = 6.511, p = 0.031) (Fig. 2C). No statistical difference was found in the duration of sternal REM between Control and Treatment Bedding (F(1,9) = 2.317, p = 0.162), nor in the amount of time spent in lateral REM (F(1,9) = 0.758, p = 0.407), sternal NREM (F(1,9) = 0.037, p = 0.853) and standing NREM (F(1,9) = 2.981, p = 0.118) between the two light conditions. There were no significant interaction effects between the Bedding and Lighting treatments for any of the sleep stages: Standing NREM (F(1,9) = 0.410, p = 0.538); Sternal NREM (F(1,9) = 4.203, p = 0.071); Sternal REM (F(1,9) = 0.012, p = 0.917); Lateral REM, (F(1,9) = 0.946, p = 0.356).

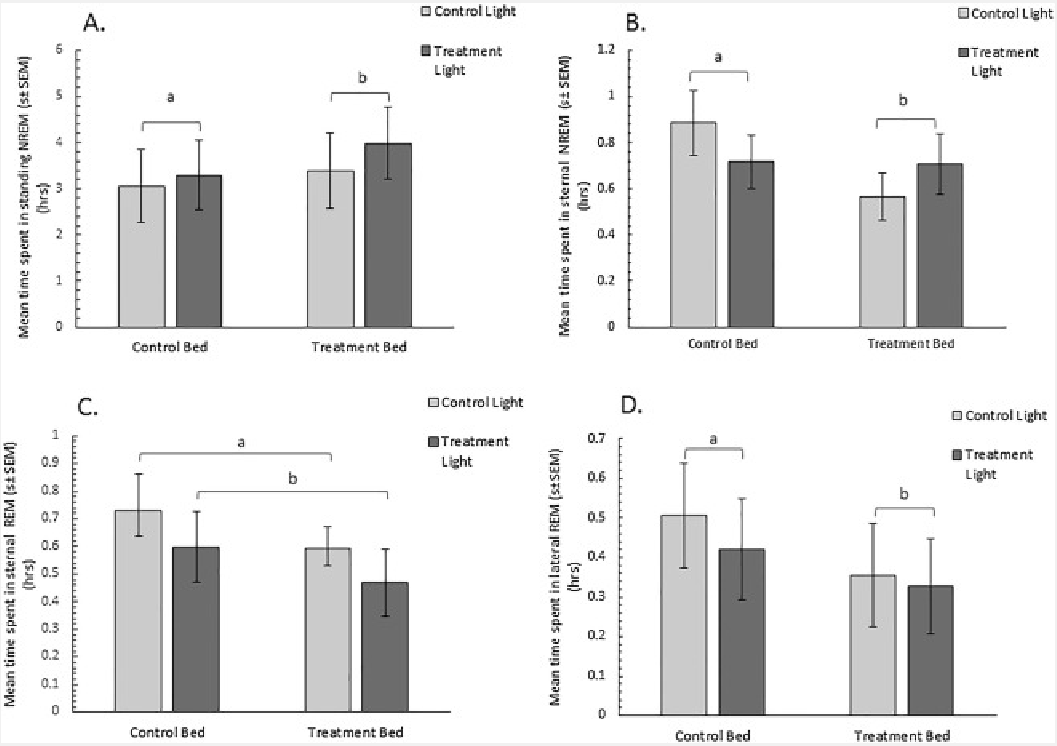

Fig. 2. Mean (±SEM) (n=10) time spent in the 4 different sleep behaviours (A. standing NREM, B. sternal NREM, C. lateral REM and D. sternal REM over 6 days of each treatment (Control Bedding [15cm], Treatment Bedding [5cm], Control Light (lux 2), Treatment Light (lux 180). Significant differences between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

<u>Changes over time</u>

The durations of sleep stage per day for each treatment are presented in Fig. 3. There was no significant interaction effect of treatment (light and bedding) with day for Standing NREM (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) = 0.135, p = 0.983). For Sternal NREM, there was a significant interaction of the Treatment Light (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 hoc tests with Bonferroni correction revealed a significant difference between Treatment Light conditions for Day 2 (p = 0.025) but no significant difference between Treatment Light conditions for other days. For Lateral REM, there was no significant interaction for the Treatment Light (F(1,9) = 3.049, p = 0.056) with day nor for Treatment Bedding (F(1,9) = 1.613, p = 0.221).

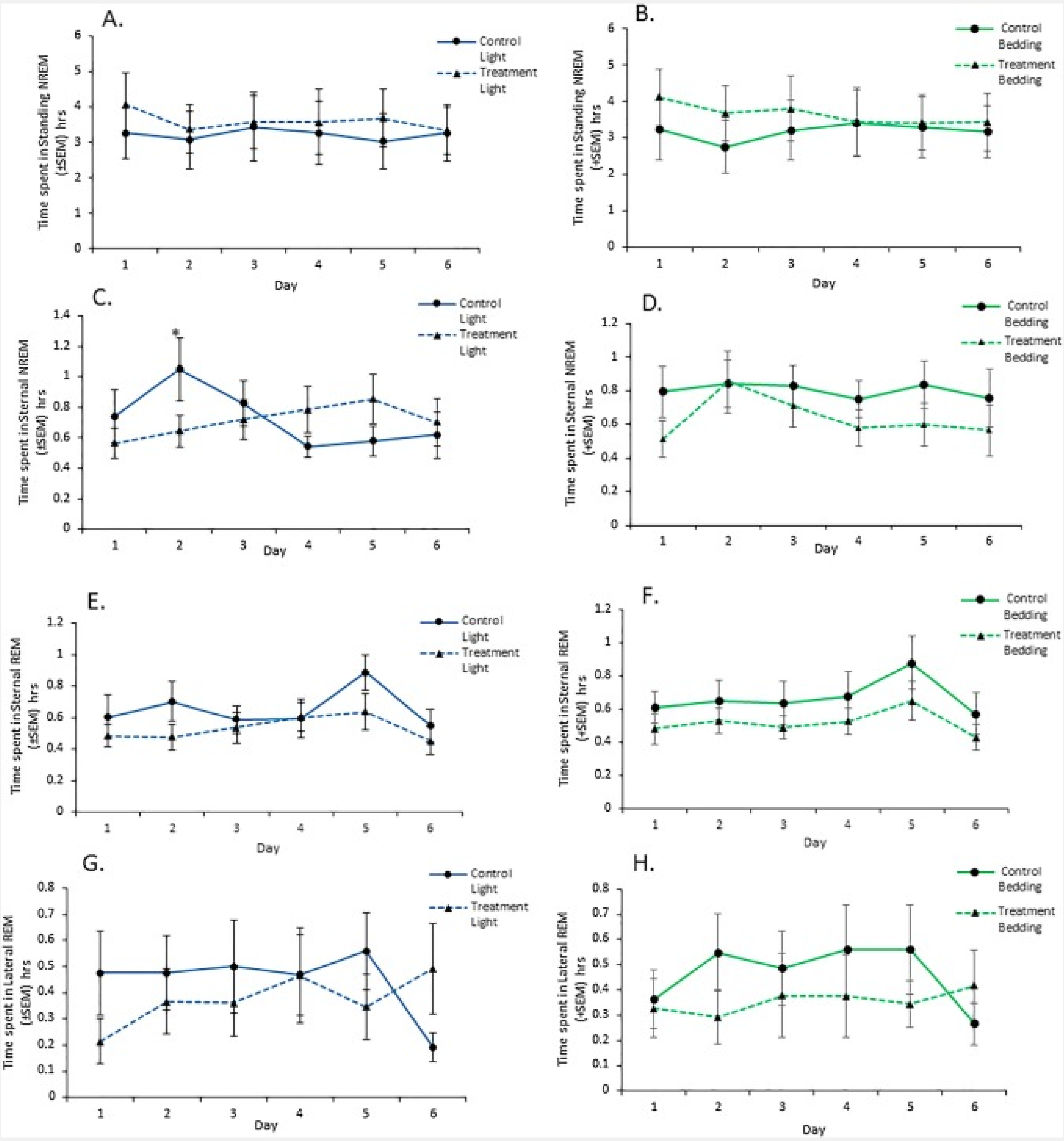

Fig. 3. Mean (±SEM) (n = 10) time spent in the 4 different sleep stages: standing NREM (A,B), sternal NREM (C,D), lateral REM and (E,F), sternal REM (G,H) over 6 days of each.

Treatment (Control Light [lux 2], Treatment Light [lux180]), Control Bedding [15 cm], Treatment Bedding [5 cm]). * Significant differences between treatments at each time point (Day)($p \leq 0.05$).

Memory

A full set of memory test results was obtained for 9 out of the 10 horses. One horse completely disengaged from the task during the second set of memory testing by standing still at the starting point inside the arena. There was no statistical difference between the combined control condition

(15 cm bedding/lux 2) and the combined treatment condition (5 cm bedding, lux 180) for training latency ($t(8) = 0.855$, $p = 0.417$), testing latency ($Z = -0.415$, $p = 0.678$) (Fig. 4A.) and mean difference latency ($t(8) = 2.064$, $p = 0.073$). There was no statistical difference between the combined control and treatment conditions for the percentage correct training responses ($t(8) = -0.684$, $p = 0.513$), percentage correct testing responses ($t(8) = 0.632$, $p = 0.545$), or difference in percentage of correct responses ($t(8) = -1.791$, $p = 0.111$) (Fig. 4B).

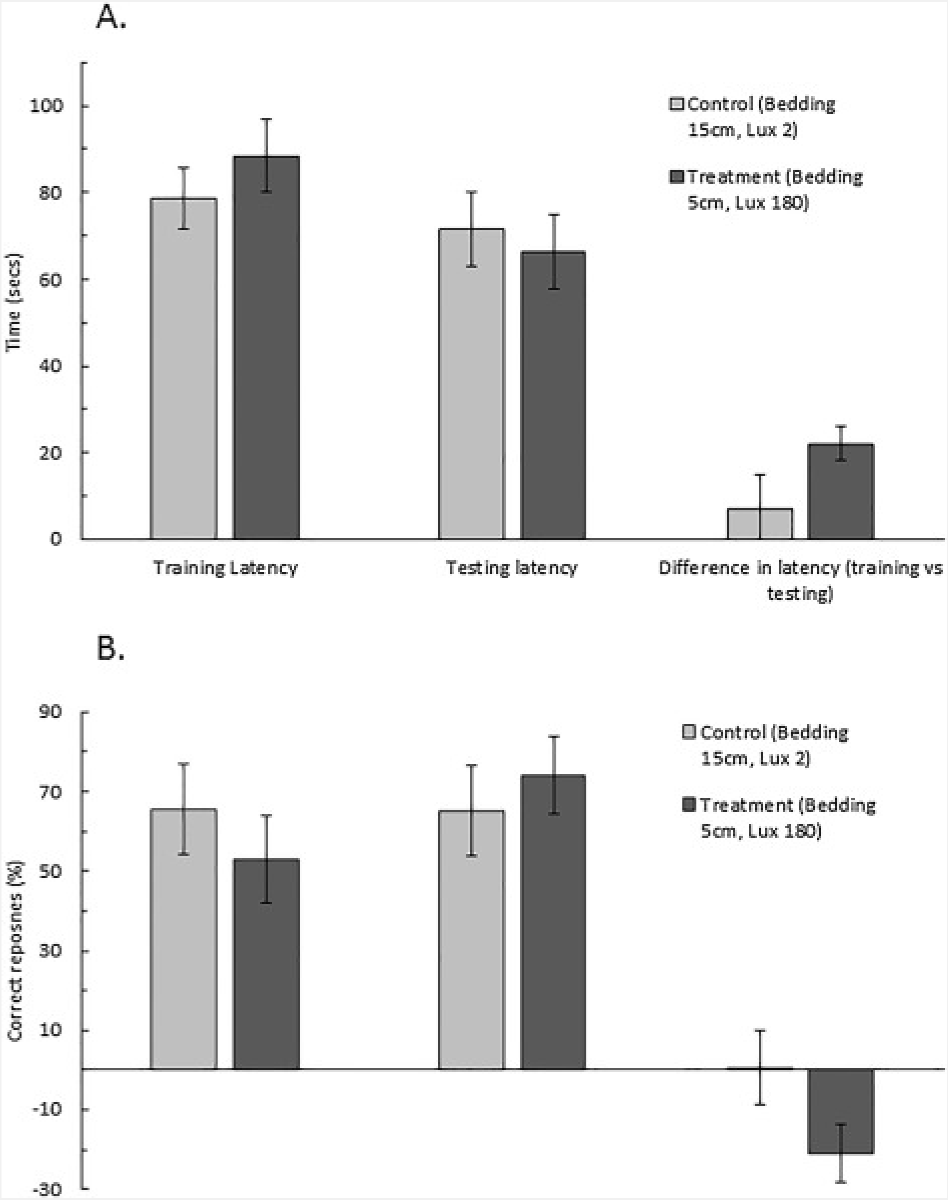

Fig. 4. A. Means (±SEM) for training latency (s), testing latency (s) and difference latency (s) for the combined Control (15 cm bedding, lux 2) and Treatment (5 cm bedding, lux 180) conditions for all horses (n = 9). B. Means (± SEM) for correct percentage of training responses (%), correct percentage of testing responses (%) and difference in percentage of correct responses (training vs

testing, %) for the combined Control (15 cm bedding, lux 2) and Treatment (5 cm bedding, lux 180) conditions for all horses (n = 9).

Discussion

Baseline sleep values and variability

Horses slept on average for a total of 5.2 ± 0.88 h/day during the Control conditions week (15 cm bedding, lights off), which is higher than has been previously reported for total sleep time (TST) for horses (Zepelin, 2000; Wohr et al., 2016). Whilst the use of behavioural observations to quantify equine sleep stages comes with a risk of over or under estimating time spent in each sleep stage, the equine sleep positions observed in this study aligned strongly with the descriptions and characteristics described in the EEG study by Williams et al. (2008). In the present study, REM sleep during the control period represented on average 23.9 % of TST (1.23 h/day), which falls within the range of 15%–27.9% of TST for REM sleep previously reported for stabled horses (Kalus, 2014; Williams et al., 2008; Dallaire and Ruckebusch, 1974a, b). NREM sleep during the control period (76.1 % of TST; 3.94 h/day) was also similar in percentage of TST to previous EEG data (Kalus, 2014). The present study observed horses for 24 h compared to previous studies of stabled horses that tend to report data from nocturnal observations only. Whilst most sleep occurs at night, stabled horses do rest during the day (Ruckebusch, 1975), and in the present study several horses engaged in sternal NREM, sternal REM and even lateral REM during the daytime period. Only 70 % of horses achieved at least one bout of REM sleep every day/night indicating inter-horse variability in sleep profiles. Previous work has suggested that sleep variability in horses may be due to differences in age, diet, daily exercise or their ability to adopt a recumbent position (Crowell-Davis, 1994; Dallaire and Ruckebusch, 1974a, b; Ruckebusch et al., 1970). In this study, the variation in age range of the horses observed in the present study was low, there were no reported injuries and all horses accustomed to the same management routine and daily exercise. Sleep (quantity and quality) in other species has also been reported to be genetically determined (Sehgal and Mignot, 2011). The majority of horses used in this study were classed as 'sports horse' types containing varying proportions of the Thoroughbred breed which may also have had an effect on time spent in the different sleep stages Considering the effect of breed on the occurrence of sleep may, therefore, be important for future equine sleep studies.

NREM sleep

Sternal NREM was significantly reduced with the treatment bedding (5 cm) whilst standing NREM significantly increased. Standing behaviour has previously been used to detect general discomfort within sub-optimal environments for both cattle (Relić et al., 2012) and horses (Chung et al., 2018).

In the present study, rubber matting was used in every stable, which may have improved overall comfort within the stable as indicated by the overall duration of TST which fell within the normal levels previously recorded for the horse. The increase in standing NREM appeared to compensate for the loss of sternal NREM and this was more apparent at the start of the treatment period (Fig. 3b) suggesting some acclimatisation to the reduced bedding substrate.

Overall, no significant effects of light were recorded for total sternal and standing NREM sleep duration, which would suggest that light contamination did not significantly influence the occurrence of NREM sleep stages. There was a significant interaction of Light Treatment with day for sternal NREM, with a significant difference between treatments at day 2 followed by a crossover trend between experimental conditions over the 6 days (Fig. 3c). This potentially suggests some initial effect of the Light Treatment on duration of NREM sleep but also potential adaptation as the treatment progressed. The artificial light/dark cycle in the Control Light condition may therefore act as a 'Zeitgeber', where artificial light appears to initially interrupt the natural patterns/ rhythm of sleep experienced under treatment conditions when left on. Previous work in other species has also shown that dim light (~ 5 lx) can have a significant effect on NREM and REM sleep patterns (Stenvers et al., 2016; Batra et al., 2020). Thus, the lights off condition (2 lx) in this study may still have contained sufficient light intensity to have a physiological effect on sleep, thereby reducing the overall comparative effect with the lights on treatment. Overall, a thinner bed reduced recumbent NREM sleep stages, whilst light appeared to have a short-term effect on the occurrence of sternal NREM specifically.

REM sleep

The Treatment Bedding resulted in a significant reduction in the average duration of lateral REM, and a trend towards reduced duration in sternal REM and total REM (lateral and sternal combined). The reduction in sternal REM was not as pronounced as lateral REM which may be explained by the latter compensating the former. There were no significant interactions of day during the Control and Treatment Bedding conditions with both REM stages consistently lower during the 5 cm Bedding Treatment for the majority of the six-day period. There was, however a peak in lateral REM on day six under the Treatment Bedding condition indicating that a longer observation period might be advantageous for future studies. The data overall confirm that recumbent positions increase when deeper bedding is used, indicative of increased comfort as has previously suggested in other studies (Werhahn et al., 2010) irrespective of bedding type (Modena and Greening, 2019). Additional attributes of bedding can also influence recumbency including texture, softness, smell, cleanliness and insulation properties (Pedersen et al., 2004; Kwiatkowska-Stenzel et al., 2016). In the present study, dirty and wet bedding was removed every morning and evening, however, the reduction in bedding depth may have exacerbated wetness and made the Treatment Bedding more unappealing in this respect. Throughout the study, some of the horses ate their bedding, as seen previously ((Greening et al., 2013)), which may have affected bedding depth towards the end of the 24 h.

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

The influence of the environment on memory test completion

Although no significant effects of the treatment condition (lights on, 5 cm bedding) were apparent for any of the parameters measured during the spatial memory task, there was a slight trend towards increased mean difference latency (training vs testing) for treatment versus the control condition. The latter was predominantly due to horses under treatment conditions taking longer to complete the task during the training phase as opposed to the testing phase. Memory function following some disruption to sleep stages as previously described, therefore, did not appear to be impaired as initially hypothesized, which may be due to a number of reasons. Firstly, the significant reduction in overall sleep and/or specific sleep stages may not have been sufficient to affect memory consolidation due, for example, to the compensatory increase in standing NREM sleep during treatment conditions. Sleep deprivation studies that have reported effects on cognition often physically prevent study subjects from sleeping (for example, (Zhang et al., 2013)Zhang et al., 2013; (Rahman et al., 2013)Rahman et al., 2013), but this was not the purpose of this study. Secondly, the significant reduction in sleep stage duration reported in this study may not reflect the level of sleep quality that the animal is experiencing, the latter of which may not have been affected by the treatment condition. Cycling into REM sleep immediately following NREM is reported to help stabilize transformed memories (Rasch and Born, 2013). Thus, measuring sleep quality, particularly the relationship between REM and NREM cycles, may be a more useful approach to understand the interplay between sleep and memory consolidation. Recording this and additional measures of sleep quality such as sleep fragmentation, may therefore be critical for future sleep studies. Thirdly, sleep reduction is also known to have an impact on the neuroendocrine system which may have indirectly affected cognitive performance. For example, previous research indicates that ghrelin, a hormone promoting hunger, increases with sleep restriction, whereas leptin, a hormone contributing to satiety perception decreases (Morselli et al., 2010). Thus, horses with reduced sleep may have been more motivated to engage in the food-reward task. Increased levels of motivation have previously been found to compensate for deficits in cognitive performance in humans (Alhola and Polo-Kantola, 2007) and other species (Ward, Winiger et al. 2015) and this factor may need to be taken into consideration for future work. Finally, horses under control conditions appeared to perform only marginally better in the testing compared to the training phase of the spatial memory test. This may also suggest that overnight memory consolidation was not required given the cognitive demands of the task.

Conclusion

The duration of equine sleep stages was found to be affected by both the depth of bedding and lighting conditions. During sub-optimal conditions horses tended to display significantly reduced time in recumbent states for both NREM and REM sleep, which was compensated by increased time in the standing NREM stage. The impact of reduced sleep on the animal was not fully determined in this study. Although the data suggested that there might be some effect on spatial memory, a more cognitively demanding task may better differentiate the performance-related consequences of different levels of sleep duration and quality. This study specifically highlights the influence of bedding depth on sleep behaviour, and how minimal alterations can be made in the stable environment to better accommodate sleep behaviour with potentially positive outcomes for equine welfare.

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Paper 5. Towards an objective measurement of sleep quality in non-human animals; using the horse as a model species for the creation of sleep quality indices.

5.1.1 Published as (at the time of writing)

Greening, L. Allen, S. and McBride, S, D. (*IN PRESS*) Towards an objective measurement of sleep quality in non-human animals; using the horse as a model species for the creation of sleep quality indices. *Biology Open*.

5.1.2 Objectives of the study

- Determining whether a novel sleep quality metric is different to sleep quantity.
- Highlighting areas for future research, including the proposal of novel measurements.

5.1.3 Why this journal

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5.1.4 Author contributions

	L Greening	S Allen	S McBride
Conceptualisation	45%	10%	45%
Methodology	45%	10%	45%
Formal analysis	50%	0%	50%
Resources	0%	0%	100%
Data curation	0%	100%	0%
Writing – original draft	50%	0%	50%
Writing – reviewing & editing	60%	10%	30%
Visualisation	50%	0%	50%
Supervision	10%	0%	90%
Project administration	0%	70%	30%

5.1.5 Future contributions

The use of the SQI metric could have application across a range of animal species but the next step will be to determine how a low sleep score is quantified as poor sleep relative to the outcomes in terms of welfare or performance. The proposed collaborative project outlined in sections 3.4.1

and appendix 1.2.5 aiming to use EEG to validate the sleep ethogram will include metrics of sleep quality i.e. awakenings, that can be used to investigate the impacts of disturbed sleep.

5.1.6 Confirmation of acceptance

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To: Linda.Greening
Cc: shallen97@btinternet.com; sdm@aber.ac.uk; rachel.hackett@biologists.com
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Biology Open

MS ID#: BIOLOPEN/2023/059964

MS TITLE: Towards an objective measurement of sleep quality in non-verbal animal species; the horse as a model

AUTHORS: Linda Greening, Sian Allen, and Sebastian McBride
ARTICLE TYPE: Research Article

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5.2 The published article full draft (prior to author proofs)

Summary statement: Unlike humans, the quality of animal sleep cannot be assessed via verbal subjective report. This study sought to use quantitative scoring indices to measure sleep quality in a non-verbal animal species (*Equus caballus*).

Abstract

Sleep disturbance is observed across species, resulting in neurocognitive dysfunction and poor impulse control/regulation of negative emotion. Understanding animal sleep disturbance is thus important to understand how environmental factors influence animal sleep and day-to-day welfare. Self-reporting tools for sleep disturbance are commonly used in human research to determine sleep quality, that cannot be transferred to non-verbal animal species research. Human research has, however, successfully used frequency of awakenings to create an objective measurement of sleep quality. The aim of this study was to utilise a novel sleep quality scoring system for a non-human mammalian species. Five separate sleep quality indices calculations were developed using frequency of awakenings and total sleep time/total time spent in different sleep states. These indices were applied to a pre-existing data set of equine sleep behaviour taken from a study investigating the effects of environmental change (lighting and bedding) on the duration of time in different sleep states. Significant treatment effects for index scores both differed and aligned to the original sleep quantity results, thus sleep quality may be a useful alternative measurement of sleep disturbance that could be used to investigate impactful (emotional, cognitive) effects on the animal.

Introduction

Sleep disturbance in humans produces a number of deleterious effects including neurocognitive dysfunction and poor impulse controls (Fabbri et al., 2021), impaired executive functioning (Tucker et al., 2010), poor regulation of negative emotion (Mauss, Troy and LeBourgeois, 2013) and depression (Jakubcakova et al., 2012). Disturbed sleep has been shown to have a similar effect in animals, for example sleep disruption leads to significantly reduced interspecific emotion recognition in dogs (*Canis familiaris* – Bolló et al., 2020), and behavioural communication is affected in honey bees (*Apis mellifera* – Klein et al., 2010). Understanding and being able to measure sleep quality in different animal species is therefore paramount if we are to understand how environmental factors can affect animal sleep and thus impact their day-to-day welfare. To date, animal sleep research has largely focused on cumulative quantity relative to total sleep time, as opposed to the quality of sleep. This may be an important limitation because, in human studies, sleep quantity does not reflect the subjective sleep experience (for example, Bei et al., 2010). Although subjective reports of sleep quality in humans have previously been compared to objective measures of sleep quantity (e.g. sleep onset latency (SOL), total wake time (TWT), and total sleep time (TST) (Mendonça et al., 2019), these metrics are not always capable of differentiating between different levels of sleep quality. For example, measures often do not differ significantly between normal sleepers and insomniacs or individuals who continue to subjectively report poor quality sleep (Krystal and Edinger, 2008). In contrast, other characteristics of sleep architecture have been more closely linked to subjective reports of sleep quality, primarily the number of awakenings during total sleep time (Rosipal et al., 2013).

Sleep awakenings are often referred to as sleep disturbance, disruption, fragmentation, interruption, arousal and wake sequences/episodes, with each being defined differently. For example, sleep fragmentation has been defined as an increase in electroencephalography (EEG) frequency and electromyography (EMG) amplitude lasting for more than three seconds, (Stepanski et al., 1984). Meanwhile sleep interruptions have been defined as any sleep cycle with more than three minutes of continuous wakefulness for humans (Merica and Gaillard, 1986) after which the individual cannot easily revert back to sleep (Halász et al., 2004). Wake sequences in rats have also been described in similar terms but with a threshold of 300 s used to distinguish between brief wake and long duration wake episodes (Simasko 2009). Whilst awakenings have some functional significance for normal sleep (Halász et al., 2004; Chuan Lo et al., 2004), they have also been described as markers of sleep disruption representing a detrimental and harmful feature for sleep (Atlas Task Force, 1992). The occurrence of sleep awakenings between species is variable, possibly due to the variation in the functional significance of sleep (Greening & McBride, 2022) and the differences in total sleep time (Campbell and Tobler, 1984). For example, mouse sleep is highly fragmented with sleep episodes lasting less than five minutes (Toth and Bhargava, 2013), and rat sleep can be interrupted by a high number of wake episodes (38-40; <300 s) (Simasko 2009). Meanwhile, equine sleep episodes last on average 40.74 minutes (Greening & McBride, 2022) with an average of seven wake sequences (<3 minutes) per sleep cycle (Kalus, 2014) and humans have sleep episodes lasting 80-200 minutes (Le Bon et al., 2002) with an average of 0.64 wake sequences per sleep cycle (Greening & McBride, 2022). These profiles of species-specific awakenings are considered to be part of the normal sleep pattern (Chuan Lo et al., 2004). In this context, disrupted sleep is when the number of awakenings is significantly above this normal pattern but also when these awakenings transition into a state of wakefulness that subsequently affect the duration of total sleep time. Establishing criteria for sleep interruptions and awakenings in relation to total sleep time (total sleep state time) could therefore provide a useful basis for developing objective measures of sleep quality in non-human species.

The use of subdural and surface electrodes for EEG and polysomnography (PSG) has proven successful for recording sleep in a range of animals e.g. horses (*Equus caballus* – Williams et al., 2008), dogs (*Canis familiaris* – Kis et al., 2017), and cows (*Bos taurus taurus* – Hunter et al., 2021), however there are some disadvantages to this approach. For example, the continuity and accuracy of 24 hr observations can be affected by movement of superficial facial and ear musculature, loss of contact between electrode and surface of the head, and influence of sweat and other extraneous factors (Hunter et al., 2021; Zanker et al., 2021). EEG outputs are also complex and voluminous, and time consuming in their interpretation. Meanwhile, EEG equipment is expensive and technically demanding, and is often limited for use in non-human species. The alternative of behavioural sleep scoring has advantages and disadvantages, including human interpretation of sleep state, time required for sleep scoring and overlap in behaviours between different sleep states (Greening and McBride, 2022). In terms of developing a measurement of sleep quality in non-human animals, the methodological approach (EEG vs. behaviour) will have implications on the validity of the sleep quality metric. For example, in an experimental situation where changes in the environment are used to induce changes in quantity and quality of sleep, different levels of conclusion can be drawn depending on which metrics are used (Table 1). Overall EEG metrics allow for firmer conclusions, whereas behavioural metrics can create alternative interpretations of the data and thus fewer firm conclusions can be drawn. In this study, we employed a behavioural approach, for some of the reasons stated above but primarily with the aim of developing an index of sleep quality that is not reliant on specialist equipment and can be derived from the more accessible measurement of movement/ posture of the animal.

Table 1. Conclusions that can be drawn using EEG versus behavioural metrics of sleep quantity and quality in sleep disturbance studies.

Measurement		**Conclusions using EEG metric**	**Conclusions using behavioural metric**
Quantity	Quality		
No change	No change	Strong evidence that there is no change in sleep due to changes to the sleep environment.	Unable to completely discern whether lack of change in sleep behaviour is due to lack of sensitivity or inappropriateness of behaviour metric or lack of effect of changes to sleep environment
No change	Change	Quality but not quantity measures are sensitive to changes to the sleep environment. Quality measures may be superior in predicting impactful consequences of disturbed sleep.	Quality but not quantity measures are sensitive to changes to the sleep environment. Quality measures may be superior in predicting impactful consequences of disturbed sleep.
Change	No change	Quantity but not quality measures are sensitive to changes to the	Quantity but not quality measures are sensitive to changes to the sleep

		sleep environment. Quantity measures may be superior in predicting impactful consequences of disturbed sleep.	environment. Quantity measures may be superior in predicting impactful consequences of disturbed sleep or, quality measures are insensitive/inappropriate measures changes to the sleep environment
Change	Change	Both quantity and quality measures are affected by changes to the environment. Both quantity and quality measures may useful in predicting impactful consequences of disturbed sleep.	Changes in both measures offer reasonable assurance that the behavioural metric is sensitive to changes to the environment. Both quantity and quality measures may useful predicting impactful consequences of disturbed sleep.

Equine sleep has been well characterised with evidence of the occurrence of rapid eye movement (REM) and non-rapid eye movement (NREM) sleep states, and sleep fragmentation (Greening and McBride, 2022). As a prey species, the horse is typically vigilant and demonstrates high levels of wake sequences during sleep cycles. Equine sleep can also be greatly affected by changes within the environment (Greening et al., 2021) with evidence of sleep disturbances occurring within EEG profiles (Kalus, 2014). In this context, the horse may be a useful model for the study of sleep quality in a non-human species that is unable to self-report sleep experiences. Therefore, the aim of this study was to develop a behaviour-based measurement of sleep quality in the horse, which could be used more broadly as a methodological approach for other non-human species.

Results

The following presents significant results for each of the five indices. Non-significant model outputs can also be found in Supplementary Material Table 1.

Total SQI

A significant difference (F=4.942, P=0.05, η^2=0.354) in Total SQI was observed between Control Bedding (mean 7.61, SE=0.55) and Treatment Bedding conditions (mean 8.21, SE=0.66), but not between Control Light and Treatment Light conditions. No significant interactions were observed between bed and light or bed/light and day. No significant effects were observed for lighting or bedding conditions between days (Figure 1).

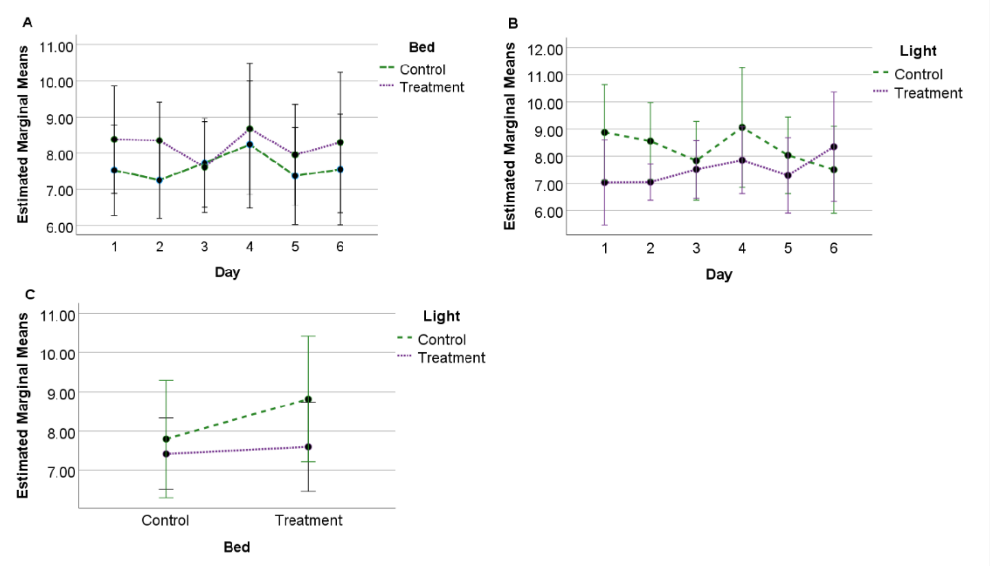

Figure 1. Estimated marginal means (s.d.) for total sleep quality index (SQI) score. Observed for 24 h across six days under each experimental condition (N=10) showing A) profile of SQI scores for bedding over six days; B) profile of SQI scores for lighting over six days, and; C) interaction between bedding and lighting conditions.

Combined SQI

There were no significant differences between treatment or control conditions for lighting or bedding, nor any significant interactions between bed and light. A significant interaction of lighting with day ($F=3.674$; $P=0.007$, $\eta^2=0.290$) was observed between light conditions. Post-hoc analysis found a significant difference ($P=0.048$) between Control Light (mean 11.85, SE=0.72) and Treatment Light (mean 10.50, SE=0.36) on day two. No significant interactions with day detected for different bedding conditions, or for interactions between days for bedding or light (Figure 2).

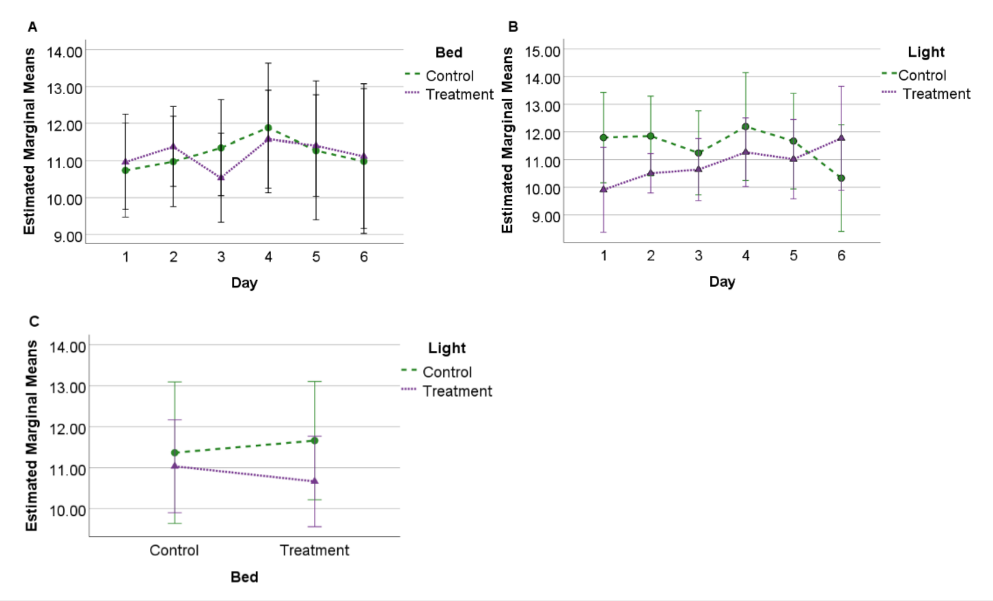

Figure 2. Estimated marginal means (s.d.) for combined sleep quality index (SQI) score. Observed for 24 h across six days under each experimental condition (N=10) showing A) profile of SQI scores for bedding over six days; B) profile of SQI scores for lighting over six days, and; C) interaction between bedding and lighting conditions.

Combined weighted SQI

There were no significant differences for treatment or control conditions for lighting or bedding, nor any significant interactions between or bed and light. A significant interaction between days for Control Light (F=2.477, P=0.046, η^2=0.216) was observed. Post-hoc analysis found a significant difference (P=0.034) between day two (mean 15.49, SE=0.91) and day six (mean 13.02, SE=1.18) (Figure 3). There were no significant differences between days for Treatment Lighting, or Control and Treatment Bedding conditions, nor were there significant interactions with day for lighting and bedding conditions.

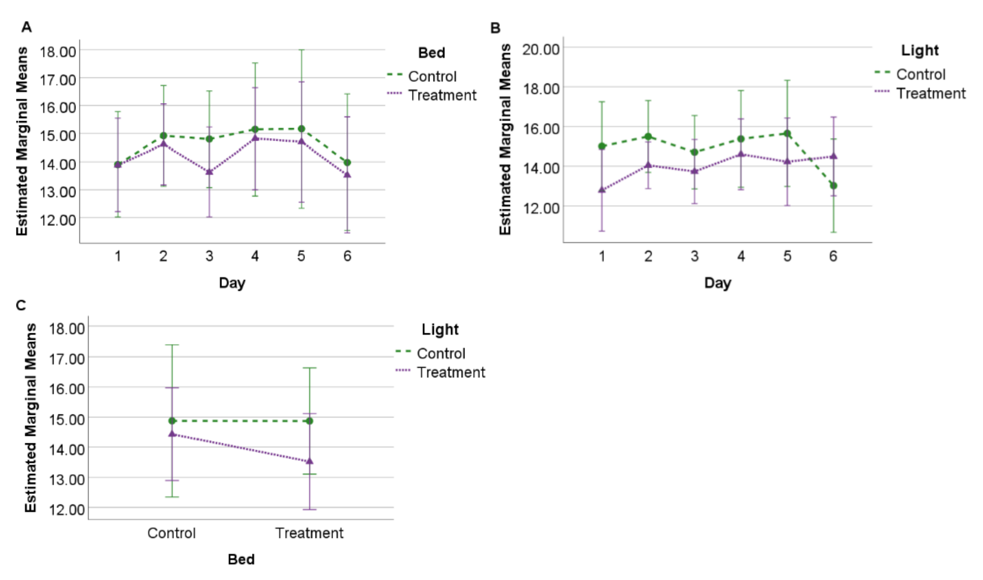

Figure 3. Estimated marginal means (s.d.) for combined weighted sleep quality index (SQI) score. Observed for 24 h across six days under each experimental condition (N=10) showing A) profile of SQI scores for bedding over six days; B) profile of SQI scores for lighting over six days, and; C) interaction between bedding and lighting conditions.

REM and NREM SQI

No significant differences between scores were reported for any variable for the REM SQI calculation (Figure 4). For the NREM SQI, there were no significant differences between treatment or control conditions for lighting or bedding, nor any significant interactions between bed and light. A significant effect of day (F=3.83, P<0.01, η^2=0.30) was observed between light conditions. Post-hoc analysis found a significant difference (P=0.02) between Control Light (9.38, SE=0.77) and Treatment Light (7.99*, SE=0.41) on day two (figure 5). There were no significant differences between days for each bedding and lighting condition.

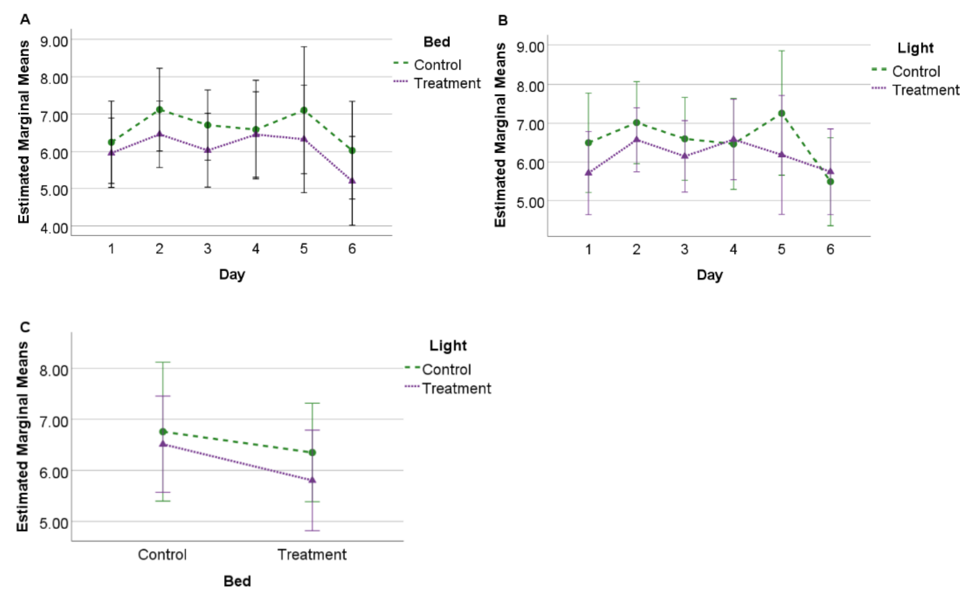

Figure 4. Estimated marginal means (s.d.) for REM sleep quality index (SQI) score. Observed for 24 h across six days under each experimental condition (N=10) showing A) profile of SQI scores for bedding over six days; B) profile of SQI scores for lighting over six days, and; C) interaction between bedding and lighting conditions.

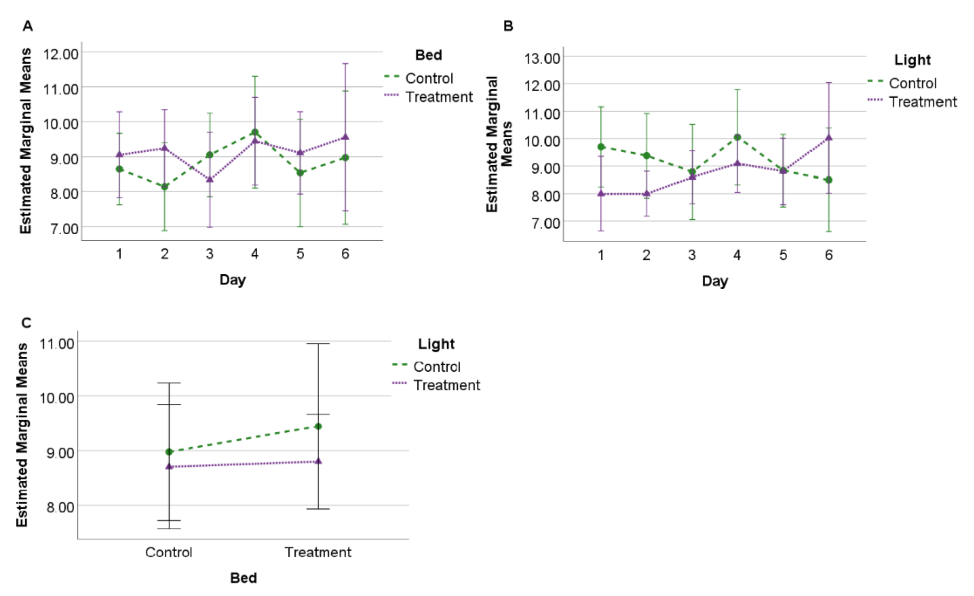

Figure 5. Estimated marginal means (s.d.) for NREM sleep quality index (SQI) score. Observed for 24 h across six days under each experimental condition (N=10) showing A) profile of SQI scores for

bedding over six days; B) profile of SQI scores for lighting over six days, and; C) interaction between bedding and lighting conditions.

Discussion

Total Sleep SQI scores were significantly lower for Control (deeper bed) compared to Treatment (thinner bed) Bedding conditions, suggesting that thinner beds encouraged greater quality sleep through reduced sleep disturbance. Comparatively, the duration of sleep behaviour was greater for standing NREM sleep under the Treatment Bedding conditions, with less time spent in sternal recumbency (NREM sleep) and lateral recumbency (REM sleep) during the Treatment Bedding (Greening et al., 2021). In this context, it is possible that sleep is more disturbed when horses are in recumbent postures, so that when this type of sleep increases in duration (as during the Control Bedding) it is also susceptible to greater interruption. However, when REM SQI was calculated by itself, scores tended to be higher on Control Bedding (although these findings were not significant, figure 4). Scores also tended to be higher for the combined weighted SQI and REM SQI on Control Bedding. Although these findings were also not significant, they suggest that fundamental differences exist between the quantity and quality metrics of sleep that require further investigation from a functional impact perspective.

Both the duration of sternally recumbent REM sleep (Greening et al., 2021) and the Combined SQI and NREM SQI (on day two) scores were significantly lower during the Treatment Light versus Control conditions. Results suggest that not only were horses engaging in significantly less sternal REM sleep but that they were experiencing greater sleep disturbance when the lights were left on overnight. Greening et al., (2021) suggested a pattern of habituation to Treatment Light within the sleep quantity data, which was also observed for sleep quality except the REM SQI. In this sense, both quality and quantity data appeared to offer reasonable assurance that the behavioural metric is sensitive to changes in the environment. However, the quality metric appears to be slightly more sensitive to environmental change than duration measurements on a day by day basis in that there was a significant interaction of Treatment Light by day for the Combined SQI and NREM SQI but not for the duration data (Greening et al., 2021).

Four indices were derived to enable differentiation between the REM and NREM sleep states and to establish whether these differed from total sleep time. The first was the product of dividing the total amount of sleep with the number of wake episodes, the second was similar but used total sleep time within sleep states, and the third index added numerical weight to REM sleep considering the importance of this type of sleep against reduced occurrence compared to NREM sleep. The third and fourth indices focused on the number of wake episodes occurring within the total amount of

REM and NREM respectively. In terms of which index has the best empirical measure of sleep quality, this was difficult to conclude given the analyses in this study. REM sleep quality differences were better accounted for within the Weighted Combined SQI and the separate REM SQI, compared to Total SQI, Combined SQI, and NREM SQI. The latter indices produced significantly different profiles under the Treatment Light conditions within this study, suggesting that sleep quality metrics which give weight to NREM sleep may be more sensitive to changes in environmental conditions cues. However, consideration should be given to the association between spontaneous collapse and REM sleep deprivation (Fuchs et al., 2018), where REM sleep orientated quality indices may be useful in this respect. Where the observed horse fails to engage in REM sleep, this in itself potentially points at other aspects of reduced welfare (Fuchs et al., 2018). Further work assessing the predictive ability of each of the indices on the impact of sleep disturbance on cognitive and emotional state will help reveal to which is the most useful from a practical perspective.

Whilst Fuchs et al., (2018) reported high inter- and -intra-individual repeatability of total sleep time profiles under standardized conditions, we observed large variation in SQIs between individual horses. This variation in sleep quality is also observed in humans where for example, recent human sleep research highlights how people react differently to the same light exposure (Chellappa, 2021 ways). Further work on animal sleep quality therefore, not only needs to establish normal levels of awakenings to determine what constitutes a high and low score within each sleep state for the species in question (Rattenborg et al., 2017), but also consider inter-individual variation. There is also opportunity to investigate the way in which compensatory mechanisms are employed by individuals, relative to how they cope with environmental changes. For example, NREM stage one sleep is not as restorative as other sleep stages (Brinkman, Reddy and Sharma, 2018), thus an increase in this sleep stage in conjunction with decreased total sleep time will not sufficiently repay the sleep debt (Wesensten et al., 1999). Future studies also need to investigate the appearance of daytime sleepiness/drowsiness in animals following overnight sleep disturbance, where increased sleepiness is known to increase the likelihood of recovery sleep in humans (Bonnet and Arand, 2003). There is also opportunity to utilise different objective measurements of sleep quality such as cumulative wake time through sleep interruptions, and sleep onset latency (SOL) to give further insights into the sleep profile. However, the true validation of both quantity and quality metrics needs to be done in relation to the functional impact of sleep disturbance on the state on the animal. For example, future work using combined metrics of sleep quantity and quality with the horse as a model, could assess whether these metrics can both identify the effects of sleep disturbing factors (such as noise and temperature) and whether these are predictive of changes in the cognitive and emotional state of the animal.

To conclude, changes to the environmental factors of light and bedding depth influenced both sleep quantity and quality. These non-invasive measurements of observable behavioural changes during the sleep state appeared to be useful measures of sleep in a non-human animal species. As an alternative to EEG measurements, this approach is transferable to other animal species but requires that normal patterns for both sleep quality and quantity on a species-by-species basis are established. The Treatment Light profiles of both sleep duration and SQIs aligned in response to treatment effects, although SQI appeared to be the more sensitive metric to subtle changes over time. Compared to sleep duration, no significant differences were detected within quality metrics of sleep response to Treatment Bedding effects which is fundamentally different to quantity metrics. Sleep quality in different sleep states appeared to differ between the various SQIs, that could also be as a consequence of differing environmental cues. Future studies are recommended to consider utilising REM and NREM SQIs due to the differences between these scores. However, further study is required to test these indices relative to the effects of sleep disturbance from practical and functional (cognitive and emotional) perspectives.

Materials and Methods

Subjects

Ten horses (six geldings, four mares; mixed breeds; average age 14.9 ±2.4 years; average height 163.5 ±7.4cm, none displaying stereotypic behaviours) had previously been observed on the equine yard at Aberystwyth University via a convenience sampling approach. During the study, subjects were routinely housed in stables (measuring 3.6 m x 3.6 m) on the yard for 24 hr Monday to Friday then turned out to pasture for eight hours on both Saturday and Sunday according to their normal routine. As standard practice in all stables, approximately two thirds of the stable floor surface was covered by rubber matting and a straw bed leaving one third as bare concrete that was cleaned as required throughout the day. Horses were accustomed to lights off at 20:00 (post final check), and a straw bedding substrate of 15 cm depth.

Experimental design

Horses were randomly assigned to two groups balanced for sex and age (Group A [N = 5] or Group B [N = 5]) using the RAND () function in Microsoft Excel, and exposed to two treatment conditions (Light and Bedding) within a two factor, crossover repeated measures experimental design. The Control Light condition involved the normal turning off (at 20:00) of fluorescent tube lighting (2 lx) overnight, whereas the Treatment Light condition maintained the fluorescent tube lighting on (180 lx) during this period. The fluorescent tube lighting was ‘warm white’ with primary spectral peaks of 490 nm, 550 nm and 625 nm. Control Bedding involved a bed of normal depth (15 cm) whilst

Treatment Bedding was maintained at a lower depth (5 cm). Each treatment lasted for six days plus a one-day wash-out period. Groups were staggered in the treatment sequence by seven days (Group B started seven days earlier and Group A finished seven days later) to facilitate the cross-over of experimental lighting conditions, where all the lights were on the same circuit thus either had to be on or off.

High-quality security infrared cameras (Reolink H.264 Digital Video Recorder and ANNKE model N28WEB) were used to record behaviour of all study subjects for 24 hr across seven consecutive days during the five-week study period, according to a predetermined ethogram (Table 2). The study was given ethical approval from the Aberystwyth University Animal Welfare and Ethical Review Board (AWERB).

Table 2. Definition of equine behavioural sleep states (Greening et al, 2021)

Behavioural sleep state		**Definition**
Standing Sleep NREM (non-rapid eye movement)		Subject is standing immobile, with the altanto-optical (poll) below the withers (with or without a resting hind limb)
Sternal Recumbency NREM		Subject is lying with the sternum in contact with the ground and legs folded beneath the body, the muzzle is not in contact with the ground
Sternal Recumbency REM (rapid eye movement)		Subject is in sternal recumbency (as described left) but showing REM sleep signs, i.e. the muzzle is clearly resting on/in contact with the ground
Lateral Recumbency (REM)		Subject is lying on its side (either lateral thoracic area parallel to and in contact with the ground), legs

		extended, head is on the floor and immobile other than for ear/eye movement

Measuring sleep duration

Sleep behaviour states were designated as being indicative of either REM or NREM sleep states based on behavioural research from previous seminal EEG studies (Greening & McBride, 2022). For example, standing sleep is indicative of NREM sleep, whilst lateral recumbency was indicative of REM sleep (table 2). Duration of sleep behaviour was recorded using continuous focal behavioural sampling over a 24 hr period for six consecutive days per experimental week for each horse. Duration of behaviour was recorded (hours) using continuous focal behavioural sampling over a 24 hr period for the six days per experimental week for each horse, that was reviewed by three observers. Several training sessions were held to ensure accurate agreement on the sleep behaviours, followed by inter-observer reliability measurement using sample behaviour footage ($R2 = 1$).

Defining sleep interruptions

Greening et al., (2021) had previously recorded behavioural duration data using spreadsheets and the ethogram in Table 2. These data were re-analysed to identify the occurrence of sleep interruptions, defined as awakenings that occurred within any sleep state lasting no more than three minutes (Merica and Gaillard, 1986). An individual horse was considered to have entered into a state of wakefulness if an interruption lasted for more than three minutes. For each horse per day, this yielded a frequency count of interruptions lasting <3 minutes, that were further categorised according to which sleep state they occurred in.

Development of the sleep quality indices (SQI)

Four different sleep indices were developed using frequency of sleep interruptions, total sleep time and total rapid eye movement (REM) and non-rapid eye movement (NREM) sleep time. For all equations, a higher number of sleep interruptions leads to a lower index score that is indicative of poorer sleep quality.

Total SQI

SQI derived from the total sleep time (TST) divided by the number of sleep interruptions using the equation below:

Total SQI = TST (duration in minutes)/ total no. interruptions +1

Adding one to the no. interruptions eliminated the occurrence of zero data points.

Combined SQI

Recognising that different levels of disturbances could occur during different sleep states, the Total SQI equation was adjusted to differentiate data for each behavioural sleep state:

Combined SQI = (total duration lateral REM/ no. interruptions in lateral REM +1) + (total duration sternal REM/ no. interruptions in sternal REM +1) + (total duration sternal NREM/ no. interruptions in sternal NREM +1) + (total duration standing NREM/ no. interruptions in standing NREM +1)

Weighted combined SQI

Considering that the horse on average spends approximately 75% of total sleep time in a state of NREM sleep (Greening and McBride, 2022) but that REM sleep may be equally functional from a sleep quality perspective, the second SQI was further adapted to give equal weighting to the REM component of sleep:

Weighted combined SQI = (total duration lateral REM/ no. interruptions in lateral REM+1) x3 + (total duration sternal REM/ no. interruptions in sternal REM+1) x3 + (total duration sternal NREM/ no. interruptions in sternal NREM +1) + (total duration standing NREM/ no. interruptions in standing NREM +1)

REM and NREM SQI

Finally, the different sleep states were subdivided into separate specific equations to determine whether different sleep quality profiles were discernible amongst the two different sleep states.

REM SQI = (total duration lateral REM/ no. interruptions in lateral REM +1) + (total duration sternal REM/ no. interruptions in sternal REM +1)

NREM SQI = (total duration sternal NREM/ no. interruptions in sternal NREM +1) + (total duration standing NREM/ no. interruptions in standing NREM +1)

Data Analysis

Each index score was square-rooted to normalise data and meet the assumptions for parametric statistical analysis. To assess the effects of light and bedding conditions on sleep quality, index scores calculated over 6 days of each treatment were tested using a two factor, repeated measures general linear model within SPSS 29©. Data are presented using estimated marginal means in order to give the average of the response variable for a factor, adjusted for the means of other factors in the model. Day, bedding and light were set as within-subject factors with no random factor and the model was set at full factorial. Interpolation graphs were generated by SPSS 29© which automatically created error bars with +/- 2 standard error. Post-hoc analysis utilised Bonferroni (significance set at $P<0.05$). With significant findings, effect size was determined by calculating partial eta squared (large effect η^2= >0.14).

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Greening, L. (2021) Tender is the night: How equine husbandry can facilitate sleep behaviour linked to improved performance and welfare. *<u>Invited speaker</u> at the Spotlight on Welfare Equine Performance Conference, hosted online by Bishop Burton College, July 2021.*

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Greening, L. (2023) Sleep quality measurements. *Webinar for the Animal Welfare Slack Workspace for the International Society of Applied Ethology.* (https://youtu.be/He790IwngTs) online March 2023

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From Field to Stable: Equine Nocturnal Acclimatisation

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INTRODUCTION: Horses experience seasonal changes in management practices, where for example moving from overnight turnout to overnight stabling is common practice (Wylie et al., 2013; Minero and Canali, 2009). There is little evidence of the influences of these changes on the horses, specifically in relation to nocturnal behavioural profile (Williams et al., 2008; Ruckebusch, 1975; Ruckebusch et al., 1970) even though sleep is recognised as an important factor linked to wellbeing. This study aimed to investigate whether differences in the occurrence of sleep-related behaviour were observed between the first nights of overnight stabling (period one) and six weeks later (period two), and whether familiarity with the environment influenced this further.

METHODS:

- Riding school horses, mean age 9 years (±4years), mixed breed/sex/height, were randomly assigned to group A (familiar stable) and group B (unfamiliar stable)
- Continuous focal sampling between 7pm and 7am for two consecutive nights in each observation period, using Gamut OBK20B DC12v 500mA and Hikivision 4 Channel HDMI CCTV Digital Video recorder.
- Differences in the duration of sternal recumbency (SR), lateral recumbency (LR, see plate 1), stand sleep (SS), stand alert (SA) and ingestion (IN) between period 1 and 2/ groups A and B were analysed using Wilcoxon Signed Rank (P<0.05).

Plate 1: Lateral recumbency

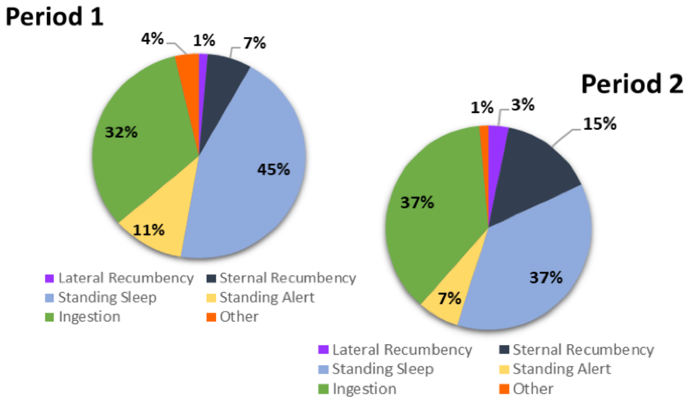

Figure 1. Overall average proportion of the nocturnal time budget observed for the first nights stabling (period 1) compared with six weeks later (period 2).

RESULTS: Significant differences were found between period one and two (figure 1) for the average overall duration of LR (Z=-2.783, p= 0.000), SR (Z=-3.845, p=0.000), SS (z=-2.427, p=0.015), and SA (Z=-3.771, p=0.000).

Environmental familiarity appeared influential, with statistically significant differences (p<0.05) for all behaviours between period one and two, observed as a trade off between standing sleep and recumbency for group A. This pattern was not replicated by group B, with statistically significant differences (p<0.05) observed against SA (Z=-2.803, p=0.05) and SR (Z=-2.803, p=0.05) only.

DISCUSSION: Overall horses appeared to acclimatise to overnight stabling within a six week period, subject to a routine that maintains the horse in a familiar stable. Increases in the duration of sleep related behaviours/ decreases in standing behavior were observed. Previous exposure to the stable appears influential; the trade-off between standing and sleep-related behaviour was only apparent for horses in an unfamiliar stable relative to stand alert/ sternal recumbency, suggesting that these horses were less inclined to adopt a recumbent sleep position possibly due to the fact that, as a prey species, instinctively it renders them vulnerable.

IMPLICATIONS: Consideration of time required for acclimatisation to changing nocturnal environments (overnight turnout to stabling) may be required, due to the apparent effect on the nocturnal behavioural profile. Further work is required to establish the impact of this on the diurnal behaviours and overall well-being of the stable-kept horse.

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Understanding sleep-related behaviour (and lack of) as a measure of welfare using the horse as model.

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EQUINE SLEEP AND RELATED BEHAVIOUR:

- As a large prey animal the horse engages in sleep typically for 2-9% of the 24 hour time budget.
- Non-REM sleep occurs whilst standing or during sternal recumbency, whilst REM sleep typically occurs when the horse adopts sternal or lateral recumbency (e.g. plate 1).
- A recumbent position renders the horse vulnerable and thus this position is commonly observed between 0000 and 0500h.
- A number of factors in the stable environment influence the occurrence of sleep (figure 1) to potentially cause a sleep debt.
- There is little understanding of how sleep debt affects equine performance and welfare.

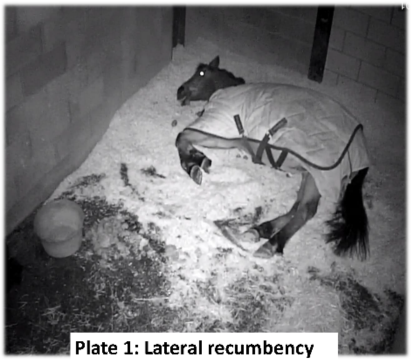


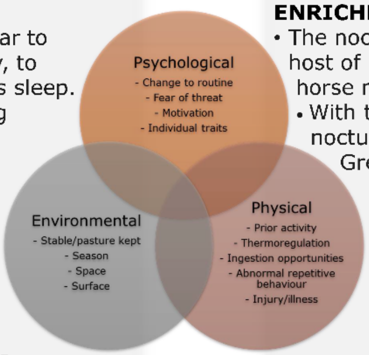
Plate 1: Lateral recumbency

ACCLIMATISATION:

- Group-kept horses at pasture appear to organize occurrence of recumbency, to maintain vigilance whilst individuals sleep.
- Upon transfer to over-night stabling shared vigilance is lost.
- This appears to cause a significant change in the time budget which alters as horses adapt to the stable environment (Table 1; first week of overnight stabling [Period 1] to six weeks later [Period 2]) (Fewings & Greening, 2018).

Table 1. Changing duration of behaviour as proportion of 12hr observation period (7pm to 7am) *P<0.05

Behaviour	Period 1	Period 2
Lateral recumbency*	1%	3%
Sternal recumbency*	7%	15%
Stand rest*	45%	37%
Stand alert*	11%	7%
Ingestion	32%	37%
Other	4%	1%



Psychological
- Change to routine
- Fear of threat
- Motivation
- Individual traits

Environmental
- Stable/pasture kept
- Season
- Space
- Surface

Physical
- Prior activity
- Thermoregulation
- Ingestion opportunities
- Abnormal repetitive behaviour
- Injury/illness

Figure 1. Factors influencing sleep

ENRICHMENT OPPORTUNITIES:

- The nocturnal environment can present a host of novel auditory stimuli which the horse must learn to habituate to.
- With the addition of music in the nocturnal environment (Hartman & Greening, 2018) horses performed significantly less **behavioural switching** (phase 2 [music] versus phase 1 [pre-music] and phase 3 [post-music]).
- Changes in time budget also suggest music was linked to acclimatisation (Table 2).

Table 2. highest average duration of behaviour between phases of music exposure

Behaviour	Highest	Lowest
Lateral recumbency	Phase 3	Phase 1
Sternal recumbency	Phase 3	Phase 2
Standing	Phase 2	Phase 3
Stand alert	Phase 1	Phase 3
Ingestion	Phase 2	Phase 3

FURTHER STUDY:

- Does the time budget adjust during the day to accommodate a potential loss of overnight sleep?
- Does the time budget adjust to specifically balance the amount of REM sleep (linked to recumbent behaviours)?
- What are the long term implications of playing music overnight?
- Could music be used to help reduce the potential sleep debt associated with changes to the nocturnal environment?

REFERENCES:
Fewings, V. & **Greening, L.** (2018) From field to stable – using equine nocturnal behaviour to observe acclimatisation to changing environments. *Poster presentation*

[redacted]

The welfare implications of horse owner perceptions & knowledge of equine sleep

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INTRODUCTION:

This study aimed to document what understanding horse owners of different experience have about equine sleep compared to current knowledge in the field of equine sleep research, and to establish whether owners consider sleep important relative to welfare and performance. In addition this study used social media to communicate findings to participants with the aim of increasing awareness and knowledge of equine sleep for horse owners.

METHODS:

- An online mixed methods questionnaire created on Google Forms was piloted before distribution via social media platforms from 13/11/2019 (closing 31/03/2020).
- In total 582 responses were gained via convenience and snow-ball sampling.
- Thematic analysis was used to identify the higher level themes emerging from answers describing signs that horse owners used to detect sleep.
- Single Factor ANOVA tested differences (P>0.05) between participant groups separated by years of horse ownership/experience (1-5 years, 6-15 years, 16-25 years, 26+years).

RESULTS:

Existing research suggests that horses sleep on average for 3 to 4 hours [1, 2]. In the current study, 50% of respondents thought their horse slept for $\geq$4 hours whilst 17% were unsure how long their horse slept for (Fig. 1), 29% reported never considering the amount of sleep that their horse achieved (Fig. 2), and 55% did not attribute lethargy to lack of sleep (Fig. 3).

No significant differences (P>0.05) were found between participant groups for: the number of hours respondents thought horses slept for; how often they considered the amount of sleep the horse had achieved; whether they attributed lack of sleep to lethargy in the stable/during exercise.

Higher level themes included 1) behavioural indicators e.g. mood/ alertness, and 2) signs of recumbency e.g. stable stains/bedding on body/rug and flattened bedding. A small proportion (11%) of participants a) assumed their horse slept without looking for signs b) were unsure of signs c) didn't answer.

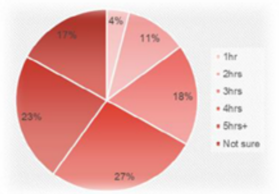

Figure 1. % participants grouped by the no. hours they thought horses slept for in a 24 hour period

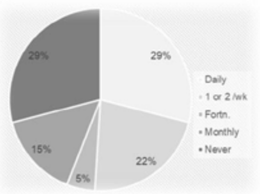

Figure 2. % participants grouped by how often they considered the amount of sleep their horse achieved

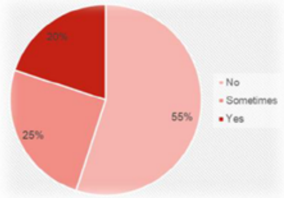

Figure 3. % participants grouped by whether they attributed lethargy to lack of sleep

CONCLUSIONS:

Results suggest uncertainty amongst participants about the amount of sleep their horse 1) achieves and 2) requires. Some participants did not consider whether sleep occurred. Years of experience as a horse owner did not influence responses. Sleep does not appear to be consistently considered by horse owners relative to equine wellbeing, despite its function relative to body maintenance and memory consolidation[3].

The findings from the study are currently under public consultation (please see '**Nocturnal equine behaviour group**' https://www.facebook.com/groups/753699465073014 for live presentation of this process).

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