An investigation into rest patterns in the horse (*Equus caballus*) and their potential use in welfare assessment

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For Donal, my rock.

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Abstract

It has been well documented in humans and other mammals that rest abnormalities act as an indicator of physical or mental dysfunction. Horses present an ideal model species when examining the interaction between rest patterns and distress. As a prey species that relies on fleeing for predator avoidance, horses have evolved to sleep for short periods and will only assume a recumbent position when they feel safe enough to do so. Therefore, the recumbent position in horses may act as an indicator of psychological state, and consequently, have the potential to be used as a welfare measure in horses. This thesis examines which features of sleep can be used as a measure of horse health and welfare, both by researchers and horse owners. A questionnaire designed to sample horse owner attitudes towards rest in horses, received 714 responses ith details on 976 horses. We found that while most horse owners recognised the importance of laying down in horses (94.38%), few owners reported monitoring horse rest (18.07%). Owners were significantly more likely to monitor rest if they had some form of equine specific qualification ($P<0.05$) but there was no significant relationship with years of experience with horses and likelihood of monitoring rest ($P>0.05$). Analysis of behaviour of 14 horses over 3-7 consecutive nights, in relation to environmental and individual characteristics showed that time spent feeding was negatively associated with time spent resting at 95% CI (-0.15, -0.04). Additionally, the personality trait of neuroticism was positively correlated with variation in time spent resting. Increased variation in time spent recumbent on a night-by-night basis was correlated with higher levels of the personality trait Neuroticism ($P<0.05$) and there was some evidence that the occasional absence of recumbency some nights may indicator sleep disorder in horses, although this requires further investigation. An analysis of long term nightly behaviour using 30 minutes scan samples of 11 horses over 18-28 nights was

compared to hair cotrisol levels and syncrony of lying with horses in neighbouring stables. There was no significant correlation/relationship between hair cortisol and time spent resting, suggesting that hair cortisol may not be an effective biomarker for sleep disorders in horses. Finally, horses in stables did not show signs of synchronised lying behaviour which has been observed in group housed horses. These findings suggest that horse rest patterns have the potential to be used as a welfare measure and should be included in Quality-of-Life assessment. Future work can draw on these findings to develop educational resources for the equestrian community around how to best monitor rest and which aspects of rest reflect welfare in horses.

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Chapter 1 Literature review

1.1 Introduction

The science of animal welfare requires the ability to monitor and evaluate an animal's health and affective state (Mata, 2021). Monitoring physical health can be straightforward and is continuously improving with the development of new technology, whilst the ability to measure affective state is more complex. Animal carers and scientists are faced with the often complex task of understanding what an animal is feeling based on observable indicators such as physiological and behavioural measures (Visser et al., 2008). It is well documented that the domestic environment and captivity can result in stress and therefore, negative affective state in many species, including the horse (Dawkins, 2008; Lecorps et al., 2021; Mcgreevy et al., 1995). While horses were domesticated some 6000 years ago, they maintain their foraging and social behaviours that evolved to survive in the wild. Meanwhile, there are numerous behavioural disorders found in the domestic horse population, usually caused by limits to space and social contact and inappropriate feeding (Goodwin, 2007). While many welfare measures accurately assess the effects of intense short-term stressors, they become less reliable when measuring low intensity, longer term stressors faced by animals who are unable to cope with their environment (Lesimple, 2020; Whitham and Miller, 2016). For example, increases in core temperature measure via Infrared thermography (IRT) of the eye and changes in heart rate and heart rate variability (HRV) have been used to indicate a stress response (Squibb et al., 2018). However, these measures would indicate emotional arousal associated with fear resulting in the flight response is unlikely to be maintained for long periods of time. Physiological measures are variable and highly influenced by other processes in the body such as physical activity (Mormède et al., 2007; Pawluski et al., 2017). Behavioural observations can also be unreliable, they often require a trained observer in that species (Whitham and Miller, 2016) and there is wide variation in how individuals of the same species react to sub-optimal conditions (Ijichi et al., 2013a; Koolhaas et al., 1999). Where possible, best practice is to collect a number of physiological and behavioural indicators of stress to look for trends, however, it is not unusual for results to be contradictory (Harewood and McGowan, 2005; Pierard et al., 2015; Whitham and Wielebnowski, 2013). While no single indicator of stress is likely to be the 'silver-bullet' measure of animal welfare, the more indicators researchers have to build a picture of an animal's experience, and therefore their affective state, the better they are able to describe and improve welfare (Whitham and Wielebnowski, 2013). Rest patterns have been shown to have close links with physical and mental wellbeing in humans (Doufas, 2017; Lo Martire et al., 2020) and thus provide an ideal candidate as an additional measure in Quality of Life (QoL) assessments in horses.

1.1.1 Potential of sleep as a welfare indicator

Sleep is of interest to welfare scientists both for its potential as a measurable indicator of welfare, as stress often leads to abnormal sleep patterns, but also as a cause of poor welfare, as sleep deprivation is a stressor (Meerlo et al., 2008). Sleep is defined as a rapidly reversable state of quiescence with an elevated arousal threshold (see section 1.2), and often a species specific posture (Zepelin et al., 2005). While pinpointing the exact moment of awake, drowsiness or sleep can be difficult, general rest patterns identified by body position and/or inactivity are recognisable even to the untrained observer in most species (Langford and Cockram, 2010).

Sleep patterns in humans are strongly linked to both positive and negative affect and psychological well-being (Langford and Cockram, 2010; Steptoe et al., 2008). It is well established in humans that stress causes a disruption to normal sleep patterns through loss or addition to overall time, fragmentation and increased variability in sleep times (Banks et al., 2005; Mezick et al., 2009). Research has shown a direct link between psychological stress and sleep quality with psychological stress being used as a predictor for sleep (Li et al., 2019). Studies have shown that individuals suffering from depression or anxiety are more likely to develop sleep problems such as insomnia (Zhang et al., 2022). The link between stress and sleep occurs at a physical level as lack of sleep has been shown to increase baseline cortisol levels (Vargas and Lopez-Duran, 2017). Zhang et al., (2022) found negative emotions and sleep share neural correlates in the brain, going some way to explain why they are often connected (Zhang et al., 2022).

Animal models, such as rodents, are frequently utilised as models to understand how psychological stressors interact with sleep, in order to explore potential treatments in this area (Pawlyk et al., 2008). Immobilisation of mice is believed to mimic a psychological stressor, as the animal does not experience any pain. Immobilisation of mice in a plastic tube for 1-3 hours resulted in increased sleep times, and it is possible that the increase in sleep time represents a recovery of sleep time lost as the animal did not sleep during the immobilisation procedure (Pawlyk et al., 2008). However, the increase in sleep time is often associated with an increase in plasma corticosterone levels, suggesting the change in sleep pattern could be in response to psychological stress. When the immobilisation time is increased to 22 hours over 4 consecutive days to induce high intensity psychological stress, sleep time reduced. Therefore stress intensity is likely to be an important factor in how sleep pattern is altered (Pawlyk et al., 2008).

Lying time is considered an important aspect of dairy cow welfare and is linked to both physical and mental wellbeing and is a common feature of large scale on-farm assessments (Tucker et al., 2021). Cows are highly motivated to lie down and will lie down for an average of 10-12 hours a day. Following 3 hours of both lying and feed deprivation, cows prioritised lying down when the opportunity to both feed and lie down were reintroduced (Metz, 1985). Lying time is linked to health, as cows that are given less areas for comfortable lying, and therefore spent less time lying, were found to have higher incidences of lameness

(Cook et al., 2004; Dippel et al., 2009). Several studies have found a link between forced standing or unfavourable lying conditions and an increase in faecal glucocorticoid metabolite concentrations. This suggests reduced lying time results in activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis, an indicator of stress (Tucker et al., 2021). Cows spend a relatively small proportion of time sleeping when lying down, however, if deprived of sleep a rebound will occur where cows spend considerably more time in sleep to compensate for the loss. When deprived of sleep for 24 hours, it took cows between 2 and 4 days to return to regular sleep and lying patterns (Kull et al., 2019).

In contrast to cows, elephants spend very little time lying down, with African elephants spending just over 2 hours a day and Asian elephants spending just over 3 hours a day in a recumbent position. In elephants, sleep is the primary reason for recumbency and they will fall asleep almost immediately upon the adoption of a recumbent position (Holdgate et al., 2016). Reluctance to adopt a recumbent position in zoo kept elephants has been related to both physical and mental welfare. Two cases of suspected sleep deprivation were reported in elephants suffering from degenerative joint disease, causing difficulty in the animals getting down and back up again (Schiffmann Christian et al., 2018). A case study of an Asian bull elephant moving to a new facility showed resting behaviour changed from relatively normal rest pattern at the original facility to a complete absence of recumbent behaviour in the new facility. A similar reduction in recumbent behaviour was recorded in a female African elephant who lost her companion. Both cases are indicative of loss of sleep due to psychological stress (Schiffmann Christian et al., 2018).

The potential of rest and/or sleep as a welfare indicator for both physical and mental health is gaining popularity. The Canadian Code of Practice for Dairy Cattle recommends a minimum lying time of 12 hours a day (Rushen et al., 2009) and the New Zealand Code of Welfare for Dairy Cattle recommend 10-12 hours a day access to a dry, well bedded surface to meet the behavioural need of lying and rest (*Code of welfare: Dairy Cattle*, 2019). The Swiss Federal Council in the Animal Welfare Ordinance give minimum space allowance per horse based on wither height of the individual to allow for the performance of basic behavioural needs including areas to lie down (*The Swiss Federal Council Animal Welfare Ordinance*, 2008). However, the interaction between wellbeing and rest patterns is complex; a close examination of what is typical for the species and/or individual and the nature of the stressor is needed to accurately interpret the effect on rest patterns.

1.2 Features of mammalian sleep

Sleep can be defined as; a state of immobility, often with a characteristic change in posture and/or specific sleep site; heightened threshold for arousal with the ability for rapid reversal to distinguish it from a coma; distinctive electrographic signals; and after loss referred to as a "sleep rebound" (Datta, 2010; Siegel, 2005b). Rebound after loss suggests that sleep plays an essential role in normal biological functioning.

Sleep is not believed to be fundamentally different between mammalian species and appears to have a common underlying mechanism, suggesting it shares a common evolutionary past (Anafi et al., 2019; Tobler, 1995). However, while the underlying mechanism may be the same, the structure of sleep can vary greatly between species (Capellini et al., 2008; Tobler, 1995). Despite decades of research, the exact function of sleep is still unknown, but whatever function sleep serves, it appears to be achieved using a wide variety of sleep strategies. Even within mammals, the structure of sleep can be strikingly different depending on ecological pressures (Lima et al., 2005). Despite these differences, all mammals need appropriate, species-specific levels of sleep for good physical and mental health.

All mammalian sleep is mediated by daily oscillations in hormones controlled by the master pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus, known as the circadian rhythm (Balbo et al., 2010). A number of hormones are involved in the circadian rhythm but of particular note are the roles of melatonin and cortisol. Melatonin regulates the circadian rhythm and facilitates the transition to sleep, while cortisol is involved in the transition to and maintenance of wakefulness (Tortonese et al., 2011).

Two main stages of sleep have been identified in all terrestrial mammals and birds, they are Rapid Eye Movement (REM) sleep, sometimes termed Paradoxical Sleep (PS), and Non-Rapid Eye Movement (NREM) sleep, sometimes termed slow wave sleep (SWS) (Lima et al., 2005; Roebuck et al., 2013). The initiation of sleep or wakefulness and cycling between the two sleep states is a complex neurological task involving several regions of the brain (Breedlove, 2013). The hypothalamus controls the initiation and maintenance of NREM sleep but requires the reticular formation, a net-like structure formed by brainstem nuclei and neurons that acts as a coordinator and relay centre, to transition back to wakefulness and maintain consciousness (Breedlove, 2013; Mangold and Das, 2023; Patel et al., 2023). The pontine systems or 'pons', is responsible for the initiation and maintenance of REM sleep and inhibition of motor neurons causing the muscle atonia associated with this stage (Breedlove, 2013). This inhibits the motor neurons that control the muscles; however, it does not impact the motor neurons that control the muscles in the eyes, hence the behavioural effect of rapid eye movement from which this sleep stage gets its name (Siegel, 2003). The hippocampus and amygdala are both active during sleep; the hippocampus is involved in memory during NREM sleep and the amygdala is the emotions centre, active during REM sleep (Lerner et al., 2021; Patel et al., 2023; Rasch and Born, 2013). The internal "clock" that controls when each of these brain centres are activated or inactivated, is located in the hypothalamus (Breedlove, 2013; Murphy, 2010). The diversity of brain regions involved in each sleep stage and the distinction between each stage suggests discrete but complimentary roles of NREM and REM sleep (Datta, 2010).

1.2.1 Evolution of sleep

While all terrestrial mammals and birds have a sleep cycle that consists of NREM and REM sleep, the structure and length of these sleep cycles, termed sleep "architecture", vary significantly between species. It appears that although the presence of both phases in a sleep cycle is essential, the length and distribution of sleep bouts throughout a 24-hour period is highly flexible. Humans and most primates sleep in one single, long phase over a 24-hour period, this is referred to as monophasic sleep. However, most mammals sleep in multiple short phases distributed throughout a 24-hour period; this is referred to as polyphasic sleep (Lima et al., 2005; Murase et al., 2018; Tobler, 1995). These differences are likely due to a number of drivers. Larger mammals with large brains relative to body size and greater basal metabolic rates tend to have lower total sleep time compared to other mammals. Additionally, a longer gestation period is associated with reduced total sleep time, although none of these trends are universal and there will always be some exceptions to the rule. For example, lion have both long gestation and long sleep times with no valid explination, however in general genstation is a better predictor of sleep time than other variables (Gonfalone and Jha, 2015). Predation risk appears to be the most reliable predictor of sleep time, although this trend is more consistent in time spent in REM sleep and less so in NREM sleep. Species at high risk of predation spend less time in REM sleep than those that have less risk (Lesku et al., 2006; Lima et al., 2005). Spending extended periods of time unconscious, particularly in muscle paralysis as in REM sleep, would seem to be maladaptive for many prey species that rely on early predator detection for escape (Anafi et al., 2019). However, the fact that REM sleep persists despite strong selection pressure against it speaks to its critical importance in biological functioning (Lima et al., 2005). Lesku et al., (2006), conducted a phylogenetic analysis of sleep architecture in mammals and found that herbivores that sleep in an exposed site, such as open grassland, spend less time in REM sleep compared to species that have a protected sleep site either above or below ground level, such as cave ceilings, burrows and tree hollows (Lesku et al., 2006). Species such as bats, rats, moles and ferrets have REM sleep times of 2-6 hours compared to ungulates such as horses and giraffe who have an average REM sleep times of 0.5 hours per 24-hour period (Zepelin et al., 2005). Capellini et al., (2008) used phylogenetic comparative methods to test if predation was the main driver of sleep pattern and length in mammals. They found energetic constraints rather than predation appeared to play a greater role in sleep architecture. They proposed that the need for frequent feeding bouts is what drives the reduction in sleep time and increased sleep fragmentation (Capellini et al., 2008). However, in this model, species with a large body size were not classed as prey species which resulted in the mis-categorisation of large numbers of ungulate species. Many large ungulates including deer, antelope, camel, and most bovine and equine species are large mammals that fill a prey niche and sleep in exposed sites where they would be at risk of predation. Most of these species have adopted shorter sleep times, particularly in relation to REM sleep. Many live in social groups and display 'sentry' behaviour where some members of the group remain awake and watch for predators while others sleep (Greening and McBride, 2022). A small group of ungulates including elephants, cows, zebra and horses are able to sleep standing up. A system of ligaments called the passive stay apparatus in the fore and hind limbs lock the joints of the leg into place (Chung et al., 2018; Houpt, 1980). However, the stay apparatus can only function during NREM sleep where some muscle tone is still present. The motor neuron inhibition that occurs during REM sleep activation results in paralysis of the few muscles involved in maintaining the stay apparatus and therefore, the animal must be recumbent to achieve this stage (Burla et al., 2017; Dallaire and Ruckebusch, 1974a; Greening and McBride, 2022). This may go some way to explaining why predation risk seems to only drive the reduction in REM sleep rather than total sleep time in some prey species.

1.3 Function of sleep

The physiological distinction between REM and NREM sleep suggests there are likely to be different functions of each distinct phase. The exact function of sleep is yet to be clearly defined and is a still a hotly debated matter. It is clear that sleep plays a role in a number of biological processes including thermoregulation (Rechtschaffen and Bergmann, 2002) energy and metabolic regulation, immunity, and recovery after exercise (Halson, 2014; Samuels, 2008) and is essential to an individual's health and wellbeing (Hauglund et al., 2020; Lima et al., 2005). However, it could be argued that all these functions could be achieved in a quiet, wakeful state, and should not require a period of unconsciousness. Therefore, the primary function of sleep is likely to be brain specific (Rasch and Born, 2013). Recent research has suggested the main role of sleep is to remove waste metabolites from the brain that build up during wakefulness, however, this line of investigation is still in its infancy (Hauglund et al., 2020).

1.3.1 Sleep, memory and learning

The role of sleep in memory and learning has received extensive study over the past few decades, and has been reported in a wide number of species including humans (Walker, 2010). Initially, it was believed that REM sleep was responsible for the consolidation of memories due to the similarity between EEG signals in REM sleep and wakefulness, and at first experimental studies seemed to support this hypothesis (Rasch and Born, 2013). However, the experimental procedure that was often used in REM sleep deprivation studies to examine learning and memory in rodents was likely causing stress in addition to sleep deprivation. Common experimental techniques were the "flower-pot" method; this involved placing the rat on a small platform surrounded by water. When muscle atonia associated with REM sleep occurred the rat would fall into the water and be roused to wakefulness. Even seemingly less stressful procedures such as gentle head lifting or touching when REM sleep was initiated was likely producing a moderate stress response in most animals (Siegel, 2001). It is probable that it was the induced stress rather than the sleep deprivation that inhibited learning and memory (Siegel, 2001). When pharmaceuticals or brain lesions were used to suppress the REM sleep phase no negative effects on learning and memory were recorded in mice or humans (Rasch and Born, 2013; Siegel, 2001). In fact, a common drug used to treat clinical depression, phenelzine, is known to completely supress REM sleep and can be taken for months or years with no reported impact on learning or memory (Siegel, 2001).

More recently, the role of NREM sleep on learning and memory has been investigated in the two-stage memory model. Under this model memories are first stored in a fast-learning store (the hippocampus) before being slowly transferred to a long-term learning store (the neocortex). The quietness of the NREM sleep phase allows the brain time, without other inputs, to complete the slow learning store process. To date this hypothesis seems to be supported by experimental data in rats and some human studies (Rasch and Born, 2013).

There is a strong link between REM sleep and mental health; specifically disorders such as depression and Post-Traumatic Stress Disorder (PTSD) are almost always associated with changes in REM sleep (Cai, 2016; Genzel et al., 2015). The role of REM sleep seems to be linked more closely to emotional memory processing and mood and may explain why anti-depressant drugs often result in REM sleep suppression. Vivid and emotional dreams are usually reported after waking from a period of REM sleep, while dreams after a period of NREM sleep are more thought-like (Cipolli, 1995; Rasch and Born, 2013). The sleep to forget, sleep to remember hypothesis suggests that sleep after a negative emotional experience strengthens the memory, but reduces the emotional response over successive nights when the memory is retrieved (Walker, 2010). Failure of this decoupling process of affective state from the memory could be responsible for the development of long-term anxiety. For example, in the case of PTSD, nightmares and flashbacks of the event continue to induce the same level of emotion as the initial event itself (Lerner et al., 2021; Walker, 2010). Length of REM sleep has been correlated with the recall and strength of emotional memories and REM sleep is often associated with conditioned fear memories (Cai, 2016; Genzel et al., 2015; Rasch and Born, 2013; Smith, 1995). REM sleep is also likely to play a supporting role to NREM sleep by reorganising, strengthening and reactivating memories on a molecular and synaptic level (Rasch and Born, 2013).

1.3.2 Sleep and development

REM sleep also appears to play a central role in brain development, although again, both sleep phases likely work in unison. REM sleep is present as a higher proportion of sleep time in infants, gradually shifting in balance toward NREM with age (Knoop et al., 2021). Human infants at birth spend approximately 8 hours in REM sleep, 50% of total sleep time; this reduces to approximately 2 hours in adults, only 20% of total sleep time (Siegel, 2005a). This difference between REM sleep time is more pronounced in altricial species, but is still present in precocial species (Blumberg et al., 2020). The function of REM sleep in development is believed to be related to facial and limb twitching that is associated with this phase of sleep, spontaneous patterns of neural activity may promote brain maturation (Yamazaki et al., 2020). REM sleep provides an ideal environment for establishing connections across distant structures of the brain and development of the sensory motor system (Blumberg et al., 2020).

1.4 Monitoring sleep

1.4.1 Electroencephalogram (EEG)

In order to study the function of each sleep stage, we must have ways of identifying which stage an individual is experiencing. One of the primary techniques to measure sleep stage is the use of an electroencephalogram (EEG). When neurons in the brain excite, they create synaptic currents which in turn create a magnetic field that is detectable to an EEG via surface electrodes placed on the scalp. The EEG magnifies the signal and displays the readings in the form of waves on a screen (Sanei et al., 2007). There are 5 frequency ranges; Delta (δ) 0--<4 Hz, Theta (θ) 4-<8 Hz, Alpha (α) 8-<13 Hz, Beta (β) 13-<30 Hz (although in some literature no upper bound is given) and Gamma (γ) >30Hz (sometimes referred to as the fast beta range) (Dallaire, 1986; Williams et al., 2008). In humans NREM stage of sleep is further divided into four stages N1-N4 (Carskadon and Dement, 2005). Each stage of vigilance including REM, NREM, wakefulness and drowsiness can be distinguished in the EEG by their different frequency ranges. NREM sleep is characterised by high-amplitude, low- frequency waves and REM sleep by low-amplitude, mixed frequency waves in conjunction with muscle atonia and rapid eye movements (Lesku et al., 2006). Although different stages of vigilance have been associated with a different frequency range, these stages exist on a continuum, and at times it can be difficult to identify the exact moment of transition between different sleep stages from EEG alone. Wakefulness and REM are particularly hard to distinguish as their EEG signatures are incredibly similar, hence the term paradoxical sleep sometimes being used for REM sleep (Roebuck et al., 2013; Williams et al., 2008). Drowsiness exists as a transitional state, and for a while it was debated if this phase should be classed as a wakeful or sleep state. Ultimately drowsiness is classed as an awake state however, this categorisation is arbitrary and some believe it is a form of vigilant sleep that allows for predator detection (Lima et al., 2005; Tobler, 1995; Zepelin et al., 2005).

1.4.2 Physiology of sleep stages

Fortunately, each sleep stage is also associated with differing physiological features; NREM is associated with low metabolic and neural activity, some muscle tone, irregular respiration, and diminished thermoregulation (Carskadon and Dement, 2005). REM sleep is associated with high neural activity (likely why it resembles wakefulness in EEG), complete muscle atonia, rapid eye movements, highly irregular respiration and diminished thermoregulation (although not as pronounced as NREM) (Siegel, 2005a). These changes are measured using electrobiological signals including; Electrocardiogram (ECG) from the heart, Electromyogram (EMG) from muscles, Electrogastrogram (EGG) from the stomach, and Electroocclugram (or electrooptigram, EOG) from eye nerves (Sanei et al., 2007). It may be possible to distinguish REM sleep from EOG and EMG alone due to the rapid eye movements and complete muscle atonia that is unique to REM sleep. However, rapid eye movements are not always present in REM sleep so identifying this sleep stage using EOG is likely to result in an underestimation of time spent in this phase (Roebuck et al., 2013). EMG theoretically could identify REM sleep from other phases as muscle atonia is always a feature of this sleep stage, however, it could not distinguish NREM from quiet wakefulness. While measuring some physiological measures of sleep can be useful when monitoring sleep disorders, such as respiration when monitoring sleep apnoea, most physiological measures are too subtle or inconsistent to be used as a measure of sleep on their own (Roebuck et al., 2013). However, movement and body position have been shown to be a good estimator of sleep stage in some species such as elephants (Gravett et al., 2017) and dogs (Clarke and Fraser, 2016).

1.5 Monitoring sleep in humans and other mammals

1.5.1 Accelerometers

While electrophysiological measures such as EEG remain the gold standard for measuring sleep, attachment of multiple sensors may be physically uncomfortable and in themselves alter sleep patterns (Roebuck et al., 2013). Actigraphy measures sleep paramaters using an accelerometer device worn to track body position and movement in order to track sleep patterns over a period of weeks or months (Martin and Hakim, 2011). This model has been further incorporated in the Fitbit, Inc. type devices which also monitor Heart Rate Variability (HRV) (Haghayegh et al., 2019). These devices use algorithms to estimate sleep duration and stage and have been found to have moderate accuracy in detecting sleep stage with high sensitivity but low specificity when measuring total sleep time. This means they are accurate at detecting when sleep is occurring but are less accurate when distinguishing between quiet wakefulness and sleep (Haghayegh et al., 2019).

Accelerometer devices are being used to monitor animal movement and are becoming an important aspect of monitoring animal health and welfare. Clarke and Fraser, (2016) examined wearable accelerometers to measure dog rest behaviour. Dogs have a characteristic sleep posture with their head down allowing an accelerometer device to distinguish the degrees of tilt of the neck when the device is attached to the collar. Additionally, the accelerometer measured general movement and categorised movement as awake and stillness as sleep. The results found that it was difficult to set a tilt threshold for each dog with huge range of dog breeds of different shapes and sizes, however, an intermediate threshold of 8° to 12° was accurate for most animals. Additionally, 2 individuals obtained poor accuracy results due to unusually restless behaviour. The study concluded that triaxial accelerometers had potential to automatically monitor dog sleep behaviour but allowance would need to be made for individual variation of breed and activity level (Clarke and Fraser, 2016).

Recently Högberg et al., (2020) validated accelerometers initially marketed for cows to assess sheep lying time. The study found that although there was a slight overestimation of lying time, this type of logger was able to detect lying or standing time accurately enough to be used in sheep studies. However, the step counter function on this accelerometer was not reliable for sheep when compared to cows, as acceleration threshold set to record a step for a cow is not always met by the smaller step of sheep. This study highlights the importance of validating accelerometers to each individual species as different thresholds may be needed (Högberg et al., 2020).

While accelerometers show some promise for measuring lying behaviour in a number of species, care must be taken when attaching the device so that it does not interfere with normal behaviour. Bonk et al., (2013) found attachment of an accelerometer device to the leg of un-weaned dairy calves resulted in more leg movement. An additional limitation of a wearable device comes when attempting to monitor large herds of agricultural animals; having a device attached to each individual may not be practical or economical.

1.5.2 3D Computer vision and Artificial Intelligence

Many species can be identified as sleeping by identifying/recording a specific sleep site and/or sleep posture coupled with lack of movement. Automated 3D Computer Vision Technology has been adapted to measure basic behaviours in dogs (Barnard et al., 2016), cattle (Cangar et al., 2008), pigs (Matthews et al., 2017) and hens (Okinda et al., 2020) and has showed some promise. But the cost of such a system and technical skill required to implement it has remained a barrier to its widespread implementation (Wurtz et al., 2019).

Advancements in Artificial Intelligence (AI) may overcome problems associated with wearable devices such as accelerometers, as AI provides a non-invasive way to monitor the behaviour of large groups of animals through video recordings (Bao and Xie, 2022). Although still in the development stages, some commercial systems are available and becoming increasingly common to monitor animal behaviour as, beyond the initial training of the program, human input is low. A review of AI for farm animal welfare found AI can detect aggressive behaviour, feeding behaviour and coughing in pigs (Bao and Xie, 2022). Wu et al., (2021) used AI in the form of computer vision technology to identify the basic behaviours of dairy cows including, drinking, ruminating, walking, standing and lying. The authors note the benefits of computer vision technology when compared to wearable sensors, as the wearable sensor are vulnerable to damage and may alter the animal's behaviour. The proposed algorithm showed an accuracy of 0.976 when monitoring the behaviour of a single cow showing promise as an accurate, non-invasive way to monitor behaviour in the future (Wu et al., 2021).

While a number of reviews promote the use of technology for automated monitoring of behaviour, they also note the number of studies who tend to still rely on manual behaviour scoring (Auer et al., 2021; Fogarty et al., 2018). This is likely due to the expense of the equipment and difficulty in validating devices. However, the price of this technology is reducing, and more scientifically validated devices are coming onto the market, making research into sleep without the constraints of manually scoring videos potentially much easier in future.

1.6 Sleep in horses

Horses present a good model when examining sleep as a measure of welfare. The likelihood that horses will only adopt a recumbent position, particularly the laterally recumbent position, when entering REM sleep may make it a reliable and repeatable indicator for when this phase of sleep is occurring (Greening and McBride, 2022; Langford and Cockram, 2010; Williams et al., 2008). Horses are a prey species living in open habitat that relies on fleeing for survival; lying down inhibits their ability to escape, therefore they have evolved to do this as little as possible (Greening and McBride, 2022; Houpt, 1980). In environments where the horse does not feel comfortable, they avoid recumbency and compensate by extending time spent in standing NREM sleep. However, as both sleep phases are essential for normal functioning, this strategy is temporary and likely to result in REM sleep deprivation followed by REM sleep rebound (Bertone et al., 2015). Therefore, time spent in recumbency may act as a proxy measure for stress, and by extension, affective state. Additionally, increasing reports of sleep disorders in domestic horses suggests this may be an emerging welfare issue of yet unknown proportions (Bertone et al., 2015; Chaplin and Gretgrix, 2010; Fuchs et al., 2016; Houpt et al., 2001; Williams et al., 2008).

1.6.1 EEG in horses

Monitoring brain wave patterns is the only conclusive way to determine what phase of sleep an individual is experiencing, although interpretation of outputs requires a trained eye as REM and wakefullness signals can look similar (Williams et al., 2008b). In the case of humans, little muscle and a relatively thin skull makes EEG comparatively easy. Electrodes can be placed on the front and back of the head to detect brain wave patterns from different areas of the brain (Sanei et al., 2007). However, in other species this is not always so simple. The skull morphology of the horse makes accessing the hind brain with non-invasive EEG electrodes impossible (Williams et al., 2008). Furthermore, there is no standard electrode placement based on the equine anatomy despite the vital role electrode placement plays in influencing the outcome of data between individuals (Ree and Wijnberg, 2012). Williams et al., (2008) used electrode placement based on human EEG placement to examine sleep in horses. They found EEG was severely contaminated by muscle and movement artefacts while the horse was awake, particularly those associated with the eye, jaw and ear movements (Williams et al., 2008).

Despite this, some more recent studies appear to have overcome this problem. Recently, a mobile EEG has been developed specifically for horses (Cousillas et al., 2017). Although the device has not yet been used for sleep studies, it has been used to examine EEG profiles between horses in different housing conditions (Stomp et al., 2021). In this study researchers hypothesised that left hemisphere dominance of Theta waves would be associated with positive emotions, as it is in humans. They found that horses living in naturalistic conditions had significantly more left hemisphere Theta activity than those in restricted living conditions (Stomp et al., 2021). While further research is required to interpret the findings from this device, it remains a promising tool for sleep and welfare research in the future.

1.6.2 Accelerometers in horses

The first mention of using accelerometers to measure lying behaviour in horses in the literature was by Chaplin and Gretgrix, (2010) who used TinyTag® data loggers with a tilt switch attached to the left fetlock to measure recumbent behaviour. This type of device was manufactured to measure environmental parameters for goods in transit, although the authors reported that the device was validated but the data was unpublished so accuracy was unclear (Chaplin and Gretgrix, 2010).

The first published study to validate accelerometers for lying behaviour in horses was made by DuBois et al., (2015), who measured lying behaviour in two adult horses using the Onset Pendant G data loggers (Model No. UA-004-64; Hoskin Scientific, ON, Canada) attached to the foreleg with a bandage. This device had previously been validated to measure lying behaviour in dairy cows (Ledgerwood et al., 2010) and therefore was a good candidate to measure lying behaviour in horses. Although the sample size was small, the Onset Pendant G data loggers showed high sensitivity and specificity at >99% for lying and standing behaviours (DuBois et al., 2015).

However, despite this validation, few studies in the subsequent years used accelerometers, continuing to rely on video observations (Auer et al., 2021; Greening et al., 2021; Kjellberg et al., 2022; Oliveira et al., 2022; Raspa et al., 2020). This may be due to the cost of the device and technical expertise required for processing accelerometer data, as well as the time-consuming nature of validating different devices and attachment location.

Additionally, some research may be looking at behaviours beyond just standing or lying down that cannot be detected by accelerometers.

The development of commercially available devices that are specifically marketed to record lying behaviour in horses such as Happie© (Formally Horse Analytics, Germany) and Trackener® (London, UK) may increase the use of accelerometers in horse rest research. Trackner® was validated in 17 horses admitted to hospital for signs of colic and was found to have 81.7% agreement for sternal recumbency and 92% agreement for lateral recumbency when compared to video observation (Sinovich et al., 2021). A second study by Kelemen et al., (2021) used the Trackner® device to measure resting behaviour in horses with Chronic Orthopaedic Diseases. Scientific validation of these devices along with reductions in price are likely to result in more studies utilising this technology in future.

1.6.3 AI in horses

In horses AI has been used for lameness detection (Feuser et al., 2022), to monitor foraging behaviour (Nunes et al., 2021), and to monitor signs of health such as colic (Fraiwan and Abutarbush, 2020). Commercial systems are also available as a foaling alert system, monitoring mare behaviour before foaling. For example, Novostable© claim their video surveillance system can detect health issues, indicators of welfare and pre-foaling behaviour. While it doesn't currently detect the difference between sternal and lateral recumbency, they are currently working to include this feature (Novostable, pers. Com.). However, peer reviewed validation is still pending for many commercially available systems, therefore accuracy is unknown.

1.7 "Normal" sleep in horses

When standing, sleep in horses can be distinguished from drowsiness by the position of the poll (top of the head) in relation to the withers, where a poll higher than the withers would suggest drowsiness and the head would drop to parallel with or below the withers when transitioning to NREM sleep (Williams et al., 2008). Additionally, horses will not fully close their eye lids while in standing NREM sleep, whilst they may or may not close their eyes when sleeping lying down (Greening and McBride, 2022). To the author's knowledge, there is no research relating to the importance of the eyelids remaining open while sleeping in horses, but it is hypothesised that this allows the horse to passively survey their environment while sleeping (Rattenborg et al., 2017).

Research on horse sleep thus far has found horses in both free-living or feral conditions and in domestic situations primarily sleep at night between midnight and 4am, with napping sometimes occurring during the day (Kelemen et al., 2021; Tortonese et al., 2011). Horses are polyphasic sleepers, and throughout the night, horses will go through an estimated 5-7 sleep phases lasting about 30-40 minutes each time (Dallaire, 1986; Kjellberg et al., 2022). NREM sleep must occur before REM sleep can be achieved; NREM sleep often occurs first while standing, before returning to the drowsy stage to adopt a recumbent position. Another period of NREM sleep may occur in sternal recumbency before adopting a laterally recumbent position to achieve REM sleep (Williams et al., 2008). Total sleep time including standing sleep is estimated at 3-5 hours a day which is roughly 15% of the horses total time budget (Dallaire, 1986). Of the 3-5 hours, only 30 minutes is devoted to REM sleep (Williams et al., 2008; Wöhr et al., 2016; Zepelin et al., 2005). In the healthy horse, REM sleep occurs every night (Wöhr et al., 2016) suggesting that although it only occurs for a short period, it is still important for normal functioning.

Horses have two main recumbent positions and are very consistent in how they present these positions: sternal recumbency involves lying down with weight resting on their sternum with legs folded under the body, and this can be with their head supported (Figure 1a) or with their nose resting on the ground (Figure 1b). Lateral recumbency involves being flat out on either their left or right side with legs outstretched (Figure 1c) (Littlejohn and Munro, 1972). REM sleep is only achieved in lateral recumbency or, more rarely, in sternal recumbency with their nose on the ground in cases where there is a barrier to lateral recumbency such as physical discomfort or insufficient space (Greening and McBride, 2022; Kjellberg et al., 2022).

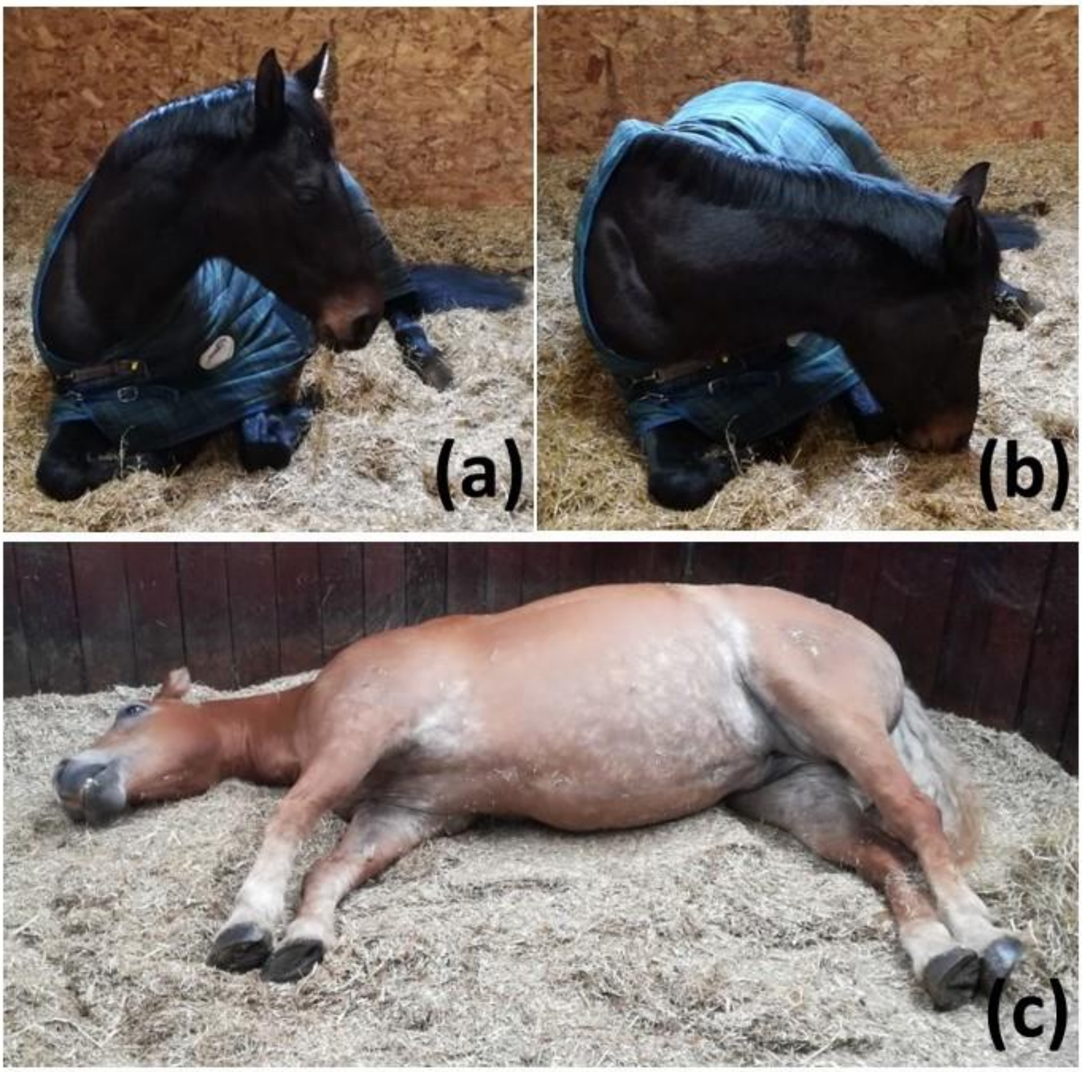


Figure 1.1: Horses can adopt 3 positions in recumbency without support: (a) sternal recumbency with head supported; (b) sternal recumbency with nose on the ground; (c) lateral recumbency.

Despite the comparatively small amount of time horses spend resting, sleep is still essential for normal functioning. Studies of free ranging horses suggest that time spent resting is not flexible when competing with other essential behaviours. For example, during the breeding season male horses will reduce time spent feeding to allow more time for reproductive activities, while time resting remains the same, suggesting a high level of behavioural resilience (Duncan, 1980). It may be that while there is a small degree of flexibility in sleep time due to a number of internal and external factors (listed in section 1.8), large deviations would only occur as a result of extreme physical or mental disruption.

1.8 Factors influencing time in recumbency

The diversity of bodily processes in which sleep plays a role, including thermoregulation (Rechtschaffen and Bergmann, 2002), metabolism and exercise recovery (Halson, 2014; Samuels, 2008) and stress systems (Lo Martire et al., 2020), means that sleep time and structure can be influenced by changes or imbalances in any one of these processes. Below is an outline of the major environmental and biological factors that are likely to have a significant impact on horse sleep patterns.

1.8.1 Bedding type

Bedding type has been of particular interest as it is likely to have a big impact on horses that are stabled for much of their time. Horses show a clear preference for straw to facilitate recumbent behaviours, showing significantly more time recumbent on straw bedding vs. shavings (Greening et al., 2013; Mills et al., 2000; Pedersen et al., 2004). Horses bedded on straw also spend more time engaged in investigative behaviours which might indicate better welfare as the straw acts as enrichment (Greening et al., 2013). However, straw has higher levels of airborne particles compared to wood shavings so the benefits of promoting recumbent behaviour would need to be weighed against the possible disadvantages to respiratory health (Fleming et al., 2008). Bedding thickness also plays a role with thicker (15 cm deep) beds promoting more time in recumbency than thinner (5 cm deep) beds (Greening et al., 2021). Similar results were found in Burla et al. (2017) although precise bedding measurements were not reported. The preference for thick straw bed is likely due to superior physical and thermal comfort.

1.8.2 Ambient temperature and rainfall

Weather is likely to play an important role in sleep quantity and quality. In humans, optimum temperature for rest lies within a narrow range of approximately 19-22°C (Pan et al., 2012). While there are no studies looking specifically at ambient temperature and sleep in horses, time budgets of free-ranging horses have shown increased lying in Spring and Summer months compared to Autumn and Winter (Duncan, 1985). It is likely that this difference in lying times is the result of a number of environmental factors including more optimum temperatures for rest in the spring and summer and favourable weather conditions including less rain, making for more comfortable lying conditions. Additionally,

it could be related to food availability with more abundant food in spring and summer resulting in less time required for foraging to meet nutritional requirements.

Murase et al. (2018) found foals spent less time lying down in wet weather. This gives some insight into how horse rest patterns can be influenced by the external environment and may help inform optimal management and housing conditions to promote healthy rest patterns in domestic horses. However, the domestic horse is likely to experience their external environment differently compared to their free ranging counterparts with the addition of stables, rugs and artificial lighting.

1.8.3 Diet

The effect of diet on behaviour is relatively well studied as diet is such a central aspect of equine health, however, rest or recumbent behaviours are not always included in these studies. One study that specifically looked at the effects of diet on sleep behaviour found that total time spent in recumbency increased by about 20% in horses fed oats compared to hay alone. However, the mean time in recumbency was not significantly different between the two groups (Dallaire and Ruckebusch, 1974b).

Another study looking at time budget differences between foals on a low or high protein diet found male foals spent significantly more time in lateral recumbency when on a high protein diet compared to those on a low protein diet. The same difference was not seen in the female foals and the authors were not able to find a clear explanation for this difference (Sartori et al., 2017). One possible explanation could be in the way each sex uses the available energy provided by the high protein diet. Colts have been found to spend significantly more time engaged in interactive play compared to fillies (Crowell-Davis et al., 1987). This increase in physical activity could lead to a higher requirement for the restorative effects afforded by REM sleep.

Studies that have examined time budgets of horses fed with hay bags/slow feeders vs. freely available hay have found that the hay bags reduced the consumption of hay by up to 20% without reducing the time spent feeding. The reduction in consumption of hay did not appear to have impacted the time spent resting in any study (Aristizabal et al., 2014; Correa et al., 2020).

These results suggest that the nutritional makeup of feed may result in alteration of sleep patterns but how the feed is presented, and the rate of consumption does not have an impact on rest. In humans, both the duration and quality of sleep has been linked to diet (St-Onge et al., 2016) and plays a significant role in metabolic disorders such as obesity, diabetes, hypertension and cardiovascular disease, although the direction of this relationship is unclear (Golem et al., 2014). Generally, a healthy diet is linked to healthy sleep patterns (St-Onge et al., 2016). In horses it is well established that a trickle fed, high fibre diet is essential for both gut and behavioural health (Richardson and Murray, 2016). Therefore, it would be unwise to alter this type of diet significantly to induce greater time performing recumbent sleep behaviour.

1.8.4 Unfamiliar environment

As a prey species, horses are sensitive to unfamiliar environments. Horses that feel vulnerable in a new environment are likely to avoid adopting a recumbent position due to the perceived predation risk (Boyd et al., 1988). Instead, these individuals are likely to by trying to meet their sleep needs with standing NREM sleep rather than lying down for REM sleep. While some studies have noted the reluctance of horses to lie down when in a new environment (Chaplin and Gretgrix, 2010; Fuchs et al., 2016; Houpt et al., 2001; Williams et al., 2008), the length of time it takes for a horse to fully habituate to a new environment is yet to be investigated. Ruckebusch et al., (1970) mentions in passing that horses require several months of habituation to a new environment before returning to regular sleep patterns, however, no formal results are presented. Considering the frequency in which horses are moved to new environments for sale, training, competition, or owner convenience, understanding how long this process is likely to take and what steps can be taken to speed up habituation is of vital importance to horse performance and welfare. Additionally, when conducting research using horses that requires moving to a new environment, most researchers do not give a justification for the acclimation period chosen to allow horses to return to normal behaviour patterns. Often horses are given a week or less to habituate to a new environment before behavioural observations begin (Chaplin and Gretgrix, 2010; Raabymagle and Ladewig, 2006; Williams et al., 2008a). If the estimation by Ruchebusch et al., (1970) is correct, the behaviours measured may not be representative of the horse's true behaviour. Additionally, if changes in social grouping are imposed along with change in environment, then this is likely to further prolong the habituation period and would need to be accounted for in any sleep research.

1.8.5 Lighting and the circadian rhythm

All species, from single -celled algae to humans have an internal biological clock in each cell allowing the organism to predict and prepare physiological functions and behaviours to meet challenges that are presented over a 24-hour period and this is called the circadian rhythm. In mammals, this clock is controlled by the suprachiasmatic nucleus (SCN) in the hypothalamus which receives photic information from the retina (Murphy, 2019). This system controls, hormone secretion, metabolism, cardiovascular activity, immunity, and many other essential functions within the body and is primarily controlled by the light dark cycle (Murphy, 2019). Melatonin is one of the hormones controlled by the circadian rhythm and plays a central role in many biological processes including the initiation of sleep. Light is known to impact melatonin production in horses, and therefore is likely to impact on sleep (Greening et al., 2021; Walsh et al., 2013). Many horses are stabled for long periods of time reducing their exposure to natural lighting conditions. Additionally, artificial lighting is often used at night, altering the light-dark cycle and disrupting circadian regulation (Murphy, 2019). The effects of light on the horse's biological process has long been known, and is regularly manipulated to stimulate breeding out of season for economic gain (Burkhardt, 1947; Walsh et al., 2013). However, the effect artificial lighting has on equine rest patterns has received less attention. Greening et al. (2021) examined the effects of fluorescent light of 2 lux (control) compared to 180 lux (treatment) lighting switched off at 8:00 pm. They found horses spent significantly less time in sternal recumbency with their nose on the ground and a non-significant trend of reduced lateral recumbency in the treatment group. Both body positions were assumed to be synonymous with REM sleep; when taken together the difference was not significant between the two groups, but the trend was towards reduced REM sleep in the 180 lux treatment. The difference between treatments seemed to reduce over the 6 day period of observation, which may suggest horses are capable of a certain level of acclimation to less optimal sleeping conditions over time (Greening et al., 2021). Despite this, steps should still be taken to reduce the disturbance of sleep by ensuring horses receive at least some natural light during the day or exposure to artificial blue light with a lux of 10 or higher which has been shown to suppress melatonin levels and imitate natural light (Walsh et al., 2013). Additionally, exposure to light should be minimised at night; the installation of incandescent bulbs with an orange/red light spectrum can be used to minimise disturbance for any checks during night-time hours (Murphy, 2019).

1.8.6 Jet lag

Horses are one of the few domestic animals that are regularly flown across time zones for competition or breeding purposes. Crossing time zones, particularly in an easterly direction, can result in a mismatch between the body's internal circadian rhythm, experienced at a cellular level, and the new solar cycle at the destination (Tortonese et al., 2011). This results in irregular sleep, gastrointestinal disturbances and problems with concentration and memory in humans (Samuels, 2008). Despite the obvious disadvantage this could have on horse performance, this is an area that has received little attention. Tortonese et al., (2011) examined the effect of simulated phase shift mimicking an eastward flight on blood lactic acid (lactate) and run time to fatigue on a treadmill as a measure of performance and hormone measurements of the neuroendocrine systems as a measure of homeostasis and stress. Measures were taken on day 1 and 14 after the light/dark phase shift. The results showed an improvement in physical performance after the phase shift, in direct contrast to what was expected based on the human literature. The horses in this study showed quick adjustment in changes in light dark cycles through molecular circadian gene expression, and appeared to have advanced aerobic and anaerobic capacities on day 1 after the phase shift (Tortonese et al., 2011). The fast adjustment to phase shift shown in this study could be related to the polyphasic nature of sleep demonstrated in horses, contrasting with the nature of human sleep which is monophasic. However, the results of this study have been questioned as performance was only measured at a single timepoint. More research on jetlag is required before conclusions can be drawn on this topic (Murphy, 2019).

1.8.7 Age

A common pattern across species, including horses, is that young individuals spend more time in REM sleep, with sleep times gradually decreasing as the individual ages (Murase et al., 2018; Yamazaki et al., 2020). As horses tend to only lie down to achieve REM sleep, time spent lying can be used to measure time in REM sleep and how that changes over a horse's lifetime. Murase et al. (2018) found foals at 1 week of age lie down between 15-35% of the time; this time in recumbency decreased to 2% of their time after weaning. A study on free roaming Camargue horses found yearlings spent more time in both lateral and sternal recumbency compared to individuals over a year old (Duncan, 1980). The first year of life is likely to be an important time period for physical and mental development in this species (Crowell-Davis, 1986). This comparatively large amount of time spent in REM sleep in early life is hypothesised to be conducive with brain maturation. The spontaneous neural activity associated with REM sleep provides ideal conditions for neurological development (Kirischuk et al., 2017). Additionally, it has been hypothesised that the twitches and jerks of the muscles in REM sleep provide sensory input, assisting in the development of the sensorimotor system (Yamazaki et al., 2020).

As an individual ages they appear to require less sleep, with arrousal during sleep episode increasing. By the age of 60 years old NREM may no longer be present for many human men, while it seems to persist longer in women, NREM will still reduce over time (Carskadon and Dement, 2005). Percentage of REM sleep remains the same into old age but will decline with the evelopment of brain dysfunction related to the elderly, such as dementia (Prinz et al., 1982). While conditions such as osteoarthritis which are more prevelent in the older horse population are likely to influence lying times (Oliveira et al., 2022), old age is yet to be identified as a contributor to reduced sleep times in horses (Kelemen et al., 2021).

1.8.8 Personality

Personality has been suggested as a tool to predict susceptibility to the development of stereotypy (Ijichi et al., 2013), the expression of pain (Ijichi et al., 2014), and behaviour (Ijichi et al., 2013; Squibb et al., 2018) in horses. The personality factor neuroticism relates to stress sensitivity, and where an individual falls on the neuroticism spectrum will dictate how quickly they will reach a stress threshold. Coping strategy relates to how the animal responds to the stress once the threshold is reached: proactive individuals will actively attempt to remove themselves from the stressor or remove the stressor in a flight or fight response. Reactive individuals tend to show passive response to a stressor, usually manifesting as a freeze response (Ijichi et al., 2013). In the case of chronic stress, it has been proposed that proactive individuals respond with coping strategies such as the development of stereotypies, while reactive individuals respond with depression and emotional blunting (Ijichi et al., 2013).

While the interaction between sleep and personality in horses is yet to be investigated, there is strong evidence that poor sleep quality is consistently associated with scoring highly for the personality trait neuroticism in humans (Allen et al., 2016; Friedman and Kern, 2014; Stephan et al., 2018). This is not surprising as neuroticism is related to higher stress sensitivity (Friedman and Kern, 2014), a tendency towards negative affective state, and worse mental and physical health which disrupts sleep quality (Allen et al., 2016). Considering the range of behaviours already shown to be related to individual personality it would be reasonable to assume that sleep is no different. Additionally, coping style may play a role in differing sleep patterns in response to stress, with proactive individuals responding with reduced total sleep time and reactive individuals responding with increased sleep time, but this is yet to be investigated.

1.8.9 Exercise

Exercise has been shown to result in small to moderate beneficial effects on sleep in humans including increased total sleep time, increased NREM sleep and reduction in sleep onset latency (time taken to fall asleep) (Kredlow et al., 2015). The positive effects of exercise on sleep have resulted in exercise being recommended as an additional treatment for insomnia in conjunction with traditional pharmaceutical treatment (Brupbacher et al., 2021). Caanitz et al., (1991) looked at the effect exercise has on rest in horses and found that horses being exercised spent significantly more time lying down than those not being exercised. However, the mean age of horses in exercise treatment was 4 ± 1 years and the average age of horses in the no-exercise treatment was 9.7 ± 1.6 years (Caanitz et al., 1991). As age is known to play a significant role in time spent resting, with younger individuals spending longer in REM sleep than older individuals (Carskadon and Dement, 2005; Duncan, 1980; Murase et al., 2018), this result could be confounded by the influence of the age differences between the treatment and control groups.

Werhahn et al. 2012, looked at lying behaviour of horses with differing levels of turnout. Six warmbloods ranging in age from four to seven years old were tested in a repeated measures design in treatments consisting of no turnout, group turnout (in pairs) and solitary turnout. Horses spent significantly more time lying down in the group turnout treatment compared with the no turnout treatment (Werhahn et al., 2012). It was not stated how familiar the horse pairs were in the group turnout, but the increase in lying time would suggest they were individuals felt relaxed around each other and were likely familiar. This, taken with the results from Caanitz et al. 1991 suggests increased exercise results in increased lying behaviour, likely due to the restorative nature of sleep.

Considering the role of the horse as an athlete, a better understanding of how exercise influences sleep is required and could be a useful tool to improve performance and welfare in the future.

1.8.10 Space allowance

Studies have shown that horses tend to spend more time lying down in a larger stable rather than a smaller stable (Chung et al., 2018; Raabymagle and Ladewig, 2006). The British Horse Society (BHS) recommends horse stables are "large enough to turn around, lie down and get up comfortably" and suggests stable sizes of 3.65 X 4.25m for large horses over 172.7 cm tall, 3.65 x 3.65m for horses, 3.05 x 3.65m for large ponies, and 3.05 x 3.05m for small ponies (British Horse Society, 2022) however, it was not clear how these sizes were calculated. Chung et al., (2018) compared lying times of horses in large stables measuring 4.5 x 3.6m, medium stables 4.2 x 3.5m and small stables of 3.2 x 3.2m. They found a significant difference between sternal, lateral and standing rest between all 3 stable sizes. Horses spent over 70 minutes in sternal recumbency in the large and medium stables but only 37 minutes in the small stables. Lateral recumbency was 9.75 and 9 minutes in the large and medium stable respectively and only 4.67 minutes in the small stable (Chung et al., 2018). It was not specified how large the horses were in the study, but they were identified as Thoroughbred so presumably fall into the "horse" category for the BHS suggesting a stable of 3.65 x 3.65m. Clearly, space allowance plays a role in lying time, more research in this area could contribute to evidence-based recommendations for stable size, alternative housing design and turnout in the future.

1.8.11 Group housing

Social housing meets the species-specific needs of horses as a social species and is becoming increasingly popular. However, careful consideration needs to be taken to ensure there is sufficient space for each individual in the group to perform essential behaviours such as sleep. Horses show synchrony of behaviour, including rest behaviour, where social cues will initiate behaviour change in the whole group. Martin et al., (2010), noted that six pasture kept mares appeared to show daily behavioural rhythms that were relatively synchronous suggesting social cues played an important role in the timing of behaviour. When these mares were then moved to stables, daily rhythms continued but synchrony diminished (Martin et al., 2010). For this reason synchrony of behaviour needs to be accounted for when considering socially housed horses, including having sufficient space to lie down. Burla et al., (2017) found enlargement of bedding areas from 1 to 1.5 the minimal dimension of littered area allowed under Swiss Welfare Legislation (calculated in proportion to horse hight) resulted in increased lying time. This difference was particularly pronounced in lower ranking individuals (Burla et al., 2017). A similar study compared control horses in 10.5m^2 single stables to the treatments in social housing where horses were given 8 m^2, 18 m^2 or 28 m^2 per horse. They found lying times significantly increased when group space allowance was increased from 8 m^2 to 18 m^2 but lateral recumbency was only equal to that of control horses when increased to 28 m^2 (Kjellberg et al., 2022). Although lateral recumbency is not essential for REM sleep to occur (Greening and McBride, 2022; Williams et al., 2008), REM sleep in sternal recumbency with nose on the ground may result in fragmentation of sleep as the onset of muscle atonia in REM sleep will cause the individual to roll onto their side if not adequately balanced on their sternum. The lack of lateral recumbency may mean a loss of time in complete relaxation and may pose a welfare concern if significantly reduced (Burla et al., 2017).

Increasing space allowance also reduced the proportion of times low-ranking horses were forced to terminate lying bouts by high-ranking horses. Burla et al., (2017) found only at 1.5 times the minimal dimension of space allowance were low ranking individuals able to self-terminate lying bouts, an important aspect of horse welfare. Kjellberg et al., (2022) found forced rising occurred 26% of the time in horses aged between 3-17 years with 8 m^2, 24% of the time in 18 m^2, and 21% of the time in 28 m^2 group housed horses. Horses in this study were still achieving 100 minutes of lying time, suggesting that forced rising was not resulting in sleep deprivation. However, group dynamics are still an important factor to consider when ensuring group housed horses are achieving enough rest and optimal welfare (Kjellberg et al., 2022). These findings highlight the need for extensive space allowance when group housing individuals. However, a space allowance of 28 m^2 per horse is unlikely to be practical in many situations, in which case some time in a suitably sized single stall may be required to meet sleep needs. Additional measures in group housing such as barriers that allow low ranking individuals to visually remove themselves from higher ranking individuals, should be investigated as a potential alternative to larger area (Burla et al., 2017) and steps taken to ensure horses housed together have established social cohesion.

1.8.12 Sentry behaviour

A recent review of equine sleep and its implications for welfare highlighted the potential importance of sentry behaviour when horses adopt a recumbent position (Greening and McBride, 2022), where one or two horses of a group will remain standing to act as a lookout for predators, while the rest adopt a vulnerable recumbent position (Fraser, 1992; McGreevy and Yeates, 2018) (Figure 2a). Although this is common, it is not universal and while some studies on feral or free-ranging horse behaviours report sentry behaviour always being observed, others report instances where all horses will lie down simultaneously (Keiper and Keenan, 1980) (Figure 2b). It is unclear if this is due to the social dynamics of the herd, the environment, or both. As many horses are kept socially isolated in individual stalls and may not be able to see conspecifics around them, understanding the importance of sentry and synchrony behaviour to an individual is paramount to welfare. The reduction in sleep time in social housing with restricted space in the above studies compared to controls in single stables suggests that in terms of sleep health, some time in a single stall is preferable to social housing without sufficient space for healthy sleep behaviour to occur (Burla et al., 2017; Kjellberg et al., 2022). It may be that space allowance is more important for healthy sleep patterns than the presence of conspecifics for sentry behaviour. However, whether the presence or absence of conspecifics improves rest behaviour may be dependent on individual circumstances and group composition. For example, when moved to a new environment a horse might find the presence of a familiar herd mate comforting which may help to induce sleep, although this is yet to be investigated.

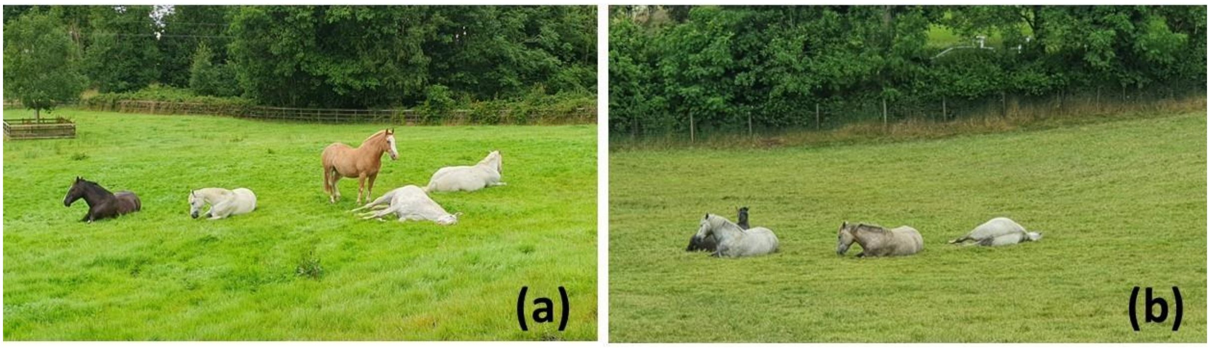


Figure 1.2: (a) Horses commonly display sentry behaviour where a single individual of a heard remains standing to better monitor for danger while others assume recumbency for sleep. (b) While sentry behaviour is common, it is not universal and there may be instances when the whole herd adopt recumbency at the same time.

1.9 Differences in sleep patterns between free-ranging and domestic horses

A recent review comparing time budgets of feral and domesticated horses has identified that both feeding and lying behaviour differ significantly when comparing time budgets between semi-feral and domestic horses, highlighting their importance when measuring welfare in horses (Auer et al., 2021). If we are to use sleep pattern as a measure of welfare, we need to determine what a normal sleep pattern looks like to recognise when deviations from this pattern are an indicator of poor mental of physical well-being. The management systems that can be employed when keeping horses in a domestic situation are likely to each impact rest in their own unique way. Therefore, it may be that the rest patterns of feral or free-ranging horses should be used as a benchmark of what is considered “normal” rest for horses. However, it is important to note that while free-ranging/feral horse rest may act as a benchmark, it cannot be considered a ‘gold-standard’ of rest, as these individuals are likely to face a different set of challenges to horses kept in domesticated systems (Auer et al., 2021). Things that disrupt normal rest patterns in free-ranging horses include adverse weather conditions, annoyance from biting insects and the risk of predation (Duncan, 1985).

There is limited research on free-living horse rest behaviour as many studies do not include nighttime observations or do not specifically measure lying behaviour. Where lying behaviour is measured, only percentage of time spent lying is reported, and some important details may be lost, however, this represents a starting point for comparison. Of the studies that did include nighttime observations and lying times, three looked at free living Camargue horses (Boy and Duncan, 1979; Duncan, 1985, 1980) and one at Prezewalski horses (Boyd et al., 1988). Additionally, some looked specifically at lying behaviour in relation to age (Boy and Duncan, 1979) and environmental conditions (Duncan, 1985). Newborn foals were found to spend approximately 33% of their time lying down, with 15% of time laterally recumbent and a further 17.9% of time sternally recumbent in free-living Camargue horses. This reduced to approximately 16% with 2.7% of time laterally recumbent and 13.2% of time sternally recumbent in pre-weaned foals (approximately 3-6 months old) (Boy and Duncan, 1979). This is in line with the assumption that younger individuals would spend more time in REM sleep. In adult horses total lying time ranged from 2.8-15.5% of the time with an average of 1.2-2.4% in lateral recumbency and 2.8-9.3% time in sternal recumbency (Boyd et al., 1988; Duncan, 1985, 1980), with more lying behaviour occurring in spring (Duncan, 1985).

Studies on domestic horses by comparison have rest time ranging from an average of 3-27.3% of their daily time budget (Chaplin and Gretgrix, 2010; Chung et al., 2018; Greening et al., 2021, 2013; Heleski et al., 2002; Pedersen et al., 2004; Raspa et al., 2020). The larger range in rest behaviour of domestic horses may speak to the diversity of management routines, and correspondingly, the diversity of types of stressors domestic horses are subject to, and the need to explore this area further (Auer et al., 2021).

1.10 Distress and sleep in horses

The interaction between distress and sleep in horses appears to be complex depending on age and/or the nature of the stressor. Some studies have noted that when adult horses are placed in a stressful situation, such as a change in stable, they will choose not to adopt a recumbent position at all, resulting in sleep loss (Fuchs et al., 2016; Ruckebusch et al., 1970; Williams et al., 2008). However, a study examining the stress response of Quarter Horse foals (4.5 months old) weaned into either social groups in a paddock or into individual stables found those in social isolation spent more time recumbent that those weaned in social groups or pairs (Heleski et al., 2002). This increase in recumbent behaviour in socially isolated, stabled weanlings was observed in conjunction with an increase in other stress behaviours such as, pawing, licking, chewing and/or kicking the stall walls (Heleski et al., 2002). Similarly, a study examining space allowance of horses reared for meat (Raspa et al., (2020) found young horses (16 ± 8 months old) spent an unusually high 27.33 ± 2.05% of their time in recumbency. Horses were kept at stocking densities of between 4-6 m^2 per horse which is extremely high compared to other studies on group housed horses (Kjellberg et al., 2022). Raspa et al., (2020) did not record stress behaviours other than stereotypy and reported that the incidents of stereotypy were relatively low. The difference in stereotypy behaviours between Heleski et al. (2002) and Raspa et al. (2020) could be related to the single stall versus social housing element of each study or it could be related to breed difference of quarter horses versus draft type horses. Draft type breeds may be predisposed to passive response to stress, which is more likely to go unnoticed by observers (Ijichi et al., 2013a).

The contrast of adult horses reducing rest times in response to stress while young horses seem to increase rest time in response to stress could be due to age, or the nature and intensity of the stressor. Dallaire and Ruckebusch, (1974b) found ponies with partial sensory deprivation (visual and auditory) showed an increase in both NREM and REM phases of sleep. It is possible that horses moved from a stimulating environment into single stables are experiencing a similar effect of sensory deprivation resulting in the reduction of information from their environment being processed, and subsequently increased sleep time.

A study looking at time budgets of Thoroughbred foals (2-60 days of age) stabled with the mare and with 7 hours turn out per day found they spent a total of 10.5 hours (43.75%) lying down. Lying times of free-living foals of a similar age were only 15-35% of their time budget (Boy and Duncan, 1979), suggesting the mostly stabled foals lying times were excessive to what is required for neonatal health. The excessive lying times of the mostly stabled foals fit the pattern of younger horses with restricted movement spending more time resting than those in more naturalistic environments. This excess time lying down could be as a result of the sensory deprivation associated with confinement to a stable (Dallaire and Ruckebusch, 1974a) and/or stress-induced apathy due to a lack of species appropriate environment (Murase et al., 2018).

Visser et al., (2008), examined the lying times of 36, two-year old Dutch Warmblood horses who were being stabled for the first time; half were housed in individual stables while the other half were in pair housing. Similar to Heleski et al. (2002), this study found a significant increase in time spent sleeping in individually stabled horses compared to those in paired housing (although they did not report exact times). Additionally, individually housed horses showed an increase in other stress behaviours including pawing, vocalisation, defecation, nibbling at stable door, vigilance and development of stereotypical behaviour (Visser et al., 2008).

These studies taken together demonstrate that both excessive time resting and reductions in time resting are indicative of poor welfare. The mechanism that determines how an individual responds to a stress could be related to age, with an increase in time spent resting in response to stress up to 2 years of age and a decrease time resting in adult horses (over 4 years old) in response to stress. Alternatively, it could be related to the nature of the stressor, as all studies on younger individuals involve restriction of movement by housing in a small stable, high stocking densities, and/or social isolation. Reports of sleep deprivation in adult horses appears to be related to an unfamiliar environment. Therefore, if using rest time as a measure of welfare in horses, both age and nature of the stressor need to be considered.

1.10.1 Stress hormones and HPA axis activation

Stress is a general term used to describe multiple responses involved in how an animal reacts to its environment. Threats could result in altered homeostasis and therefore, when stress is perceived, this results in a physiological response in the body in an attempt to maintain homeostasis (Bartolomé and Cockram, 2016; Mormède et al., 2007). When the individual perceives a threat, the HPA axis is triggered resulting in a cascade of hormones beginning in the hypothalamus with the release of corticotrophin-releasing hormone (CRH) which triggers the anterior pituitary gland to release adrenocorticotropin hormone (ACTH) resulting in the release of cortisol from the adrenal cortex (Elder et al., 2014). This is a normal, healthy, response that has evolved to allow individuals to respond to natural stressors that an animal may encounter in its life (Bartolomé and Cockram, 2016; Lo Martire et al., 2020). Cortisol plays an important role in normal biological functioning of the horse and in the short term has positive physiological functions that are essential for horse survival. For example, when responding to short term stressors such as a threat from a predator, cortisol will aid escape by increasing movement, reducing inflammation and raising blood glucose concentrations (Bartolomé and Cockram, 2016). However, problems arise when the animal is exposed to a stressor for extended periods. Most stressors encountered in a natural setting are acute, however prolonged stress exposure results in overactivation of the HPA axis which can have negative health consequences such as suppression of the immune system (Bartolomé and Cockram, 2016; Mormède et al., 2007).

Measures of 'stress hormones' cortisol or corticosterone (also known as glucocorticoids), have been used to assess both short and long-term stress in animals (Pierard et al., 2015). However, the complex nature of cortisol and its role in a number of biological processes, including daily fluctuation as part of the circadian rhythm, makes the interpretation of cortisol measurements difficult. While cortisol responses are often well described in acute stress situations, its role in chronic stress is far more complex. Results from studies looking at cortisol in response to chronic stress range from no effect, increases or decreases in cortisol and relate poorly to clear behavioural signs of stress (Pawluski et al., 2017). As mentioned earlier, activation of the HPA axis is a normal, healthy response of the body to enable a return to homeostasis. Chronic or repeated stressors may overload this system disrupting the body's normal stress response. Blunting of the stress systems in response to chronic stress is likely to result in negative consequences on both behaviour and health in the long term including depression of the immune system, reduced appetite and apathy (Carroll et al., 2017; Hall et al., 2008; Pawluski et al., 2017). There is a need for reliable measures of chronic stress that enable symptoms and underlying causes to be recognised before they manifest as long-term behavioural disorders such as the development of stereotypies or physical illness like stomach ulceration (Mormède et al., 2007). Monitoring rest patterns may be able to fill that gap as a non-invasive, objective measure of chronic stress, however, first it must be determined what normal sleep/rest patterns look like in order to identify deviations from this.

1.10.2 Sleep as a measure of physical health

Extreme sleep deprivation has a number of highly detrimental effects on the subject both mentally and physiologically including but not limited to; irritation, self-injury, stereotypy (Brylewski and Wiggs, 1999), weight loss, skin lesions and in extreme cases where deprivation continued, death (Rechtschaffen and Bergmann, 2002). However, even mild cases of sleep loss can have disproportionately negative effects on the subject. As little as 4 hours sleep deprivation in humans can lead to a heightened response to pain (Roehrs et al., 2006). Once again the effect of loss of sleep leading to heightened response to pain and pain resulting in loss of sleep make the interaction between sleep and physical health a serious welfare issue.

Recent research has examined the link between health and rest and the potential of monitoring recumbency patterns as a measure of health. In one study, a group of 83 horses were monitored to determine if age, lameness or orthopaedic disease had a significant influence on time in recumbency. There was no significant difference between horses that were grouped as 'young and lame', 'old and lame', 'old and sound' and 'young and sound', which would suggest that in this situation recumbency is not a good measure of health. However, eight horses showed symptoms of REM sleep deprivation (Kelemen et al., 2021) which may suggest that the grouping used to classify horses likely to show deviations in rest behaviour may not have reflected those at risk. A similar study looking at the influence of osteoarthritis on recumbency times found that horses with mild osteoarthritis spent more time lying down but once the disease progressed recumbency times reduced (Oliveira et al., 2022). These finding suggest recumbency can be used as a measure of the impact of osteopathic diseases on welfare in horses, but it is likely to depend on the nature and progression of the disease. While more research is required into specific disorders to understand the interaction between disease and recumbency behaviour, lack of recumbency could be used as an additional tool when vets are prescribing pain relief or when owners are making end of life decisions.

Clothier et al., (2019) suggest that lying behaviour could be used as a measure of pain in horses with musculoskeletal pathologies as an objective measure of pain informing decisions about levels of anti-inflammatory or analgesic medication to prescribe. They found horses with Angular Limb Deformities (ALD) spent significantly less time (approximately 3.5 hours) lying down than controls (approximately 7 hours) over a 3-day period. When analgesics were administered lying times of horses with ALD increased to approximately 5.5 hours over a 3-day period, which although was still less than control horses, was no longer significantly different. Lying bouts were also recorded and although treatment horses had fewer lying bouts than controls, this difference was not significant. These results suggest that the analgesics that were administered reduced the pain associated with entering the recumbent position for horses with ALD.

Pritchett et al. (2003) measured time spent resting in horses for 24 hours post colic surgery and compared with horses who had anaesthetic but no procedure and control horses that had no anaesthetic or procedure. They found time resting was significantly higher in horses post colic surgery compared to both other groups. Unfortunately, time in recumbent rest and standing rest were grouped together for final analysis so exact times were not available. Additionally, this study also found higher plasma cortisol in the surgery group compared to the control and anaesthesia group, suggesting HPA axis activation in response to this stressor/pain (Pritchett et al., 2003).

Once again, these studies demonstrate that time spent resting could be used as a measure of physical health, but the nature of the injury or illness can have contrasting effects on rest time, either increasing or decreasing time resting. More research is required on individual conditions before conclusions can be drawn on whether rest patterns can be used as a measure of health in horses.

1.10.3 Quality of Life Assessment (QoL)

Disturbances in rest pattern as both an indication of poor welfare and cause of poor welfare make it a good candidate for inclusion in QoL assessments. Currently, comfort and rest appear as a resource based measure in the housing category in validated welfare assessment protocols including the Welfare monitoring system (Viksten et al., 2017), Animal Welfare Indicators Network (AWIN) welfare assessment protocol for horses (Dalla Costa et al., 2016) and Equid Assessment, Research and Scoping (EARS) welfare assessment and monitoring tool (Raw et al., 2020). It is not practical to include rest in some welfare protocols such as the Standardised Equine-Based Welfare Assessment Tool (SEBWAT) or in Qualitative Behavioural Assessments (QBA), where assessment is based on a moment in time. However, if behavioural signs of rest disorders were more clearly quantified, rest patterns could be included in comprehensive welfare assessments as an animal-based measure. While thorough research is required to determine what features of rest behaviour best warrant inclusion in QoL assessments, it certainly shows promise as a new and informative measure in the welfare assessment toolbox.

1.11 Owner knowledge of horse sleep

Very little research has been carried out to investigate how sleep is managed, monitored or understood by those responsible for caring for horses. There has been some work investigating the high rate of anecdotal reports of sporadic idiopathic hypersomnia (excessive daytime sleepiness) and cataplexy often attributed to narcolepsy in horses

(Bertone et al., 2015; Fuchs et al., 2016). Narcolepsy is a neurological sleep disorder characterised by excessive daytime sleepiness, REM sleep disfunction and cataplexy. It has not been thoroughly investigated in horses but in humans and dogs it is associated with a lack of the hormone hypocretin in the hypothalamus and is likely similar in horses (Fuchs et al., 2016). Familial narcolepsy has been reported in some lines of Miniature and Lipizzaner horses, but otherwise is exceedingly rare. Although anecdotal reports are common, many reported cases do not fulfil the clinical signs of narcolepsy (Bertone et al., 2015). Horses that show a complete lack of lying behaviour outside of collapse episodes, who showed signs of collapse in correlation with a stressful event or other illness are unlikely to be narcoleptic (Bertone et al., 2015; Fuchs et al., 2016). The work by Fuchs et al., (2016) put out a call to owners who believed their horse was narcoleptic within Germany and received 177 responses. Thirty-nine of these were selected for detailed clinical examinations and overnight monitoring with video and EEG. Results showed none of the 39 horses examined were suffering from narcolepsy, but rather were suffering REM sleep deprivation due to "illness, ethological deficits or husbandry shortcomings" (Fuchs et al., 2016). It was unclear if the diagnosis of narcolepsy was assumed by the owners themselves or an outside party, but this label may have been responsible for a lack of veterinary intervention and delay of treatment.

Preliminary studies have found few owners consider the sleep needs of horses or suspect behavioural or performance issues could be caused by a lack of sleep (Greening et al., 2020). The propensity for horses to predominantly rest at night makes routine monitoring of this behaviour logistically difficult. Additionally, some owners may not be aware of the importance of recumbency for horses to complete their full sleep cycle. It has been suggested that normal healthy horses spend some time recumbent in REM sleep every night (Wöhr et al., 2016), however, when reviewing the literature it seems some horses have nights where they do not lie down and some do not lie down at all during the observation period. Greening et al., (2021) stated only 7 of the 10 horses used in their study lay down every night over a 6 night observation period. Chaplin and Gretgrix, (2010) noted that on several occasions, horses did not lie down in the 24-hour observation period. This study design involved repeated measures where horses were moved from one treatment to another (fully stabled, partly stables and paddocked) for a 7 day period with a 6 day acclimation period (Chaplin and Gretgrix, 2010). It may be that 6 days was not sufficient to allow horses to fully habituate to their new environment. Williams et al., (2008) carried out EEG recordings of 6 horses that were moved to the testing environment and given 5 days to habituate before 1 night of recording. They found one horse did not lie down at all for the recording period and exhibited spontaneous collapse after entering REM sleep while standing, behaviour indicative of REM sleep deprivation (Williams et al., 2008). Again, 5 days may not have been sufficient for habituation to the new environment to occur, however, 5 days is unlikely to be long enough to develop sleep deprivation to this level and it is possible this horse was experiencing some level of sleep deprivation before recording commenced. It may be that the number of horses suffering mild or severe sleep deprivation in the domestic population is greater than expected and it may be an overlooked welfare concern. It is unclear if horse owners are monitoring rest patterns and if so, how? If they are not monitoring rest patterns, then strategies for doing so must be developed and widespread education required to safeguard horse welfare.

1.12 Research aims and objectives

The importance of healthy horse sleep as an aspect of horse care has been largely ignored until recently, with initial investigations suggesting that sleep disorders in the domestic horse population may be a widespread problem of unknown proportions. There is an urgent need to determine how owners/careers and welfare scientists can practically monitor equine sleep patterns and once established, which aspects of this behaviour would indicate compromised welfare. Many external and internal factors are known to influence sleep patterns and a certain amount of healthy variation is to be expected both between and within individuals. Determining what is normal variation and what falls outside of the expected range will allow stakeholders to better identify when welfare is likely to be compromised. This thesis aims to identify which features of equine sleep can be practically monitored to distinguish normal variation in sleep behaviour from abnormal sleep behaviour that could indicate compromised welfare.

In chapter 2 I surveyed owners and carers of horses to determine i) how rest is viewed as a care priority, both as the provision of resource in the form of a dry comfortable place to lie down, and as an animal-based measure of health and wellbeing by monitoring rest patterns directly; ii) if horse owners/carers monitor rest patterns and if so, how? Observation methods were reviewed and evaluated for accuracy and practicality in a real world setting; iii) what attributes of horse owners/carers contribute to the likelihood of monitoring rest patterns; and finally, iv) evaluate whether owner reports can identify overall trends in rest patterns in horses that relate to horse height, age or season, which may highlight areas for further research.

Much of the current research on sleep is completed on a single night's observation with little to no work looking at the interplay of sleep behaviour over consecutive nights. In chapter 3 I explored i) whether there are any features of rest behaviour that are consistent over consecutive nights both within and between individuals; ii) what external factors are likely to account for normal variation in sleep patterns over consecutive nights including level of exercise and ambient temperature; iii) determine whether between or within individual variation over multiple nights can be explained by internal factors such as time spent performing other behaviours or individual personality score. These were compared to duration, frequency and night-by-night variability of recumbency behaviours to determine what features of rest pattern are likely to provide the best summary of wellbeing with minimal influence from normal variation.

Cortisol plays an important role in the sleep wake cycle and the activation of the HPA axis in response to an acute stressor. In chapter 4 I aimed to: i) Determine whether there are patterns in horse rest over the long term and whether these patterns can be linked to other behaviours within an individual; ii) examine the potential link between long term rest patterns and the secretion of cortisol within an individual; iii) investigate whether horses housed in individual stables show signs of synchrony of rest behaviour as has been reported in group and pasture kept horses.

Finally, I will conclude with a general discussion exploring the overall findings of the research highlighting how rest patterns can be used in welfare assessment by both owners and researchers, what features of rest behaviour are best to measure as an indication of horse wellbeing and how this relates to physiological measure of stress, in this case cortisol. This work represents a starting point from which to develop rest patterns as an animal-based welfare measure to be used in welfare assessment protocols by both owners and welfare scientists.

Chapter 2: Horse carer perceptions: The importance of horse rest

2.1 Introduction

Sleep is essential for biological functioning. Its role in both physical and mental health is well documented in the human literature (Banks et al., 2005; Brown, 2012; Halson, 2014) and may mean that any disruptions to normal sleep functioning are indicative of compromised horse welfare (Holdgate et al., 2016; Rushen et al., 2012; Steptoe et al., 2008; Vargas and Lopez-Duran, 2017). As is the case with humans, horses exhibit two distinct types of sleep, Non-Rapid Eye Movement (NREM) and Rapid Eye Movement (REM) sleep (Dallaire, 1986; Ruckebusch et al., 1970; Williams et al., 2008). While in NREM sleep, a system of ligaments called the stay apparatus allows horses to lock their limbs into position so they can achieve this phase of sleep while standing (Houpt, 1980). Several regions of the brain are involved in a full sleep/wake cycle; a part of the brainstem known as the pons is responsible for REM sleep. During REM sleep the inhibitory transmitters prevent spinal motor neurons from producing action potentials (Breedlove, 2013; Uchida et al., 2021). This results in complete loss of muscle tone or muscle atonia during the REM phase of sleep rendering the stay apparatus ineffective, therefore, for this phase of rest the horse must lie down (Houpt, 1980; Williams et al., 2008b; Wöhr et al., 2016). A horse that does not feel secure in their environment or that is physically unable to lie down or get up due to pain and/or discomfort while either getting into or out of the recumbent position or while in a recumbent position may adopt strategies to reduce or delay the need to adopt that body position. For example, horses can increase the time spent in NREM as a compensatory measure to reduce or delay the onset of the REM phase of sleep (Bertone et al., 2015). However, each phase of sleep performs a distinct biological function, so while it is possible for the horse to delay the onset of REM sleep, eventually, they must lie down for normal functioning (Bertone et al., 2015; Dallaire, 1986).

In recent years there has been a concerted effort by equine research scientists to disseminate equine welfare research to the wider equine community (Fenner et al., 2020; Pickering and Hockenhull, 2020). However, although sleep patterns have been shown to be directly related to welfare (Bertone et al., 2015; Holdgate et al., 2016; Rushen et al., 2012), indicators of an abnormal sleep pattern may not be recognised by carers. Narcolepsy is a neurological sleep disorder caused by inappropriate activation of the REM inhibitory transmitters during wakefulness. This is usually triggered by strong emotions and results in the sufferer experiencing muscular cataplexy usually associated with REM sleep resulting in sudden collapse (Uchida et al., 2021). Narcolepsy has been documented in equids and is associated with Miniature Horses (Lunn et al., 1993) and Lipizzaners (Ludvikova et al., 2012). However, anecdotal evidence suggests that sudden cataplexy, often described by owners as narcolepsy, is far more wide-spread (Bertone et al., 2015). In order to investigate this phenomenon researchers sent an online survey targeted German horse owners who described their horse as narcoleptic (due to unexplained collapse) with 177 respondents (Fuchs et al., 2016). When a subset of 39 horses from the study were examined by a veterinarian, underlying behavioural and physical problems resulting in REM sleep deprivation were responsible for the symptoms originally attributed to narcolepsy. In 20 of the horses examined the collapses occurred shortly after an event such as a change in stable and 34 individuals did not show any normal recumbency behaviour during observation. Additionally, the majority of collapses occurred at night and were not associated with strong emotions as is typical for this disorder. These findings led the research team to conclude that these horses were unable to achieve a recumbent position and therefore transitioned to REM sleep while standing resulting in muscle atonia followed by spontaneous collapse (Fuchs et al., 2016). This suggests there is a lack of understanding among some horse owners around monitoring sleep in horses and recognising the signs of sleep deprivation.

Both domestic and free living horses predominantly lie down at night between midnight and 4am (Auer et al., 2021; Burla et al., 2017; Fuchs et al., 2016; Kelemen et al., 2021). Due to the timing and brevity of equine sleep, it may be difficult for caretakers to monitor rest patterns. Unpublished preliminary survey results by Greening et al. (2020, conference proceedings) found 29% of respondents never considered the amount of sleep their horse achieved and 55% did not attribute lethargy to lack of sleep. The number of years the respondent had owned a horse had no significant relationship to any of the survey responses (Greening et al., 2020). This suggests that a high portion of the horse owning population, even those experienced in horse care, may not consider monitoring rest as a care priority. Where individuals do consider their horse's rest pattern, it is unclear how they would monitor rest considering the timing, brevity and body posture adopted during sleep. There is a need to further establish how horse owners prioritise rest for their horses. Additionally, if owners are monitoring rest, it is important to establish how they are doing this and determine whether it is an effective strategy which could be developed into a standardized measure. This information can then be used to establish education programs for horse owners to improve horse welfare.

The aims of this study were to gain an understanding of horse owners' and caretakers' understanding of the importance of sleep as part of holistic horse care; identify where knowledge and practice is effective at monitoring equine sleep for dissemination to the wider equine public, and where there are gaps in understanding where education could be targeted.

The research objectives were:

i) Determine how rest is viewed as a care priority, both as the provision of resource in the form of a dry comfortable place to lie down, and as an animal-based measure of health and wellbeing by monitoring rest patterns directly.

ii) Establish whether horse owners/carers monitor rest patterns in their horses and if so, how?

iii) Identify what attributes of the horse owners/carers contribute to the likelihood of monitoring rest patterns in their horses.

iv) Evaluate whether owner reports can identify overall trends in rest patterns in horses that relate to horse height, age or season, which may highlight areas for further research.

H_O: There is no correlation between horse owner/caretaker attributes and their likelihood of monitoring their horses rest pattern.

H_a: There is a correlation between horse owner attributes and their likelihood of monitoring rest patterns.

2.2 Method

2.2.1 Ethics

This study was approved by the Nottingham Trent University School of Animal, Rural and Environmental Sciences human ethics procedure (Reference number ARE916). Respondents were over the age of 18 and no personal or sensitive information was collected. Data was stored anonymously according to GDPR legislation. Animal subjects were not tested or manipulated in any way for the purpose of this study.

2.2.2 Survey

An anonymous online survey was designed and distributed in Joint Information System Committee (Jisc) Survey Builder (Joint Information Systems Committee, UK) (for full survey see appendix I). The survey was developed through a series of four pilot stages for clarity and content with both horse carers and non-horse carers. Initially the survey was pilotted to three academics in the field of equine science; the second stage was to 10 non-equestrian researchers who provided technical testing of the survey tool; the third stage was to five equine students and staff to more closely assess content; and finally the fourth stage was to a small group of five equine owners, not necessarily from an academic background, before the final release. The target demographic for this survey were horse carers and owners aged 18 or over that were responsible for the care of one or more horses. The survey could be completed up to four times by an individual respondent for different horses.

Participants were recruited online via snowball sampling using a web-link to the survey which was posted on equine specific Facebook pages and shared by various individuals on other platforms including Twitter and Instagram. The link to the survey could also be shared via email but I am not aware of whether it was shared using this method. The survey was available from 3 December 2019 until 1 February 2020. After the initial page, which contained privacy information and a participant consent form (appendix I), the questionnaire contained three main sections. In section 1 participants filled out three questions answering information relating to their demographics including gender, age and country of residence. The survey was made available internationally, and where a sufficient number of respondents were present, analysis could be undertaken by country. Section 2 contained six questions about experience with horses including years of experience, formal qualifications relating to horses and horse care and if the respondents were actively competing in any equestrian discipline. This was to determine whether, considering the importance of sleep and athletic performance, those competing were more likely to monitor horse rest. In the third and final section, the survey contained 17 questions relating to horse care including questions relating to sleep. In total the survey contained 29 questions: 18 multiple choice, four select all that apply options, three short answer, two ranking and two free text answer questions.

2.2.3 Data preparation

A total of 714 people responded to the survey with data on 976 horses. Data were organised and cleaned using Microsoft Excel. Five responses, 1 donkey, 1 mule and 3 horses that were less than 1 year old were removed from the survey as their rest patterns are likely to significantly differ from that of adult horses due to their underlying biology, and were an insufficient sample size for comparison, leaving data on 971 horses and 714 horse owners and carers.

Horse breed was a 'select all option' answer with a list of common breeds and common crosses or "types", for example, Cob type. Breeds were retrospectively classed as 'common' if there were more than 30 cases reported by respondents to the survey. Owners could select more than one breed where crosses were known or select other and give a free text answer. As respondents could choose multiple breeds, common crosses such as Irish Draught and Thoroughbred were given their own category. Where it was a cross between a common and less common breed, the horse was added to the category of the common breed as a cross. Where the horse was a rare breed or a cross of two or more rare breeds, they were added to the 'other' category.

Only one respondent reported their horse as being greater than 182.9 cm therefore this individual was combined with the 175.3 - 182.9 cm category which was changed to horses that were greater than 172.7 cm. There was a low number of respondents for the less than 91 cm category (22), so this was combined with the 94 - 121.9 cm category to produce a 121.8 cm or less. This left seven categories for horse height.

When reporting the number of occasions that the owner had observed their horse lying down in the winter and summer months, responses were lower in the lying down 3 - 5 and

6 - 8 categories. This may have been because most owners would not count the number of times their horse was lying down but rather are relying on a rough guess of not very much, a medium amount, or a lot. Therefore, these two categories were combined to create four overall categories of never (0 times), rarely (1 - 2 times), sometimes (3 - 5 and 6 - 8 times combined) and a lot (more than 8 times).

When asked how often they saw their horse lay down, 60 respondents selected 'Don't know' for winter and 54 selected 'don't know' for summer. This result is important, however, as no further follow up questions were asked relating to why the respondent answered 'don't know' to this question, these responses were removed for the analysis. This left 917 responses for how often their horse would lie down in the summer and 911 responses for how often their horse would lie down in the winter.

All statistical analysis were carried out in R version 4.0.3 (R Core Team, 2020). As data was categorical a Pearson's Chi Squared Goodness-of-Fit Test was used to determine what features of caretaker or horse demographics were associated with monitoring sleep and healthy sleep patterns. Where multiple comparisons were made, a Bonferroni's correction was applied to limit type 1 error rates.

2.3 Results

2.3.1 Human and horse demographics

Respondents were primarily from the UK (n = 607, 85.0%), followed by the USA (n = 27, 3.78%), Ireland (n = 25, 3.5%), other parts of Europe (n = 19, 2.66%), Australia (n = 17, 2.38%) and other countries (n = 19, 2.66%). The majority of respondents were female (n = 694, 97.1%), followed by 16 (2.2%) male and 5 (0.6%) other or prefer not to say. Age of respondents followed a bell curve with 89 (12.5%) 18 - 24 year olds, 127 (17.8%) 25 - 34 year olds, 160 (22.4%) 35 - 44year olds, 153 (21.4%) 45 - 54 year olds, 142 (19.9%) 55 - 64 year olds, and 43 (6%) over 65 years old. More than half the respondents (n = 365, 51.1%) had >20 years' experience with horses.

Of the 971 horses that were analysed ages showed a normal distribution with 44 (4.5%) 2 - 4 year olds, 219 (22.55%) 5 - 9 year olds, 306 (31.51%) 10 - 14 year olds, 199 (20.49%) 15 - 19 year olds, 119 (12.26%) 20 - 24 year olds, and 84 (8.65%) 25 years or older.

Horse height was skewed towards taller horses with 300 (30.90%) 15.1-16hh being the most common category, followed by 245 (25.23%) horses at 14.1 - 15hh, 220 (22.66%) horses 16.1 - 17hh, 83 (8.55%) ponies 13.1 -14hh, 41 (4.22%) ponies 9.1 - 12hh, 37 (3.81%) horses greater than 17hh, and finally 23 (2.7%) ponies 12.1 - 13hh.

Thoroughbred or Thoroughbred cross was the most common breed with 173 (17.8%), followed by cob type at 143 (14.73%) and Sport Horse type with 129 (13.3%) responses. Friesian or Friesian cross had the least number of responses with only five horses.

Just over half of the horses in the survey had no known medical conditions (n = 529, 54.69%). Of those that did have a medical condition arthritis was the most common (n = 146, 15.14%), followed by laminitis (n = 100, 10.30%). One hundred and thirty respondents (13.39%) indicated their horse had some other type of medical condition including Head Shaker Syndrome, Equine Asthma, Navicular Syndrome or Chronic Obstructive Pulmonary Disease (COPD).

2.3.2 Respondent attitudes on the relative importance of main care priorities

When asked how important respondents found a number of management choices all respondents (n = 714, 100%) felt forage was either important or somewhat important, 703 (98.46%) respondents felt turnout was important or somewhat important, 701 (98.18%) felt social contact was important or somewhat important, 674 (94.38%) reported having a comfortable place to lie down was important or somewhat important followed by exercise (n = 576, 80.67%), grooming (n = 494, 69.19%), and rugging (n = 434, 60.78%) (Figure 1). Of the 714 respondents 15 (2.1%) considered rest somewhat unimportant or unimportant and one person was unsure.

When asked to rank the importance of rest against exercise, grooming and rugging, 401 (56.16%) respondents ranked rest as the most important, followed by exercise (n = 350, 49.02%), rugging (n = 162, 22.69%) and grooming (n = 58, 8.12%).

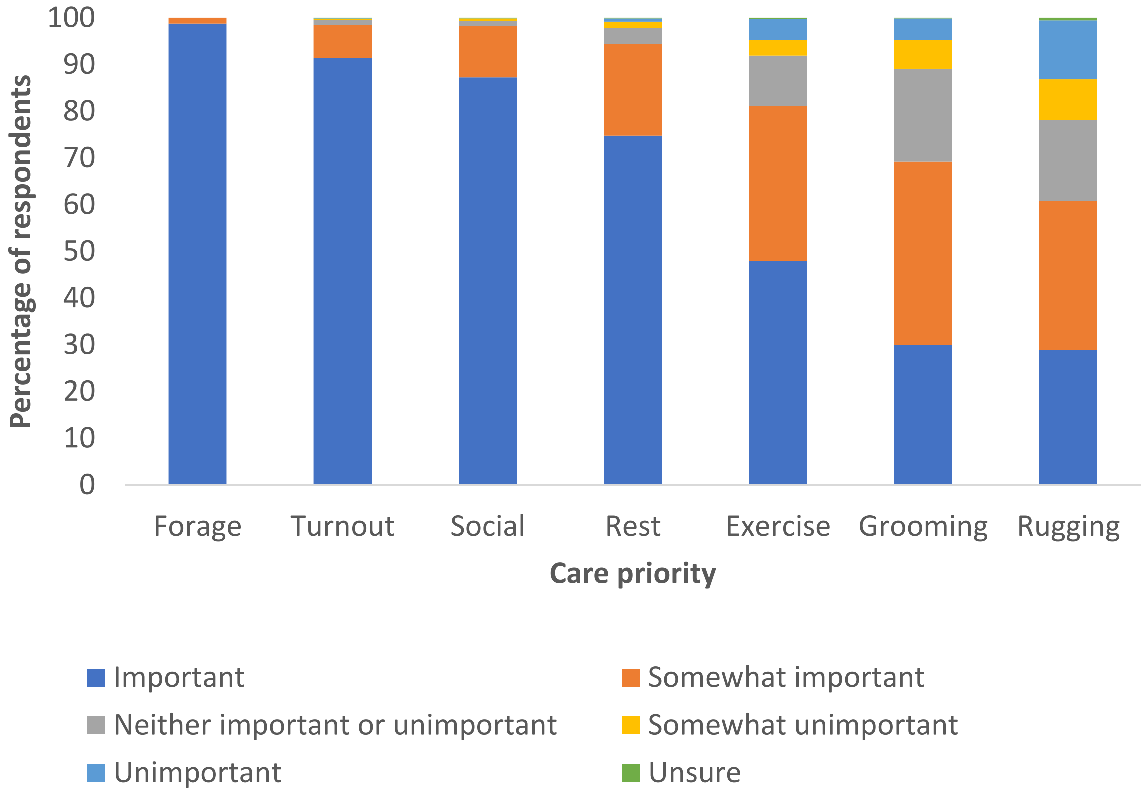


Figure 2.1: The percentage of respondents who felt each care priority was important, somewhat important, neither important or unimportant, somewhat unimportant, unimportant or unsure for horse wellbeing in general. Respondents were able to label all aspects of care as important or unimportant as there was no ranking system.

2.3.3 Frequency and method of monitoring of rest patterns

Of the 714 respondents 344 did not answer the question 'Do you monitor your horses rest pattern and if so, how?'. Of the 370 respondents who did answer, 241 (65.14%) did not monitor rest patterns and 13 (3.51%) indicated they did monitor rest patterns but did not expand on how. Figure 2 shows the breakdown of how the 116 (16.3%) respondents who answered how they monitored rest patterns.

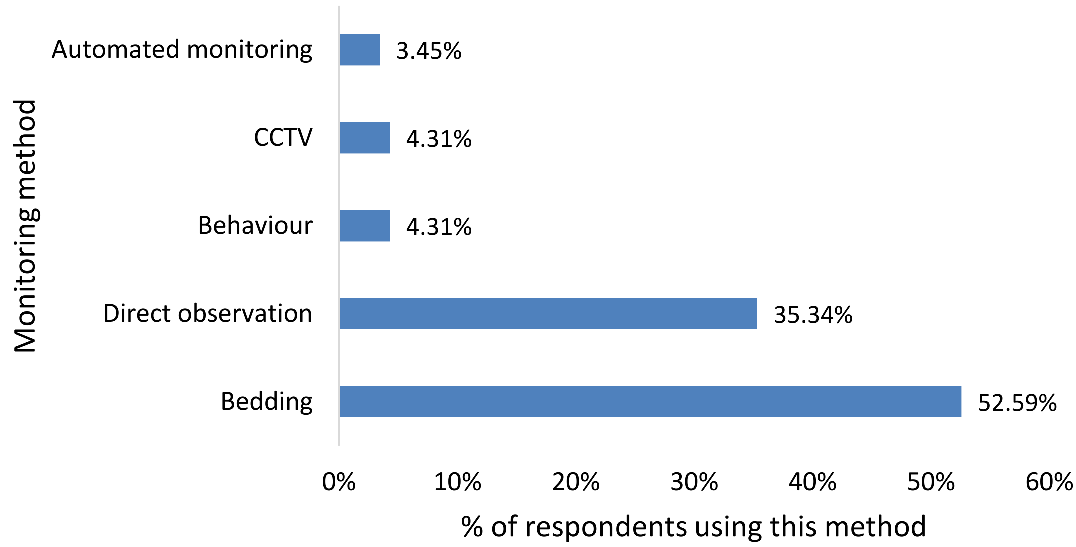


Figure 2.2: Breakdown of how respondents reported they monitored rest behaviour (N = 116, 16.3%).

Height of the horse showed no significant relationship between observed lying behaviour in either summer ($X^2(18, N = 917) = 19.529, P = 0.36$) or winter ($X^2(18, N = 917)=24.229, P = 0.148$). Figure 3 shows the percentage of respondents who observed horses of different age groups lying in the summer months. Respondents of horses aged 25 years or older were statistically more likely to report never seeing their horse lying down in the summer months ($X^2(15, N = 917) = 29.14, P = 0.015$) compared with caretakers of horses in all other age groups. This difference was not observed when reporting on the winter months (X^2(15, N = 917) = 24.001, P = 0.065).

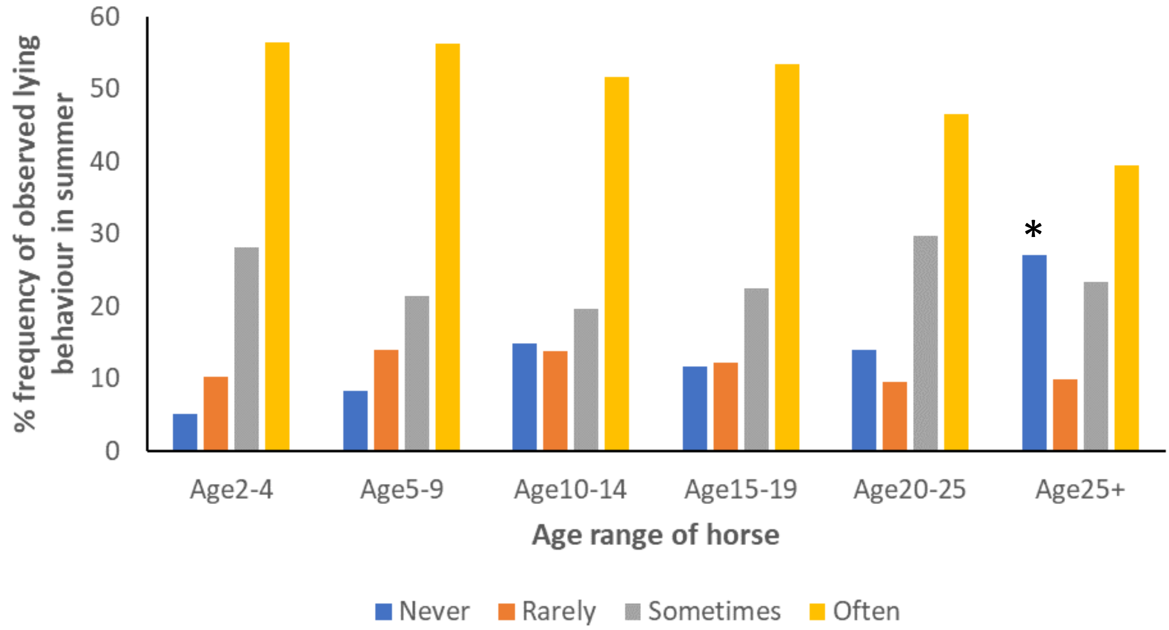


Figure 2.3: Frequency of observed lying behaviour in the summer months for horses in each age group. * indicates a significant difference (P < 0.05) in frequency of observation compared to all other age groups.

2.3.4 Factors influencing whether respondents were likely to monitor rest

Of the 370 respondents who in answered if they monitored rest or not, there was no significant relationship between years of experience (X^2(5, N = 370) = 8.159, P = 0.148) or if the respondent competed or not (X^2(1, N = 370) = 0.163, P = 0.686) and if the respondent reported monitoring rest patterns. Discipline was not analysed due to the number of respondents who competed in multiple disciplines making distinctions between disciplines unreliable. However, if the respondent had formal training (N = 404, 56.6%), compared to no training (N = 310, 43.4%), they were significantly more likely to monitor rest patterns (X^2(1, N = 370) = 6.5299, P = 0.01). Similarly, if they considered themselves a professional (N

= 99, 13.9%) then they were also significantly more likely to monitor rest patterns (X^2(5, N = 370) = 16.36, P < 0.01) when compared to non-professional (N = 615, 86.1%) respondents. An initial chi-squared test showed a significant difference in the training level and likelihood to monitor rest patterns (X^2(5, N = 370) = 16.36, P < 0.01), however, post hoc comparisons using t Test with Bonferroni correction reviled no significant difference (P > 0.05) between training level, likely due to the loss of statistical power and reduced sample size.

2.4 Discussion

The findings of this survey indicate that while most horse owners and caretakers recognise that sleep is important for the health and welfare of horses, very few actively monitor rest as part of their management routine. Higher rates of rest monitoring among respondents with equine qualification highlight the importance of education in fostering a more holistic approach to horse care.

2.4.1 Importance of horse rest compared to other care priorities

Although rest was viewed as an important care priority for the majority of horse owners/carers, there were respondents who indicated it was not as important as forage, social contact and turnout. Forage, Friends and Freedom, the 3 F's, has become a popular phrase when referring to the basic needs of horses. The 3 F's have grown in popularity since it was first mentioned in a 2012 blog by equine behaviourist Lauren Fraser for the International Association of Animal Behaviour Consultants (IAABC) (Fraser, 2012). A simple Google search in July 2022 shows the phrase was mentioned over 1 million times. The phrase was coined as a simple message to horse caretakers to instil the fundamental needs of a horse for good horse welfare. The results of this survey suggest the 3 Fs message has been effective, with most or all of respondents indicating access to forage, social contact and turnout as important or somewhat important. Having a comfortable place to lie down also ranked highly with 94.38% of respondents ranking it as ‘important’ or ‘somewhat important’. However, 2.1% of respondents ranked a comfortable place to lie down as ‘unimportant’ or ‘somewhat unimportant’. In all these cases these individuals ranked the 3 Fs as ‘important’ or ‘somewhat important’. This may suggest that these individuals believe the 3 Fs are the only requirement for good welfare rather than recognising other requirements beyond the 3 Fs are also essential to horse welfare. While the 3 Fs are integral to good horse welfare, they only represent a foundation on which to build good horse welfare. Sleep represents a biological need in all mammals and failing to provide provisions for individuals to achieve their full sleep cycle is likely to compromise welfare (Axelsson and Vyazovskiy, 2015; Burla et al., 2017; DuBois et al., 2015; Meerlo et al., 2008).

2.4.2 Frequency and method of rest monitoring

Respondents reported a range of methods employed to monitor rest patterns; the method used dictates how much detail on rest pattern can be inferred. Most respondents look for signs in the bedding, either depressed areas of bedding/ground or bedding/dirt on the horse, as an indicator of whether the horse lay down the previous night. This is likely an effective method to determine nightly rest patterns and was a key finding from this survey. Being aware of whether the horse is lying down each night is important for the detection of early signs of stress or illness that may influence rest patterns. Wöhr et al. (2016) noted that although horses spend a relatively short period of time lying down compared with humans, it is normal for them to lie down every night. Checking for signs in the bedding each morning is a quick, simple, low tech and effective way to monitor if the horse is lying down every night. This technique should be included as a part of the curriculum for all basic horse care education as a rough indicator health and welfare. However, while this method gives information on whether a horse is lying down or not, it does not give information about the duration or type (lateral or sternal) of recumbency. Whether the horse is spending more time lying down, or is having a reduction in lying time cannot be identified by using this method to monitor rest patterns. Oliveira et al. (2022) found that horses with mild osteoarthritis spent more time in recumbency, however as the disease progressed horses with severe osteoarthritis spent less time recumbent. While monitoring signs of recumbency in bedding is inexpensive and gives a rough picture of recumbency patterns, caretakers of high-risk individuals such as geriatric horses may want to consider investing in automatic monitoring devices or cameras for more detailed information on rest patterns.

Just over 4% of respondents indicated they monitor rest patterns by direct observation. Direct observation over time can give a rough indication of changes in behaviour. For example, if a caretaker is used to seeing their horse in recumbency at a particular time they may notice if this behaviour changes. While this may be effective as a general measure of recumbency, it has several drawbacks. Both feral and domestic horses predominantly sleep at night, between midnight and 4 am (Auer et al., 2021; Boyd et al., 1988; Burla et al., 2017; Pedersen et al., 2004), therefore, it is not practical for most caretakers to observe the horse at this time. Some horses may have a tendency to lie down during the day; in this case the owner must be present to observe the behaviour. For caretakers who do not keep their horse at home, for example in a livery yard, or who work away from home either full or part-time, observation is not a practical option. Additionally, while direct observation may give a rough overall picture of rest patterns for some horses it may not provide feedback on the rest behaviour before welfare is compromised. Horses are more likely to lie down in the day during summer months (Duncan, 1980), and it may take several weeks or months before a reduction of recumbency behaviour is identified by the owner, by which time sleep deprivation is likely to have already occurred (Fuchs et al., 2016; Williams et al., 2008b). Symptoms of sleep deprivation can be very general including poor performance, poor weight maintenance and poor attitude, and may go unnoticed or attributed to other factors. Signs of severe sleep deprivation include drowsiness followed by partial or full collapse which may or may not result in abrasions over the dorsal fetlock area (Bertone et al., 2015). By this stage horse welfare is likely to have been compromised for a number of weeks or months.

Just over 4% of respondents monitor horse rest using CCTV. This provides the potential for detailed and highly accurate monitoring of rest patterns, as a horse lying down is a very clear behaviour to the observer and this method provides information on both frequency and length of recumbency bouts if the footage is analysed. However, collecting this detailed information is likely to be very time consuming and beyond what is practical for most horse caretakers. Random scan observations may be possible and provide the observer with enough information to determine if the horse is experiencing sleep disturbance to a level that is likely to compromise welfare. Additionally, CCTV is likely to only be available in stables, and may be costly to set up, particularly if a power source is not available where the horse is kept. These factors may prevent the widespread use of CCTV to monitor rest patterns, although it is still likely to be used as the preferred tool in research due to the detail it captures, particularly in group housed situations.

Recent developments in Artificial intelligence (AI) have seen some commercially available products on the market that automatically monitor horse behaviour, for example Novostable marketed by Novosenso©. This product claims to monitor a wide range of behaviours including recumbency and send alerts to an app when horse behaviour diverts from what is considered normal. While this technology is likely to be invaluable to large stables with high-risk horses such as breeding yards, the cost is currently prohibitive for most horse owners. Additionally, as with regular CCTV, this type of system is only effective when horses are in single stalls. While systems such as Noldus Tracklab© are attempting to develop a tracking system for group housed horses, this system is still in development and, as with the Novostable, prohibitively expensive.

A small number (3.45%) of respondents indicated they use some form of automatic rest monitoring system such as Orscana by Arioneo®. This system attaches to the horse's rug and contains both a pedometer and accelerometer to monitor activity levels and body orientation. It then sends this information to an app that gives the percentage of time spend in recumbency each night. This information is easy to use, quick to access, relatively cheap when compared to installing CCTV, and has the added advantage of being suitable for use on horses turned out or in social housing. While some models must be attached to a rug, other brands may come with a harness system to attach directly to the horse. As this technology becomes more popular and retail price reduces, this may provide a convenient option for monitoring horse rest, although accuracy is yet to be assessed.

When asked how frequently owners observed lying behaviour in their horse there was a significant association between the frequency of observed lying behaviour in summer and horse age but no difference in winter months. Horses are more likely to have bouts of lying during the day in summer due to the warmer, dryer conditions (Duncan, 1985). This increases the chance that owners will see their horse lying down during these months compared to the winter months when lying behaviour is likely to exclusively occur at night when owners are not present. It may also be that owners pay more attention to their horses lying habits in spring and summer if they are monitoring horses for signs of conditions like laminitis which is more prevelent at that time of year. Respondents reported that horses that were 25 years old or older were more likely to never be seen lying down in summer compared to all other age groups. While general owner observation is not as reliable as detailed rest observation, it can be useful as a rough indicator of more advanced changes in rest patterns. In this case the lack of lying behaviour observed in horses 25 years and over may be an accurate representation of what is occurring in that age group. Older horses are likely to also be spending less time lying down in winter months, but as horses from all age groups are predominantly lying down at night, owners are less likely to notice the reduction in lying time. Arthritis was reported as the most common, known medical condition in the horses surveyed (54.69%). Advanced arthritis is more likely to impact older individuals and is known to reduce lying times in horses (Oliveira et al., 2022). Closely monitoring the rest patterns of these individuals can give valuable information that can be utilised to determine treatment options such as levels of pain relief required, or to be used as part of a quality-of-life assessment to make end of life decisions.

2.4.3 Factors influencing whether rest is monitored

Although providing somewhere comfortable to rest was identified as an important care priority, less than half of respondents indicated that they monitor rest patterns. There was no significant relationship between the number of years owning a horse or if the person competed or not and whether the participant monitored rest patterns or not, which agreed with the findings of Greening et al. (2020). However, respondents that had a formal equine or animal care qualification or worked professionally in the equine industry were significantly more likely to report monitoring horse rest patterns. This suggests that individuals with formal qualifications, no matter what that qualification is, are likely to have a more comprehensive approach to horse monitoring and management. This shows the value of education in raising awareness of the importance of sleep for horses, which then translates to changes in management practices. Only 10 individuals indicated that they work professionally in the equine industry but do not hold equine and animal care related qualification, therefore it seems either those hiring for the industry are asking for formal qualification or those working in the industry are gaining formal qualification to complement their work experience. Education corresponds to a positive effect on perceptions of farm animal welfare in farmers of sheep, pigs and cattle (Balzani and Hanlon, 2020). Although most equine training does not specifically cover monitoring rest patterns, it is possible that training encourages horse caretakers to consider a more holistic approach to horse care. Although monitoring horse rest is an important part of monitoring overall wellbeing, determining what aspects of rest owners need to be monitoring is the next step in using rest patterns as a measure of welfare.

2.4.4 Conclusions

Although providing a comfortable area for rest was considered a high priority for most respondents, a small number of respondents felt it was not important when compared with the 3 Fs of forage, friends and freedom. While the 3 Fs are integral to physical and mental wellbeing in horses, they are not the only provisions that need to be considered to ensure good welfare. Individuals with formal qualifications are significantly more likely to monitor rest patterns while years of experience had no significant relationship with likelihood to monitor rest patterns. However, most respondents do not monitor rest patterns in horses. Incorporating information about the problems caused by disrupted rest for horses and how to integrate horse rest monitoring into their regular horse care routine using the best available method should be included in all horse care and management education programs. Further research is required to provide owners with more detailed information about what aspects of sleep behaviour are best to monitor and what potential factors would cause normal disruptions to rest patterns so distinguish them from abnormal rest patterns.

Chapter 3: Factors influencing duration and frequency of rest patterns in riding school horses over consecutive nights

3.1 Introduction

Sleep plays an essential role in most physiological and cognitive functions (Halson, 2014) and has the potential to provide information about the health and wellbeing of an individual (animals: Langford and Cockram, 2010; humans: Steptoe et al., 2008). However, rest patterns can be influenced by a variety of internal and external factors making the interpretation of these patterns difficult. When compared with other species, horses spend relatively little time in recumbent rest (Zepelin et al., 2005). Horses are one of the few species with the ability to sleep while standing, however, this is only true of Non-Rapid Eye Movement (NREM) sleep. To achieve the Rapid Eye Movement (REM) phase of sleep, and therefore their full sleep cycle, they must lie down (Ruckebusch et al., 1970; Williams et al., 2008b). Horses mostly sleep at night (Bertone et al., 2015; Keiper and Keenan, 1980) and will spend 3-5 hours in total in recumbent rest with as little as 30 minutes of this dedicated to REM sleep (Dallaire, 1986; Zepelin and Rechtschaffen, 1974). This is in contrast to healthy, young adult humans who spend an average of 8 hours per night sleeping with approximately 2 hours of this time dedicated to REM sleep (Carskadon and Dement, 2005). The reduced time horses spend resting is likely related to the fact that they are a prey species and adopting a recumbent position makes them vulnerable to predation, therefore they have evolved to spend minimal time in this position (Belling, 1990; Houpt, 1980). Ruckebusch et al., (1970b), noted that animals introduced to a new environment may avoid recumbent rest until they are sufficiently habituated to return to normal rest patterns.

Ambient temperature is known to significantly impact sleep in humans with room temperatures that are too low or too high leading to disrupted and shortened sleep (Pan et al., 2012). While there are no studies looking at ambient temperature specifically affecting the sleep patterns of horses, observations of free-living horses showed increased lying times in warmer months, particularly in spring, which could be related to changes in air temperature, light or food availability (Duncan, 1985). This gives some insight into how horse rest patterns can be influenced by the external environment and may help inform optimal management and housing conditions to promote healthy rest patterns in domestic horses. However, the domestic horse is likely to experience different external environments compared to their free ranging counterparts, with the addition of stables, rugs and artificial lighting.

The link between both the quality and quantity of sleep, and athletic performance is a factor carefully considered by human athletes (Halson, 2014; Pilcher and Huffcutt, 1996). Despite the wide use of horses for sporting activities this association has not been fully explored in horses. Tortonese et al. (2011), examined the effects of jetlag on athletic performance and associated hormones in racehorses and found that jetlag seemed to improve athletic performance in these horses. The authors suggest these surprising results may derive from the fact that horses are polyphasic sleepers with a flexible sleep wake cycle. Horses depend on light : dark cues and this enables them to rapidly reset their molecular clockwork when moved across time zones. In comparison, humans are monophasic sleepers with a robust sleep-wake cycle that is more dependent on the circadian rhythm, which is slower to adjust to sudden light : dark phase shifts. Tortonese et al. (2011) also found that daily locomotion patterns also adjusted within the first day of the phase shift. This change in locomotory pattern was accompanied by an improvement in athletic performance in a standard incremental exercise test for the first day after the phase shift, although this benefit was only seen on the first day of the shift and performance returned to normal when tested again on day 14 post phase shift (Tortonese et al., 2011). The authors attributed this improvement in athletic performance to alterations in prolactin secretory patterns on the first day, where prolactin usually has a bimodal pattern over a 24-hour period; the phase shift in this study caused a steep, single peak in the first day after the phase shift. Prolactin is a versatile hormone involved in more than 300 different biological actions. Of particular interest is its correlation with dopamine in the brain and the role this plays in the feeling of fatigue and can positively or negatively affect endurance (Arfuso et al., 2021; for full review see: Rojas Vega et al., 2012). Tortonese et al. (2011) noted that the significant increase in prolactin after the phase shift was not accompanied by a significant change in cortisol levels the way it is in human studies, leading the authors to conclude that horses do not find the light phase shift stressful in the same way humans do. However, measurements were only taken on day 1 and 14 post phase shift and therefore this does not give a complete picture of how horses respond to sudden changes to the light : dark cycle. Further research is required in this area.

Caanitz et al. (1991) found horses that are exercised spent more time resting in the 2 - 7 hours post exercise compared to horses that were not exercised. This is probably explained by the restorative nature of rest allowing the body compensation for the energy expended during the exercise period (Caanitz et al., 1991; Halson, 2014). Studies on human athletes showed sleep deprivation can have a significant effect on mood (for review see: Fullagar et al., 2015). While limits to athletic performance are often thought of in terms of physical limits caused by muscular fatigue, central fatigue - that is the feeling of exhaustion - develops in the central nervous system. The release of serotonin in the brain can positively or negatively impact endurance independent of the body's physical limits (Arfuso et al., 2021). Increases in serotonin levels can decrease motivation, cause feelings of lethargy and loss of coordination. Conversely high levels of dopamine in the brain lead to higher levels of motivation and motor behaviour (Arfuso et al., 2021). Some studies have found sleep deprivation had relatively little impact on physical measures of performance, however, participants reported they felt they reached exhaustion up to 20% faster compared to when they were not sleep deprived (Martin and Chen, 1984; Temesi et al., 2013). This is not surprising as sleep deprivation is known to stimulate the release of serotonin and downregulate dopamine in the human brain (Grossman et al., 2000; Volkow et al., 2012). Thus, the detrimental effects of sleep loss may be difficult to assess through physiological measures alone, but can dramatically impact regulation of mood and emotional responses important for performance (Meerlo et al., 2008).

The relationship between stress and time spent resting is likely complex and results have been conflicting. Some studies examining horse behaviour in different housing and social environments found horses spend more time lying down and displayed more stress related behaviours in socially isolated stables compared with groups of horses at pasture (Chaplin and Gretgrix, 2010; Heleski et al., 2002; Murase et al., 2018). In contrast, other studies found reduced resting times due to the stress associated with being introduced to a new environment (Fuchs et al., 2016; Ruckebusch et al., 1970; Williams et al., 2008). It may be that the nature of the stressor and nature of the individual interact to dictate the impact of stress on individual rest patterns (Lo Martire et al., 2020). Increased resting times in stabled horses could be explained, at least in part, by the sensory deprivation experienced in stables. Ponies with partial sensory deprivation showed increased NREM sleep (Dallaire and Ruckebusch, 1974a), a similar pattern to what is seen in other animals including humans (Velluti, 1997). Most studies comparing horses kept in stables versus pasture include other changes like availability for activity and social interaction, which makes pinpointing the exact cause of difference in rest pattern difficult (Chaplin and Gretgrix, 2010; Heleski et al., 2002). Studies in rats have shown that rest patterns are altered in different ways depending on the type of stressor. For example, immobilisation resulted in a significant decrease in sleep efficiency and NREM and REM sleep throughout the time of recording. Forced swimming resulted in a reduction of NREM and augmented REM only during the first day (Papale et al., 2005). These differences could be related to the controllability and predictability of the type of stress (Lo Martire et al., 2020; Papale et al., 2005).

High between-individual variation in total time spent resting has been found in a number of studies investigating rest patterns in horses (Chaplin and Gretgrix, 2010; Ruckebusch, 1972). A few early studies examined between and within-individual variation in horse rest times and found that within-individual variation was less than between-individual variation, although sample sizes were small ranging from 1 to 5 individuals (Dallaire, 1986; Dallaire and Ruckebusch, 1974b; Ruckebusch, 1972). More recently, Wöhr et al., (2016) reported a certain level of repeatability of equine sleep behaviour and different 'sleep types' emerging from their data, but are yet to publish further details. In human studies a number of sleep parameters can be measured in relation to sleep behaviour including, time taken to fall asleep, known as sleep onset latency (SOL), total sleep time (TST), sleep fragmentation and self-reported sleep quality. High variation in sleep duration and fragmentation over multiple nights has been found to be associated with negative life stressors, negative affective state (Mezick et al., 2009). The variation is likely due to homeostatic rebound where one or multiple nights of reduced and fragmented sleep will lead to longer, solid sleep the next night to recover sleep that was lost. This cycle can repeat indefinitely as long as the individual is experiencing negative affective state (Bei et al., 2016; Mezick et al., 2009). This highlights the importance of measuring multiple nights' sleep as a single night may not be representative of an individual's sleep pattern. Additionally, it is important to measure not just the mean of different sleep parameters, but also the interindividual variability, in order to fully capture deviations from normal (Bei et al., 2016). Measuring within- and between-individual variation of different sleep parameters in horses may give an indication of affective state, providing some information about the health and well-being of that individual. However, firstly we must identify what a normal level of within individual variation is to determine if deviations from the normal range is an indicator of poor welfare. Secondly, we must identify what the best parameters are to measure in this species to capture that variation and finally, what is the likely cause of that variation. Horses adopting different rest strategies based on their current health or welfare may have been the underlying driver of the ‘sleep types’ noted by Wöhr et al., (2016).

Many species show consistent differences in behaviour that are stable over time, and this is referred to as personality. The separate traits of ‘neuroticism’ characterised by an individual’s stress sensitivity, and ‘extroversion’ characterised by behavioural expressiveness, are of particular interest in horses (Squibb et al., 2018; Yarnell et al., 2015). Individual variation in resting patterns in humans is common and there is strong evidence that personality plays a role in sleep quality (Allen et al., 2016; Friedman and Kern, 2014; Stephan et al., 2018). For example, neuroticism is consistently linked to poor sleep quality whereas high conscientiousness and extroversion are associated with better sleep quality in some studies (Stephan et al., 2018). Stress and sleep have a bi-directional relationship making it difficult to isolate cause and effect. The two main neuroendocrine systems involved in triggering a stress response are the hypothalamic-pituitary-adrenal (HPA) axis and sympatho-adrenal system, and these are both suppressed during sleep (Meerlo et al., 2002). Increased activation of these stress systems beyond normal wakeful levels can lead to sleep deprivation; likewise, sleep deprivation can lead to activation of these systems causing stress. Individuals that score highly for neuroticism are more likely to experience stress due to increased sensitivity to stress, and are therefore more likely to have altered rest patterns than individuals that score low on this trait (Lo Martire et al., 2020; Meerlo et al., 2008).

Rest patterns could be a useful tool in assessing an individual’s wellbeing, however, these patterns can be impacted by a number of internal and external factors. This study aims to identify internal and external factors that contribute to normal variation in rest. Defining the upper and lower limits of normal changes in rest can allow recognition of abnormal changes. Identifying these factors will provide a clearer picture of what contributes to the high level of variation in time spent resting between individual horses.

The research objectives of this study were to:

i) Determine if there are any features of rest behaviour that are consistent over consecutive nights both within and between individuals.
ii) Establish what external factors are likely to account for normal variation in sleep patterns over consecutive nights including level of exercise and ambient temperature.
iii) Determine if between or within individual variation over multiple nights can be explained by internal factors such as time spent performing other behaviours or individual personality score.
iv) Explore if different 'sleep types' can be identified using parameters that will most clearly identify within-individual variation of recumbent patterns using different types of cluster analysis.

H_O: There is no correlation between individual horse attributes or environmental conditions and time spend in recumbent rest.

H_O: There are no features of rest behaviour that are consistent over consecutive nights either between or within an individual.

H_a: There is a correlation between individual horse attributes or environmental conditions and time spend in recumbent rest.

H_a: There are features of rest behaviour that are consistent over consecutive nights either between or within an individual.

3.2 Methods

Additional material from this chapter that was explored but not presented due to technical issues or unsuitability for robust scientific analysis can be found in Appendix VIII.

3.2.1 Ethical statement

This study does not contravene any UK legislation and was granted ethical approval by the School of Animal, Rural and Environmental Sciences at Nottingham Trent University animal ethics procedure (Reference number: ARE389). Institutional and national guidelines for the care and use of animals were followed at all times.

3.2.2 Animals and facilities

Riding school horses (n = 14) from the Nottingham Trent University (NTU) Brackenhurst Equestrian Centre were used in this study. A convenience sample was used, consisting of all horses available in the same housing who were well established in their stable (had been resident in that stable for >6 months) at the centre. These consisted of three mares and 11 geldings with a mean height 162.63 ± 6.22 cm (16 ± 0.6hh) and mean age of 15.29 ± 4.01 years (Appendix II). Horses were fed 2% ideal body weight in hay twice a day 7:00am and 5:00pm (0.5% in the morning and 1.5% in the afternoon), adjusted for weight when required (underweight horses fed slightly more and overweight horses slightly less as determined by monthly weight monitoring on weight scales and Body Condition Scoring (BCS)). In addition, pasture cubes and Ossichaff© with supplements specific to individuals

(Appendix II) were fed twice a day. Horses were housed individually in box stalls measuring 3.5m wide, 4.1m deep and 2.5m tall walls, with bedding of chopped straw between 5-20cm deep on top of rubber mats. Stables opened onto a central corridor where horses could see other horses. Additionally, stables had windows on all sides including “chat hatches” in adjoining walls which consisted of a barred window (Fig. 1). This allowed neighbouring horses to see and smell but not touch each other. Horse 14 (H14) had a closed chat hatch as this horse’s behaviour was aggressive towards other horses, particularly at mealtimes. However, he was able to see other horses over the stable door (Fig. 1).

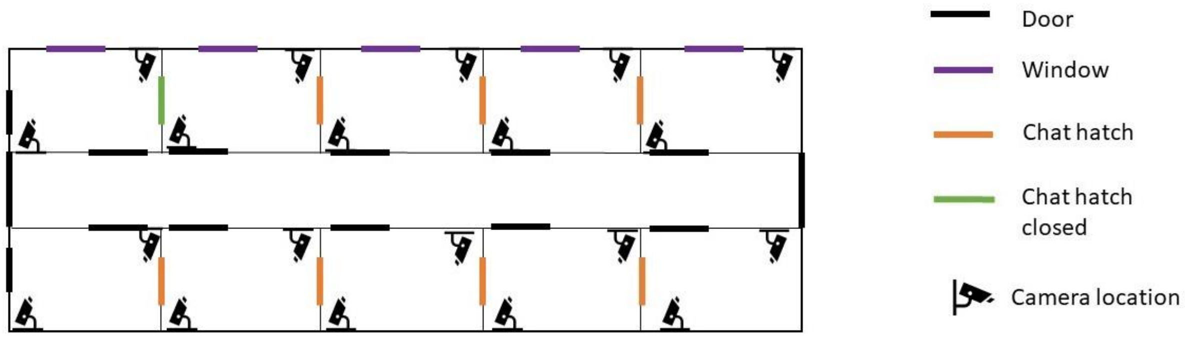


Figure 3.1: Stable block layout including camera positions for the two cameras in each stable.

Horses were kept in stables from Monday to Friday and ridden by students for 1-2 hours per day in either jump/flat lessons or lunged/put on the walker if there were no lessons that day. Exercise was kept as consistent as possible. On weekends horses were turned out in social groups of 2-3 individuals. Exercise was coded as from 0 to 8 based on the level of exercise they received that day (Table 1).

Table 3.1: Exercise type and duration monitored during monitoring period.

Code	Exercise type	Exercise duration
0	No Exercise	0
1	Put on walker or lunged	30 minutes or less
2	Turned out in field	5 or more hours (very low intensity)
3	Flat work or hacking out	30 minutes or less
4	Flat work lesson	1 hour
5	Two Flat work lessons	2 hours
6	Jumping lesson	1 hour
7	Two Jumping lessons	2 hours
8	One jump and one flat work lesson	2 hours

3.2.3 Behavioural analysis

Each horse had Foscam® (TrueTech Dist Ltd., UK) video cameras installed in their stables for 1 week from Monday to Monday between the dates of 12 February 2018 until 23 March 2018. Two cameras in each of two stables at a time were installed at 2.5 meters up the wall to ensure there were no blind spots to capture both standing (Fig. 2a) and recumbent rest behaviours (Fig. 2b). At the end of the week cameras were moved to the next two stables for the next week of recording and so on until all 14 horses had one week of video data captured.

Figure 3.2: (a) Camera mounted at the back of the stable, angled to capture standing behaviours at the front of the stable. (b) Camera mounted at front of stable and angled down to capture recumbent behaviours.

Usual weekend management varied between horses; 11 horses were turned out 5 hours a day (10am-3pm) and 3 horses turned out for 48 hours over Saturday and Sunday. Horses were filmed at all times when in the stable over a 1-week period. Due to the horse's management routines and technical problems, 7 horses were recorded for 7 nights, 3 horses for 6 nights, 1 horse for 5 nights, and 3 horses for 4 nights (where 1 individual was recorded for 3 consecutive nights and 1 non-consecutive night) (Appendix III). This resulted in a total of 84 nights recorded for 12 hours each night producing a total of 1008 hours of footage. Daytime recordings (7am-6pm) were used for scan samples every 10 minutes to determine if any recumbent rest was occurring in the stable during the day. No daytime recumbent rest was shown by any horse while in their stable. Additionally, two horses were recorded during their daytime turnout period to determine if recumbent rest behaviours were displayed while in the field. No recumbent rest behaviours were observed. Videos of each subject were then analysed using continuous observations over the night-time (6pm-7am) period and the duration of behavioural states scored according to a behavioural ethogram (Table 2). Video observations were behaviour scored using The Observer® XT Version 12 (Noldus Information Technology, Leesburg, VA, USA).

Table 3.2: Ethogram of behavioural states coded during behavioural analysis of video recordings (Adapted from Caanitz et al., 1991; Raabymagle and Ladewig, 2006).

Behaviour	Description
Standing Active	The horse is doing an activity, either drinking, walking, foraging, stereotypy, defecate/urinate or stretching/scratching for more than 3 seconds. During an activity, the horse may pause for a short period (<2mins) to look or listen to something without changing their position or shifting their weight, but the horse is clearly alert.
Standing Inactive	The horse is not doing any of the above-mentioned activities, meaning they are standing still, either in a resting state or observing/staring at something for a prolonged period (>2mins). The horse may move their head, scratch or stretch for a few seconds and slowly shift weight.
*Feeding	Muzzle either in feed bucket or within ~30 cm of hay pile, net or bedding, may lift head but showing visible chewing.
Getting down	Interim period when horse is preparing or in the process of lying down or rolling. Gradual flexing of all four limbs, forelegs go down first as trunk sinks to the ground.
Getting up	Interim period when returning to a standing position after rolling or lying in a sternal or lateral position. Upper foreleg extended first and raises the forequarters followed by the hind limbs to raise the trunk.
Sternally recumbent (noting left or right side)	Forelegs bent underneath the thorax, spinal column held in lateral arc and in some cases carried around the side of the body and lying with weight on side. Head is supported and not touching any surface.

Sternally recumbent with head unsupported (noting left or right side)	Forelegs bent underneath the thorax, spinal column held in lateral arc and in some cases carried around the side of the body and lying with weight on side. Head is unsupported and resting on the ground or leg.
Laterally recumbent (noting left or right side)	Flat on side with all four legs extended. Neck, head and muzzle touching the ground, leg movements can also occur with distal limbs. Head and body may be against the stable wall propping them up but no muscle tone to support neck.
Rolling	From either a lateral or sternally recumbent position rolling onto side or back and possibly all the way over while rubbing head and neck on the ground.

*Horses are fed hay in piles on the ground. With infrared mode activated on the camera it was impossible to distinguish between bedding and hay. Therefore, time spent eating designated feed and eating bedding were pooled together.

Of the 1008 hours of footage analysed, 3.52 hours were categorised as "other" where behaviour could not be seen or did not fit the categories of the ethogram accounting for 0.35% of observation time.

3.2.4 Ambient Temperature

Ambient temperature was recorded by a weather station located less than 0.5km from the stables. Temperatures were taken every 15 minutes from 6pm to 7am and averaged over the night. Weather station temperatures should correlate with the temperature experienced within the stables however, they will not represent the exact temperature due to the more sheltered environment within the stable yard.

3.2.5 Personality

A validated subjective 5 factor personality questionnaire (Ijichi et al., 2013) was completed for each horse by two members of yard staff who worked with the horses on a daily basis and were in charge of their day-to-day care. The questionnaires measured the personality dimensions of agreeableness, neuroticism, extroversion, gregariousness with people and gregariousness with horses. Observers placed an 'x' on a continuous line based on where they felt the horse sat between two opposing adjectives (Appendix IV). The continuous line was then divided into a 5-point Likert Scale and scored as 1-5 where 1 was the horse showed low levels of that behaviour and 5 was the horse showed high levels of that behaviour in accordance with the method used by Ijichi et al. (2013). Neuroticism was chosen as the main personality dimension to focus on for this study due to its association with stress sensitivity and the effect that may have on rest patterns. There was no biological basis for other personality dimensions to have an impact on rest patterns. Results from the two observers were analysed using Interclass correlation (ICC) agreement score estimates, and their 95% confidence intervals were calculated using R statistics (R Core Team, 2014) based on a mean-rating (k = 2), absolute-agreement, 2-way mixed-effects model. Agreement between the 2 observers had an ICC = 0.75 ($F(114,114) = 6.97$, $P<0.001$) for all personality dimensions together, and agreement on neuroticism alone had an ICC = 0.8 ($F(22,23) = 9.06$, $P<0.001$). Agreement between the two observers rate as 'good' reliability in the guidelines outlined by Koo and Li (2016).

3.2.6 Statistical analysis

All statistical analyses were carried out in R (R Core Team, 2014) and figures 4 and 5 produced using the package ggplot2 (Wickham, 2016).

To examine relationships the between night interactions in daily activity, ambient temperature and behaviour a model was fitted in a Bayesian framework using Integrated

Nested Laplace Approximation (R-INLA) (Rue et al., 2017) and best-fitting models were identified using Watanabe-Akaike Information Criterion (WAIC) (Vehtari et al., 2017) and a Gaussian General Linier Mixed Model (GLMM) was fitted to this data.

To examine the relationship between overall behaviours and external factors a Gamma distribution General Linear Model (GLM) was used to determine which variables impacted variability in rest time represented by the coefficient of variation (SD/mean*100) of total lying rest time (sternal and lateral recumbency taken together).

Response variables included age, average time standing resting, walking, feeding, sternal recumbency, lateral recumbency, frequency of lying bouts, average ambient temperature and the average score for neuroticism taken from the two observers. Due to the small sample size and the lack of biological significance of other personality dimensions only neuroticism was included in the final analysis. Backward manual model selection where predictors were removed based on their significance and Akaike Information Criterion (AIC) was computed at each step to select the most robust model.

Cluster analysis, an unsupervised learning algorithm, was carried out to determine if there was potential underlying patterns or intrinsic grouping of individuals that share features of their sleep strategy. The analysis was carried out on frequency of lying bouts and SD of total lying time to capture within-individual variability in lying behaviour. Three different clustering techniques were used for comparison all using the mclust package in r (Scrucca et al., 2016):

1. K-mean clustering algorithm groups data by identifying a centroid (initially at random) and assigns data points with the nearest mean to that cluster.
2. Hierarchical clustering using complete linkage method initially treats each observation as a distinct cluster before repeatedly merging the 2 most similar clusters creating a dendrogram.

3. Model based clustering uses an algorithm that assumes there is a finite mix of underlying probability distributions, with each cluster corresponding to a different distribution.

3.3 Results

Over the nights recorded, all horses lay down between 0 and 6 times per night with a mean ± SD of 3.09 ± 1.05. Out of the 14 horses recorded, seven had three or more rest bouts per night. Three individuals had at least one night where they did not spend any time in recumbent rest at all (for summary of rest bouts per night see appendix III). Most horses showed a preference for either the left or right side; six horses had a left side bias including one horse than spent 100% of recumbency episodes on their left side, six had a right side bias, and two horses showed no side bias (Fig. 3).

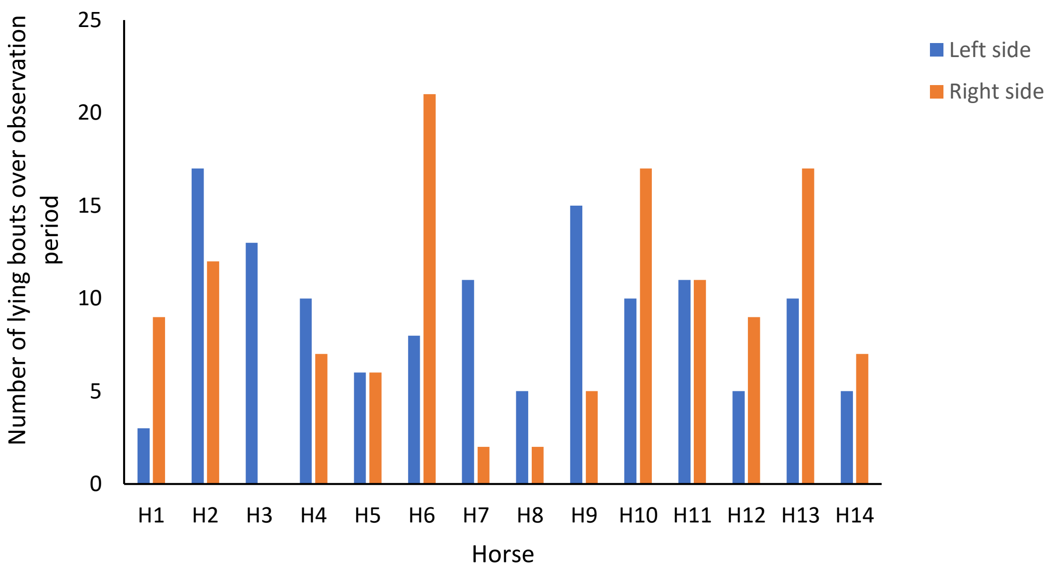


Figure 3.3: Frequency of recumbency bouts on the left versus right side for each horse (n=14) over all nights recorded.

When averaged over all nights recorded, these horses spent 146.5 ± 38.94 minutes lying down per night with 99.35 ± 27.75 minutes sternally recumbent and 47.2 ± 23.7 minutes in lateral recumbency. While recumbent, horses spent on average 55.09% ± 23.17 on their left side and 44.92% ± 23.17 on their right side. Horses spent on average 44.10 ± 26.39 minutes in a single rest bout (from getting down until getting up, may include both sternal and lateral recumbency). All lying bouts occurred between 7:20pm and 6:30am but most frequently between 10pm and 5am. When all horses are grouped the standard deviation (SD) of lying time over the recording period was 17.81 minutes. However, both H01 and H05 showed SDs of over an hour at 81.25 and 68.26 minutes respectively (Fig. 4). This individual variation is better represented by the coefficient of variation (CV = SD/mean*100). The CV of total lying time for all horses was 35.34. H3 had the lowest CV of 11.81 and H8 had the highest CV of 81.81.

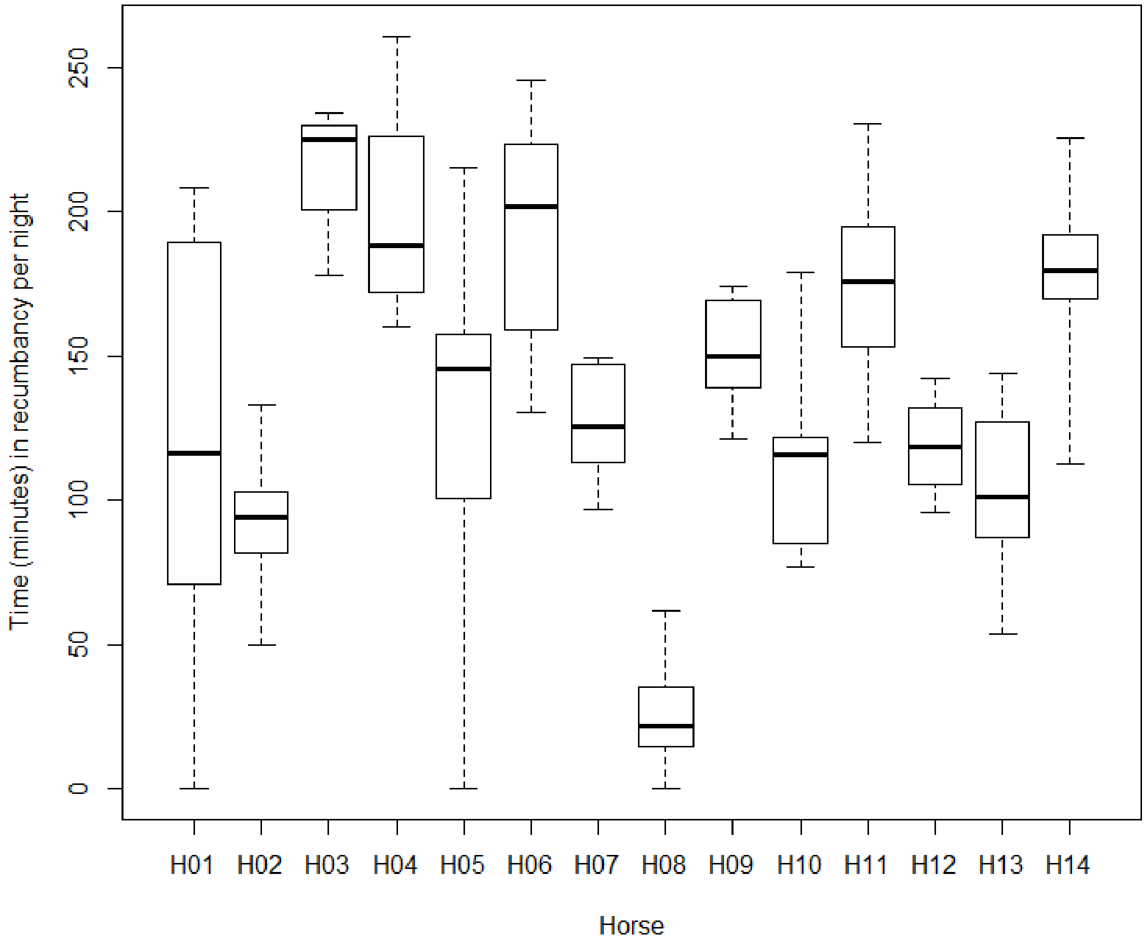


Figure 3.4: Time (minutes) spent by horses (n=14) in recumbency per night over all nights recorded.

Generalised Linear Mixed Model (GLMM)

Of the variables analysed, the best fitting model showed a statistically important negative association between lateral recumbency and time spent feeding (Table 3 & Fig 5).

Table 3.3: Posterior mean estimates of time spent lying down with head unsupported in horses (n=14) modelled using a Gaussian GLMM with temporal dependency with individual horse included as a random term. CrI is the 95% Bayesian credible interval. Credible intervals that do not contain zero indicate statistical importance.

Model parameter	Posterior mean	Lower CrI	Upper CrI
Intercept	28.87	11.52	14.20
Time feeding	-0.09	-0.15	-0.04

3.4 Discussion

The high variation observed in horse rest patterns comes from both within- and between-individual variation, although some individuals show more within-individual variation than others. On average the horses in this study spent more time lying down than has been found in other studies of partly or fully stabled horses, with lying accounting for 9.5% of their time budget compared with an average of 4% (Chaplin and Gretgrix, 2010; Chung et al., 2018; Pedersen et al., 2004). Dallaire, (1986) reported stabled horses resting for significantly longer than pasture counterparts with rest accounting for 21% and 13% respectively, although no other behaviours or the welfare status of individuals in the two groups were reported, so it is not possible to determine what factors may have accounted for this difference. This paper is highly cited when referring to normal rest patterns in horses, that is between 3-5 hours rest per night. However, we must be cautious when determining what ‘normal’ rest patterns are (Dallaire, 1986). It is possible that both a lack of, and/or an excess of lying behaviour could indicate a welfare issue.

Most horses showed a preference for lying on one side or the other but only one horse spent 100% of the observation period recumbent on one side, and this was believed to be due to an old injury (Appendix II). Preference for lying on one side could be related to motor laterality producing asymmetries. Both laterality and lameness manifest as asymmetries in motor function. If extreme lying side preference could be linked to either laterality or lameness then it may act as a useful tool for diagnosis and/or rehabilitation. As the goal in equestrian sport is for the horse to perform symmetrically (Byström et al., 2020), further work could investigate if exercises designed to improve symmetry in the horse result in a change in lying side preference in those that show a strong side bias.

In the current study, we found that on a nightly basis, horse rest patterns were influenced by how much time they spent feeding. This is likely to represent the time it took for the horse to eat rather than the amount the horse ate as all horses had finished their designated forage feed by the morning. If an individual spends more time feeding, then they are likely to spend less time resting.

Individuals that showed high levels of variation in rest pattern had significantly higher scores for neuroticism. This demonstrates that, at least in this group of horses, high variability in rest pattern is associated with neuroticism and feeding behaviour.

Average ambient temperature and average intensity of exercise showed positive associations with variability of rest pattern. Although these associations were weak they warrant further investigation as both factors are known to impact rest in humans (Carskadon and Dement, 2005; Kredlow et al., 2015).

The findings of this study suggest that, at least in this group of horses, neuroticism is associated with overnight resting patterns. There is evidence in the human literature that high neuroticism is associated with poor sleep quality (Allen et al., 2016; Stephan et al., 2018). Neuroticism is associated with increased sensitivity to stress (Friedman and Kern,

2014) and negative affective state (Stephan et al., 2018). Stress and sleep are inextricably linked, however, the relationship is not straight forward. Allen et al. (2016) found that neuroticism has a curvilinear relationship with sleep duration; that is, people that had higher levels of neuroticism tended to report both longer and shorter sleep duration. This highlights the importance of using the correct metric when measuring a behaviour. Chaplin and Gretgrix (2010) examined activity and lying behaviour in horses in different housing treatments and reported high levels of individual differences with some individual horses not lying down at all during the observational period. However, the authors reported no significant difference between treatments in total lying time, frequency of lying bouts, minimum and maximum number lying bouts. It may be that variability in rest pattern over time would more accurately assess individual differences between treatments.

Type of feed and time spent feeding are significant welfare factors for horses. Free ranging horses will spend 12.5 ± 2.5 hours feeding or foraging for food each day (Ellis et al., 2015). Stabled horses are often not provided with enough forage to maintain chewing for this length of time, a factor which likely plays a role in the development of stereotypies and the prevalence of stomach ulcers in stabled populations of horses (Nicol et al., 2002). Horses in this study were fed 2% bodyweight daily in forage and spent an average of almost 4 hours feeding during the observation period. Additionally, horses were bedded on straw which would have provided additional foraging opportunities.

Variability of rest time showed a weakly positive relationship with exercise intensity where higher mean intensity was associated with increased variability. Caanitz et al. (1991), found horses spent more time lying down when exercised compared to horses who were not exercised, this is likely related to the role sleep plays in physical and mental restoration (Caanitz et al., 1991; Halson, 2014b). However, sleep can influence exercise, the mood modulating effect sleep has on the brain can lead to a change in performance in human athletes (Arfuso et al., 2021), but exercise has only a mild to moderate effect on sleep (Kredlow et al., 2015). In this study, an effect may be more noticeable where exercise intensity is increased as most horses were only experiencing mild to moderate exercise. A larger sample size with a uniform and more intense exercise pattern would be needed to determine if the results seen here are supported.

Variation of rest time was also weakly related to ambient temperature where variability increased as temperature increased. As many domestic horses are rugged overnight in the stable it may be that on warmer nights horses rest patterns are being disturbed by overheating (Brown and Twigg-Flesner, 2018). Future research using temperature gauges under the rug to measure surface body temperature would be useful to further examine this relationship.

This study was initially designed to capture fine scale behavioural measures such as facial expression and eye movements, however as the study progressed it became clear that fine scale movements were too difficult to detect using the CCTV recording method used here. Rest behaviour patterns that are easier to detect such as time in lateral vs. sternal recumbancy, frequency of bouts and, importantly, variability over multiple nights were not only easier to detect but are yet to be thoroughly examined in the literature. Where multiple nights are recorded, most research on horse sleep averages values across several nights. This aggregation of data may result in loss of important information (Chung et al., 2018a; Greening et al., 2013; Ninomiya et al., 2008). Variations in nightly patterns of individual lying behaviour are easy to detect in contrast to fine scale behavioural measures and may be a good proxy measure for physical or psychological distress.

When trying to interpret the meaning behind patterns of behaviour, such as variability in rest behaviour, it is useful to look for correlations in both physiological measures and behaviour measures or scoring techniques (Pierard et al., 2015). No physiological measures were taken to determine the exact cause of differences in rest behaviour in this study. However, these results are supported by findings in the human literature where high levels of neuroticism results in disturbed rest patterns, this is believed to be mediated by the tendency for neurotic individuals to experience a higher rate of distress and negative emotions (Allen et al., 2016a; Montoliu et al., 2022; Stephan et al., 2018). Future work could include the addition of physiological measures of emotional response to look for trends that would suggest an underlying emotional state. However, most accepted measures of a physiological response to a stressful situation are measuring HPA axis activation through, for example, heart rate variability, eye temperature, and hormonal responses in the form of salivary or faecal cortisol metabolites (Pierard et al., 2015). These measures will lessen over time, even if the triggering stimulus is maintained, making them poor measures for chronic stress (Mormède et al., 2007). Sleep disturbance is likely caused by chronic stressors, for which there are less reliable physiological measures. However, Meerlo et al. (2002) suggested sleep deprivation appears to result in a special kind of physiological response low level but constant HPA axis activation that is resistant to blunting. If this is the case then validating rest patterns against a long term measure of HPA activation, such as hair cortisol (Russell et al., 2012), could provide further support for the link between rest patterns and underlying emotional state.

Additionally, 4-7 nights recording might not be long enough to capture inter-individual patterns; longer period of recording might show the “sleep types” mentioned in Wöhr et al., (2016). The results of the cluster analyses showed that in two out of the three analyses, three groupings of individuals could be determined in this data set. It must be stressed that this type of analysis is designed to be carried out on much larger sample sizes and therefore caution must be exercised when making generalisations from these results alone. However, it may be that the group with low lying frequency and high variation in rest represents horses that are experiencing some type of psychological stress resulting in continual sleep loss and rebound over consecutive nights. Homeostatic sleep rebound is where the loss of sleep, particularly REM sleep, results in a rebound period where sleep increases in subsequent nights (Bertone et al., 2015; Carskadon and Dement, 2005). Sleep rebound has been recorded in cows where 24 hours of sleep loss took 2-4 days of increased sleep to return to normal sleep patterns (Kull et al., 2019). In humans, variation in sleep pattern has been linked to repeated sleep rebound effect as a result of negative affective state (Mezick et al., 2009). This highlights the importance of avoiding averages of sleep time over multiple nights, as important patterns might be lost.

Horses displaying low frequency and low variability do not appear to be experiencing sleep rebound, although it may be that the rebound was not captured during the observation period. Oliveira et al. (2022) found horses with advanced osteoarthritis decreased their frequency and time in recumbency, believed to be caused by pain during joint flexion required during recumbency. While some level of compensation for lack of REM sleep can be achieved by increased NREM sleep, this has limits and eventually REM sleep deprivation will occur (Burla et al., 2017).

Finally, high frequency and low variability may represent a normal sleep pattern where horses are not experiencing any physical or psychological barriers to lying rest. It would be of value to repeat this analysis on a much larger sample size to ascertain if this finding is repeatable.

Due to the sheer number of factors that influence sleep patterns in the human population, little attention has been paid to between or with-individual variation of sleep. However, some self-reporting studies have looked at sleep variation in the human population and found differences between male and female subjects, where variation in sleep patterns is greater in females than males, independent of average total sleep time (Russell et al., 2012). There are also differences in race which are likely driven by socio-economic background and associated stressors (Dillon et al., 2015). This suggests that “sleep types” are not driven by genetic factors alone but instead deviations from what is considered normal rest patterns represent different sleep strategies in response to psychological or physical discomfort. If sleep type is driven by stressful life events and negative affect, it may be useful in identifying vulnerable groups in horses as it is with humans.

3.4.1 Conclusion

Rest accounts for a small but essential proportion of most horses’ time budgets and is likely to play an important role in an individual’s wellbeing. For this reason, it is important to understand what drives variation in individual rest patterns and in doing so further understand the potential to use rest as a measure of welfare in horses.

This is the first study examining external and internal factors that influence inter and intra-individual variation in rest pattern over multiple nights. Individual variation in rest patterns over multiple nights in individual horses are related to differences in personality, namely different levels of neuroticism. Whether this difference is explained by differing sensitivity to stress associated with this personality dimension and resulting affective state is yet to be demonstrated, but human literature suggests this may be the case. Neurotic individuals may act as a type of ‘canary in the coal mine’ when assessing the suitability of various management systems. Furthermore, timing and type of feed is likely to play a role in the time budget allowances for rest and should be taken into careful consideration for any research looking into rest behaviour in horses. Although level of exercise and ambient temperature had only a weakly positive relationship on the rest patterns of horses in this study, further investigation into these factors is needed to determine the influence they may have on rest patterns. Although no distinct types of sleep patterns were identified in this study, possible analyses to identify sleep types were explored and predictions of the features of those sleep types were suggested as part of a preliminary cluster analysis.

Further work is required to identify if individual sleep patterns are consistent over time or vary according to the external factors. This study represents an important first step to understanding what internal and external factors could potentially impact sleep patterns in horses.

Chapter 4: Long term rest patterns and the relation to hair cortisol measures and social facilitation of lying behaviour in stables

4.1 Introduction

The link between sleep and stress has been well documented (Auer et al., 2021; Balbo et al., 2010; Davenport et al., 2008; Meerlo et al., 2002; von Borell et al., 2007). The Hypothalamic-pituitary-adrenal (HPA) axis plays an important role in homeostatic metabolic processes such as the circadian rhythm, and is a major mediator of the neuroendocrine stress response by regulating circulating levels of glucocorticoids (Balbo et al., 2010; Bartolomé and Cockram, 2016; Mormède et al., 2007; Pierard et al., 2015). The most common glucocorticoid in both humans and horses is cortisol which is commonly used as a biomarker for stress (Pierard et al., 2015; Zhang et al., 2020). There is a clear bidirectional relationship between activation of the HPA axis and sleep disruption (Lo Martire et al., 2020; Meerlo et al., 2008; Minkel et al., 2012). High cortisol levels have been linked with several aspects of sleep disorders either caused by health conditions or excessive stress (El Mlili et al., 2021). When sleep deprivation acts as a stressor, a slow and gradual increase in glucocorticoid levels occurs. This is in contrast to an acute stressor which results in a sharp increase in glucocorticoid levels (Meerlo et al., 2002). Repeated or chronic exposure to a stressor will often result in a diminished HPA axis response, however, sleep deprivation results in low level but constant HPA axis activation with no signs of HPA axis habituation (Meerlo et al., 2002). Traditionally, faecal, salivary and plasma cortisol have been used to measure elevated cortisol levels as an indication of HPA axis activation in horses (Pierard et al., 2015). These measures are also more sensitive to factors that are not related to stress such as circadian rhythm. These measures of cortisol may be useful for acute stress situations resulting in transient peaks in cortisol levels but may not be practical for measuring chronic HPA axis activity (El Mlili et al., 2021; Pierard et al., 2015; Russell et al., 2012). Hair cortisol is emerging as a promising biomarker to measure chronic HPA axis activation, and may provide a suitable measure of the slow, gradual increase of cortisol that is associated with sleep disruption (El Mlili et al., 2021; Meerlo et al., 2008; Zhang et al., 2020).

Stress is a general term used to describe multiple responses an animal may exhibit in reaction to their environment. Stress can be categorised as either eustress where the affective state of the animal is generally positive or distress where the affective state is negative and has a negative effect on the body (Ghassemi Nejad et al., 2022). Stimuli that are perceived as a threat can result in altered homeostasis and therefore, stress is the body's response to maintain homeostasis (Bartolomé and Cockram, 2016; Mormède et al., 2007). When the body perceives a threat, the HPA axis is triggered resulting in a cascade of hormone release, beginning in the hypothalamus with the release of corticortrophin-releasing hormone (CRH) which triggers the anterior pituitary gland to release adrenocorticotropin hormone (ACTH) resulting in the release of cortisol from the adrenal cortex (Elder et al., 2014). This is a normal, healthy, response that has evolved to allow individuals to respond to natural stressors that an animal may encounter in their life (Bartolomé and Cockram, 2016; Lo Martire et al., 2020). Cortisol plays an important positive role in normal biological functioning of the horse including increasing movement, reducing inflammation and raising blood glucose concentrations (Bartolomé and Cockram, 2016). However, problems arise when the animal is exposed to a stressor for extended periods. Most stressors encountered in a natural setting are acute in nature, whilst prolonged stress exposure results in over activation of the HPA axis which can have negative health consequences such as suppression of the immune system (Bartolomé and Cockram, 2016; Mormède et al., 2007).

One of the functions of the HPA axis is its involvement in the circadian rhythm and release of cortisol follows a rhythmic 24 hour cycle (Balbo et al., 2010; Meerlo et al., 2008). All animals are subject to physiological, biological and behavioural cycles initiated by environmental cues such as light availability and internal systems controlled by the clock gene (Murphy, 2019). This provides an adaptive function to increase chances of survival when faced with fluctuations in food availability, reproductive success and risk of predation (Lo Martire et al., 2020). Many of the physical structures that play a central role in the circadian rhythm also play a role in stress responses. Therefore, even minor disruptions to the sleep-wake rhythm could result in an activation of the stress response (Lo Martire et al., 2020). There are some reports that horses lack a diurnal rhythm of plasma cortisol, but this is likely due to confounding factors in the studies that report this, such an underlying stressful situation or variations in feeding times (Munk et al., 2017). In horses, if there are no underlying stressors and metabolic requirements are maintained then cortisol concentrations should gradually reduce throughout the afternoon, reaching their lowest level in the middle of the night before steadily rising again throughout the early morning and peaking around midmorning before repeating the cycle (Bohák et al., 2013). Although not yet fully understood, it is clear that glucocorticoids such as cortisol play an important role in the sleep wake cycle of both horses (Bohák et al., 2013) and humans (Banks et al., 2005; Brown, 2012; Elder et al., 2014; Lo Martire et al., 2020).

When examining the effects of chronic stress, hair cortisol has the advantage of representing the cumulative effects of long term cortisol exposure, smoothing out daily fluctuations due to circadian rhythm or effects of short term, acute stressors (Jolivald et al., 2023; Zhang et al., 2020). Additionally, hair sampling does not involve aversive procedures and therefore, should not trigger a stress response during sample collection, or if it does, it will not affect cortisol levels in the hair due to the time lag for hair cortisol build up. Hair samples can be collected retrospectively of a potentially stressful event occurring and are easily stored and transported (Russell et al., 2012). However, while hair cortisol maintains many advantages over other methods of cortisol extraction when measuring chronic stress, it must be remembered that the role of cortisol in the body is not straightforward. Cortisol can be influenced by a number of internal and external factors and does not distinguish between eustress and distress. For this reason, results should be taken in context with other behavioural or biological markers (Jolivald et al., 2023).

Results from Chapter 3 suggested that horses may display different “sleep types”, in terms of total sleep time (TST) and sleep time variability over multiple nights. However, observations were only available for a week or less, so long term patterns may have been missed. Longer term observations are needed to determine if these patterns are consistent long term. While continuous behavioural observations produce the most complete data when examining animal behaviour, it requires a large amount of time and labour which can be prohibitive when investigating long term behavioural patterns. Scan sampling provides a viable alternative to continuous behavioural observations, allowing the observer to accurately estimate time budgets of the animal in question over long time periods with comparatively little time and labour input (Ross et al., 2019). The success of this method is likely dependent on the scale of the behaviour being measured; short, infrequent behaviours are likely to be missed when compared to frequent behaviours that occur over a greater time scale (Ross et al., 2019). Sleep and other night-time essential function behaviours such as feeding represent behaviours happening over a long time period and therefore scan sampling is likely an appropriate method of recording these behaviours (Daigle and Siegford, 2014; Pullin et al., 2017).

Synchrony of behaviour states in a group of animals has been proposed as a measure of welfare in a number of species including cows (Keeling et al., 2021), sheep (Muhammad et al., 2022) and chickens (Asher and Collins, 2012). Asynchrony within a group increases the likelihood that a group will break up, as individuals that are active while others are resting are likely to move to a new location, leaving resting group members behind. Group living and behavioural synchronisation have a number of adaptive advantages in the wild, in particular reducing predation pressure, which has resulted in group living and synchronous behaviour being widespread throughout the animal kingdom (Duranton and Gaunet, 2016). Horses show synchrony of lying behaviour when kept in group housing (Hartmann et al., 2012) and at pasture (Martin et al., 2010) but little research has examined the synchronisation of behaviour in stable kept horses. Martin et al. (2010) reported that synchrony of behaviour of six mares kept at pasture appeared to diminish when they were moved to stables although hormonal rhythms throughout the day remained at a lower intensity.

The aim of the present study was to determine if there were any long term patterns that could be seen in horse rest behaviour and if it was possible to identify different sleep types; if so, whether these strategies could be linked to differences in hair cortisol measurements; and finally, to determine if horses in individual stables exhibited synchrony in their rest behaviour, as has been reported in group housed and pasture kept horses.

The research objectives were to:

i) Determine if there are patterns in horse rest over consecutive nights for a month and if these patterns can be linked to other behaviours within an individual.

ii) Examine if there is a link between any features of long term rest patterns and the hair cortisol within an individual.

iii) Investigate if horses housed in individual stables show signs of synchrony of rest behaviour as has been reported in group and pasture kept horses.

H_O: There are no features of long-term rest behaviour that are correlated with hair cortisol levels in horses.

H_a: There is a correlation between features of long tern rest patterns and cortisol levels.

4.2 Methods

This was an opportunistic study undertaken due to the Covid-19 pandemic and subsequent lockdown. Details of work interrupted as a result of the Covid-19 lockdown can be found in Appendix IX.

4.2.1 Ethical statement

This study does not contravene any UK legislation and was granted ethical approval by the School of Animal, Rural and Environmental Sciences at Nottingham Trent University animal ethics procedure (Considered Level 1 and submitted to appropriate repository). Institutional and national guidelines for the care and use of animals were followed at all times.

4.2.2 Animals and facilities

Riding school horses (n = 11) from the Nottingham Trent University (NTU) Brackenhurst Equestrian Centre were used in this study. These consisted of four mares and seven geldings with a mean height 162.63 ± 6.22 cm and mean age of 15.29 ± 4.01 years, six of these horses participated in the study from Chapter 3 and are identified in Appendix V. Horse 11 was used for the synchrony study but not the hair cortisol study due to a hogged mane making mane hair sample collection impossible. Horses were fed, housed and exercised in the manner outlined in Chapter 3, that is, they were fed 2% ideal body weight in hay twice a day 7:00am and 5:00pm (0.5% in the morning and 1.5% in the afternoon), adjusted for weight when required (underweight horses fed slightly more and overweight horses slightly less). In addition, pasture cubes and Ossichaff© with supplements specific to individuals (Appendix V) were fed twice a day. Horses were housed individually in box stalls measuring 3.5m wide, 4.1m deep and 2.5m tall walls, bedding was chopped straw between 5-20cm deep on top of rubber mats. Stables opened onto a central corridor where they could see other horses. Additionally, stables had windows on all sides including "chat hatches" in adjoining walls which consisted of a barred window.

Horses were kept in stables from Monday to Friday and ridden by students for 1-2 hours per day in either jump/flat lessons or lunged/put on the walker if there were no lessons that day. On weekends horses were turned out in social groups of 2-3 individuals from 10am-3pm.

In the time period between data collection for Chapter 3 and Chapter 4 new, high-resolution video cameras (Darkfighter©, Hikvision Digital Technology Co., China) were installed in each of the stables (Figure 1). These cameras eliminated blind spots and removed the need for multiple cameras per stable as was seen in Chapter 3.

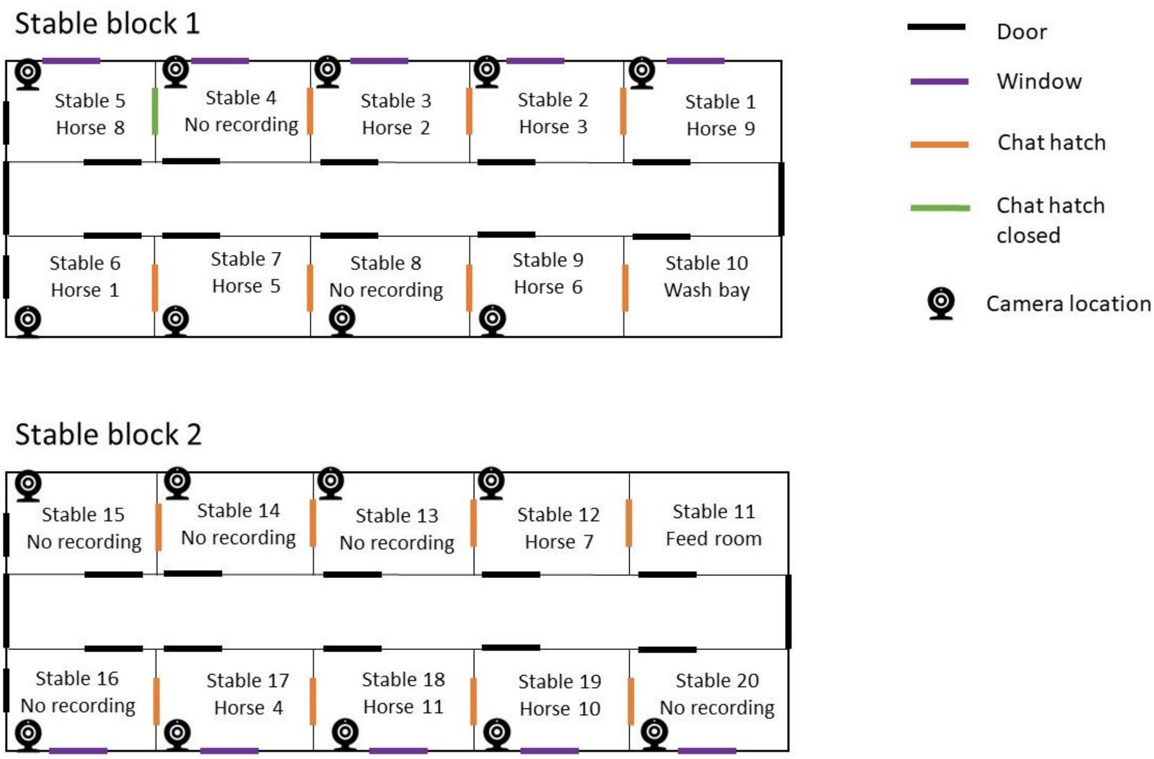


Figure 4.1: Stable block layout including stable number, horse location and camera positions for the single camera in each stable.

The cameras recorded all hours the horse was in the stable from 1st February to 28th February 2020. This resulted in three horses recorded for 28 nights, two horses recorded for 21 nights, four horses for 20 nights and one horse for 18 nights (see appendix VI). Video footage from 7pm to 7am was analysed and scored according to a behavioural ethogram (Table 1). A subsample of videos from nights 9 to 13 were used for the synchrony of behaviour analysis as this was a time period when all horses were in their stables at the same time.

Table 4.1: Behavioural ethogram for instantaneous scan samples (Adapted from Caanitz et al., 1991; Raabymagle and Ladewig, 2006)

Code	Behaviour	Description
0	Not visible	Horse removed from stable for either turn out or exercise (this includes preparation time when horse is being tacked or rugged up) OR horse is in stable but not able to see what they are doing i.e. head is over the door and unsure if standing rest or alert.
1	Standing alert	The horse is doing an activity, either, drinking, walking, defecate/urinate, looking around or stretching/scratching for more than 3 seconds. Eyes are fully open, head up and ears pricked or active.
2	Feeding	Muzzle either in feed bucket or within ~30 cm of hay pile, net or bedding, may lift head but showing visible chewing. This includes foraging in bedding as it can be difficult to distinguish between bedding and hay.
3	Standing not alert	The horse is not doing any of the above-mentioned activities, meaning they are standing still, either in a resting state or observing/staring at something for a prolonged period (>2mins) in a relaxed state. The horse may move their head slightly, stretch for a few seconds and slowly shift weight while in this state.
4	Sternally recumbent	Forelegs bent underneath the thorax, spinal column held in lateral arc and in some cases carried around the side of the body and lying with weight on side.

5	Laterally recumbent	Flat on side with all four legs extended. Neck, head and muzzle touching the ground, leg movements can also occur with distal limbs. Head and body may be against the stable wall propping them up but no muscle tone to support neck.
6	Rolling	From either a laterally or sternally recumbent position rolling onto side or back and possibly all the way over while rubbing head and neck on the ground.
7	Stereotypy	Repetitive, invariant behaviour with no obvious goal or purpose, including but not limited to windsucking, weaving, box walking, head-bobbing, pawing or self-mutilation.

4.2.3 Scan sampling of instantaneous behaviour validation

To determine how often scan sampling of instantaneous behaviours had to occur to accurately capture horse behaviour, samples of 12-hour videos from six stabled horses (three of which were subsequently used in this study) were randomly selected from the previous study in Chapter 3 which were continuously behaviourally scored using the above ethogram (Table 1). The proportion of each behaviour for each horse was calculated as a percentage of time observed performing that behaviour for the continuous observations, and the percentage of observations performing each behaviour for the 15 and 30 minute scan samples as per the method in Daigle and Siegford (2014). A Pearson's correlation showed R^2=0.913 and R^2=0.957 for 15- and 30-minute samples respectively. The results showed that 15 and 30-minute interval scan sampling showed a high correlation with continuous sampling observations. In fact, in this sample 30-minute intervals showed a higher level of correlation than 15 minutes intervals, therefore 30-minute intervals were used for behavioural data collection in this study.

4.2.4 Synchrony of lying behaviour protocol

Scan samples of lying behaviour were examined to identify when lying behaviour occurred at the same time as a horse on either side or directly across from the observed horse. Video were re-analysed to determine the exact time that bout of lying behaviour was initiated and this was compared to the neighbouring horse on each side and stable directly opposite. Lying bouts that occurred within 15 minutes of each other were highlighted as a potential synchronised bout and compared as a proportion to overall bouts.

4.2.5 Hair cortisol protocol

Mane hair samples were taken from the base of the neck of each horse on the 27th April 2020 by a member of the yard staff team. Jolivald et al. (2023) found that the location on the neck that samples are taken can have a confounding effect on cortisol results, therefore mane samples for this study were all taken from the same location at the base of the neck. Horses were caught in the field and restrained using a headcollar before the sample was taken. Once the horse was restrained a section of hair was tied with a small rubber band and cut with scissors from as close to the skin as possible. Staff were instructed to take a minimum of 10 strands of hair and in all cases took a sample of 2 or 3 times this amount. Once collected the samples were placed in envelopes with the horse's name and date before being stored at room temperature until laboratory analysis.

Sample preparation

Hair sample preparation and analysis was based on the hair cortisol extraction protocol outlined in Meyer et al. (2014). This protocol was carried out using human and monkey hair and contained the most detail about sample preparation and analysis. Minor changes were made to the protocol where equipment available in the laboratory was different to that used in the paper (see details below). The procedure was carried out by the author with assistance from another PhD student Aurelie Jolivald who were both trained in the procedure, with further assistance from staff members Dr Samantha Ward and Dr Sam White.

Sample fragmentation

Cortisol concentrations have been shown to decrease along the length of the hair shaft (Duran et al., 2017), so the 3 cm segment for analysis was taken from the area of the sample that had been nearest to the skin. Previous reports of hair growth rates were used to determine segment length based on previous work that estimates 0.56 cm/week growth, which would imply the sample contained approximately 5-6 weeks of growth (Schlupp et al., 2004). The samples were taken leaving approximately 0.5cm of hair on the horse and there would be an estimated further 0.5cm present under the skin before reaching the root. These uncollected areas need to be considered when calculating the timeline.

Sample washing and drying

To remove potential contaminates such as sweat, dirt and skin cells, samples were washed and dried. Washing was carried out by placing fragmented samples into 15 ml polypropylene centrifuge tubes with 5 ml of high-performance liquid chromatography (HPLC)-grade isopropanol (Fisher Scientific, Waltham, Massachusetts, US) and the tubes repeatedly inverted and rotated at a rate of 30 rpm for 3 minutes using a rotator. The isopropanol was then decanted into a waste container and the procedure repeated two more times to ensure contaminates were removed. The hair was then left in the fume cabinet for three days to ensure the isopropanol was fully evaporated and samples dry.

Sample grinding

A precision balance and tweezers were used to measure out 60 mg of representative hair from the original fragmented sample down to four decimal places and placed in 2 ml reinforced tubes with screw caps. Three 2.4 mm metal beads were added to each tube and loaded onto the finger plate of the bead mill. Samples were ground to a fine powder using a bead mill (Bead Mill 24, Fisher Scientific, Waltham, Massachusetts, USA). Grinding the samples well increases the chances of extracting enough cortisol for EIA analysis due to the increase in surface area (Burnett et al., 2014).

Grinding times and speeds were altered from the Meyer et al. (2014) protocol due to differences in bead mill performance. Bead mill time and speed were determined by previous studies (Jolivald, 2022) that showed samples processed for 10 minutes at 6m/s obtained satisfactory powdering.

Cortisol extraction

Methanol (Fisher Scientific, Waltham, Massachusetts, US) was used as a solvent to extract cortisol as it penetrates deep into the hair matrix maximising cortisol yield. For health and safety reasons, samples were processed in a fume hood where 1.5 ml of high-performance liquid chromatography grade methanol was pipetted into each sample tube. Samples were then transferred to a rotator and rotated at 30 rpm for 22 hours at room temperature. Sample tubes were then placed in a centrifuge (SLS Flowgen bioscience mini centrifuge, UK) for 10 minutes at 10,000rpm. Under the fume hood, 1.0 ml of supernatant was pipetted off the top of the sample, without disturbing the pelleted hair at the bottom, and transferred into 2ml Eppendorf tubes.

Solvent evaporation

The Eppendorf tubes were then placed uncapped in a block heater set at 40°C under the fume hood to evaporate the methanol solvent. A sample concentrator (Stuart SBH130D/3 and SBHCONC/1, Cole-Palmer, USA) was used to blow nitrogen gas (Fisher Scientific, Waltham, Massachusetts, US) over the open Eppendorf tubes to accelerate the evaporation process. Needles were set just through the neck of the sample tubes and did not disturb the methanol and cortisol solution. Samples were left for approximately two hours with regular visual checks until the tubes were fully dry.

Sample reconstitution

Meyer at al. (2014) recommend reconstituting sample cortisol extracts using an appropriate volume of EIA assay buffer (assay diluent) depending on anticipated cortisol values. As hair cortisol concentration is generally low, relatively low cortisol values were anticipated, therefore the volume used in this case was 0.2ml to increase sensitivity. A vortex mixer (Lab Dancer, IKA) was used to homogenise samples for 5 seconds before freezing the samples at -20°C.

Enzyme immunoassay

The reconstituted samples were assayed using a commercially available high-sensitivity enzyme immunoassay (EIA) kit (Expanding Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit, Salimetrics, USA). These kits were originally developed to assay salivary cortisol however, they have been validated for hair cortisol assays (Davenport et al., 2006) and have been frequently used for this purpose (Albar et al., 2013; Jolivald et al., 2023; Kroshko et al., 2017). In accordance with manufacturers guidelines the kits were stored in a fridge at 2-8°C upon receipt until 2 hours before the assay was performed. The reconstituted samples were assayed following the manufacturer's protocol (Salimetrics, 2019).

In preparation for the EIA assay the microtiter plate and reagents were bought to room temperature 1.5 hours before the procedure began and 2 hours before assay was performed. Buffer was prepared by diluting 5ml of Wash Buffer Concentrate ten-fold in room temperature deionized water. Plate layout was drawn using the suggested layout in the Salimetrics protocol including standards, controls, blanks, non-specific binding wells and samples which were run in duplicate (Figure 2). 25µL of each standard, control and sample were pipetted into appropriate wells. Pure assay diluent of 25µL was added to the Zero and Non-Selective Binding wells. The enzyme conjugate was then diluted by adding 15µL of enzyme conjugate to 24ml of tube Assay Diluent. The Conjugate tube was then centrifuged for 2 minutes at 500rpm to bring the liquid to the bottom. The diluted conjugate was then immediately mixed and 200µL added to each well using a multichannel pipette. The plate was then mixed on a plate rotator (PMS-1000i, Grant Instruments, UK) for 5 minutes at 500 rpm and left to incubate at room temperature for 1 hour. The content of the plate was then emptied over a sink and washed using the wash buffer four times by pipetting 300 µL of wash buffer into each well and then discarding the liquid into the sink. After each wash the plate was blotted with paper towel before turning upright. Then, 200µL of TMB (Tetramethylbenzidine) Substrate Solution was added to each well using a multichannel pipette and mixed on a plate rotator for 5 minutes at 500rpm. The plate was then incubated in the dark at room temperature for 25 minutes.

After incubation 50µL of stop solution was added to the wells with a multichannel pipette and mixed on a plate rotator for 3 minutes at 500rpm until all the wells had turned from green to yellow. The bottom of the plate was then wiped dry with a damp, lint-free cloth for the plate reader (Multiskan FC, Thermo Scientific, US) to read at an absorbance of 450nm. The raw absorbance results from the plate reader were then exported onto a PC for further analysis.

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	3.00 Std	3.00 Std	Ctrl-L	Ctrl-L	SMP-7	SMP-7	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
B	1.00 Std	1.00 Std	Ctrl-H	Ctrl-H	SMP-8	SMP-8	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
C	0.333 Std	0.333 Std	SMP-1	SMP-1	SMP-9	SMP-9	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
D	0.111 Std	0.111 Std	SMP-2	SMP-2	SMP-10	SMP-10	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
E	0.037 Std	0.037 Std	SMP-3	SMP-3	SMP-11	SMP-11	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
F	0.012 Std	0.012 Std	SMP-4	SMP-4	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
G	Zero	Zero	SMP-5	SMP-5	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
H	NSB	NSB	SMP-6	SMP-6	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY

Figure 4.2: Layout of 96 well microtiter plates used for cortisol EIA assays of prepared horse (n=11) hair with six standards (green), Zeros or blanks (blue), Non-Specific Binding wells (yellow) control low (light orange), Control high (dark orange), 11 samples in duplicate (grey) and 48 empty wells (white).

4.2.6 Data Analysis

Variation in hair cortisol was investigated using a General Linear Model (GLM) where the response variable was cortisol and explanatory variables were percentage of scan samples spent standing alert, standing resting, feeding, and lying down (sternal and lateral recumbency combined). Variation between individuals in age and height was accounted for by including them as fixed factors in the model. Due to the nature of time budget data (horses who spent more time lying in rest spent less time standing at rest and vice versa) the explanatory variables were collinear and therefore, standing rest and lying time were each removed and separate models were run for each variable. In model 1 lying time was included and in model 2 standing rest was included. After data collection had been completed, individual H10 was diagnosed with a metabolic disorder known to influence cortisol levels. This individual was identified as a statistically significant outlier (Cortisol = 5.26, Grubb's test: G = 2.280, U = 0.364, p = 0.028) and therefore removed from the data set. Additionally, H9 was identified as an outlier in time spent standing alert (Grubb's test: G = 2.585, U = 0.175, p = 0.001) and the analysis was run both with and without this individual with no difference to the model outcome. As this was a potentially biologically important outlier it was decided to retain this individual for the purpose of this chapter. The response variable of cortisol concentration was continuous and a Shapiro-Wilk test determined that this variable had a normal distribution (W = 0.854, P = 0.066) after the cortisol outlier from H10 was removed. R statistical program (R Core Team, 2020) was used to carry out a backward manual model selection to select the most robust model.

4.3 Results

Out of the nights recorded, horses spent an average of 39.27 ± 12.55% of interval observations in standing rest, 11.6 ± 5.81% of observations in sternal recumbency, 3.17 ± 3.44% of observations in lateral recumbency, 33.26 ± 8.46% of observations feeding and 7.48 ± 4.26% of observations standing alert (see Table 2 for breakdown by individual). Only one horse (H3) displayed stereotypical behaviour during the observation period and this horse spent 6.29% of observations engaged in windsucking. In total, these horses spent 53.81 ± 7.34% of the observations resting with 14.54 ± 7.90% in lying rest (either lateral or sternal recumbency). Most horses were recorded as lying down in sternal recumbency every night with the exception of H1 who was not observed lying down for 7/20 nights observed. The average hair cortisol result for this group of horses was 2.03 ± 1.42 pg/mg before the outlier was removed and 1.68 ± 0.91 pg/mg after the outlier was removed.

Table 4.2: Summary of cortisol measurements and percentage of time spent in each activity per horse over the observation period.

ID	Nights recorded (out of 28)	Cortisol (pg/mg)	Nights with no lying	% time resting (standing + recumbent)	% time standing rest	% time sternally recumbent	% time laterally recumbent	% time lying (sternal + lateral)	% time feeding	% time alert	% time performing stereotypical behaviours
H1	20	2.12	7	64.80	61.8	2.8	0.2	3	23.8	7.8	0
H2	21	0.82	0	62.48	53.52	7.05	1.9	8.95	24.38	7.43	0
H3	21	0.68	0	58.48	49.71	4	4.76	8.76	27.81	5.9	6.29
H4	28	2.04	0	51.43	39.57	11.14	0.71	11.86	36.71	6.71	0
H5	20	1.32	0	52.4	28.2	17.8	6.4	24.2	35.8	7	0
H6	28	0.94	0	59.14	43.43	15.29	0.43	15.71	29.43	5	0
H7	20	1.3	0	47	27	18.4	1.6	20	37.8	4.8	0
H8	28	2.38	0	45.57	32	10.14	3.43	13.57	44.43	9.14	0
H9	18	3.48	0	53.78	24.67	18	11.11	29.11	24.44	18.44	0
*H10	20	5.26	0	43	32.8	9	1.2	10.2	48	2.6	0

Table 4 1

***Outlier removed from analysis due to health condition resulting in elevated cortisol concentrations.**

Figure 3 shows percentage of time each horse spent engaged in different behaviours over the observation period. Horse 1 showed the least amount of time lying recumbent with 0.2% time in lateral recumbency and only 2.8% of the time in sternal recumbency. Conversely this individual spent more time in standing rest than any other horse (61.8%) and the least amount of time feeding (23.8%) than any other individual. Horse 1 was also observed to spontaneously collapse at one time point at 0300 on day 24 and was observed swaying at two other time points during the observation period (figure 4). Horse 9 spent more time standing alert than any other horse (18.44%) compared to the rest of the study sample average of 6.72%. This horse spent the second least amount of time feeding (24.44%) and the least amount of time in standing rest (24.67%), but the most amount of time in lateral recumbency, 11.11% and the second most amount of time in sternal recumbency (18%).

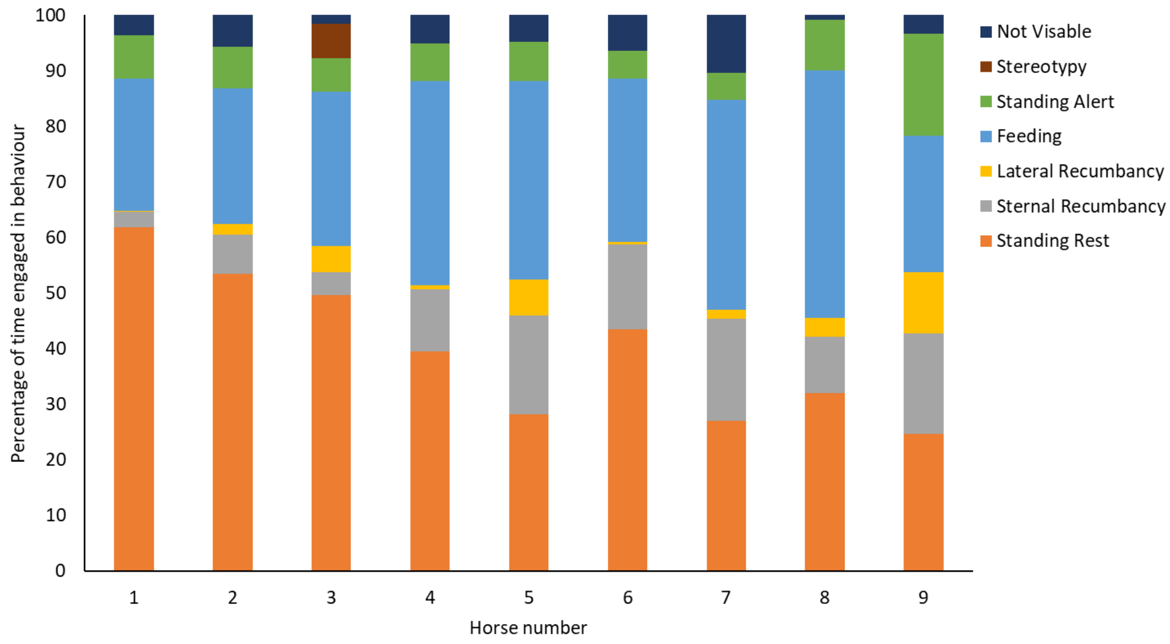


Figure 4.3: Percentage of observations each horse (n=9) spent engaged in each activity in the observation period (18-28 nights).

Figure 4.4: H1 spontaneous caudal collapse at 0300 observation point on day 24 of observation.

The GLM showed no significant relationship between hair cortisol and the behaviours standing alert, standing rest, total lying time or feeding and there was no significant effect of random factors Age and Height on hair cortisol (table 3 and figure 5).

Table 4.3: Model 1 (Standing rest omitted) - summary of GLM modelling hair cortisol concentrations in relation to behaviour with horse age and height as fixed factors.

	Estimate	Std. Error	t	p
(Intercept)	7.913	16.182	0.489	0.673
Standing alert	-23.438	38.446	-0.610	0.604
Lying	-4.892	15.333	-0.319	0.780
Feeding	0.123	0.740	0.167	0.883
Age	-0.027	0.173	-0.155	0.891
Height	-0.030	0.090	-0.329	0.773

Table 4.4: Model 2 (Lying time omitted)- summary of GLM modelling hair cortisol concentrations in relation to behaviour with horse age and height as fixed factors.

	Estimate	Std. Error	t	p
(Intercept)	11.241	16.936	0.664	0.575
Standing alert	-23.568	38.990	-0.604	0.607
Standing rest	2.764	8.963	0.308	0.787
Feeding	0.066	0.612	0.109	0.923
Age	-0.048	0.201	-0.237	0.835
Height	-0.057	0.111	-0.516	0.658

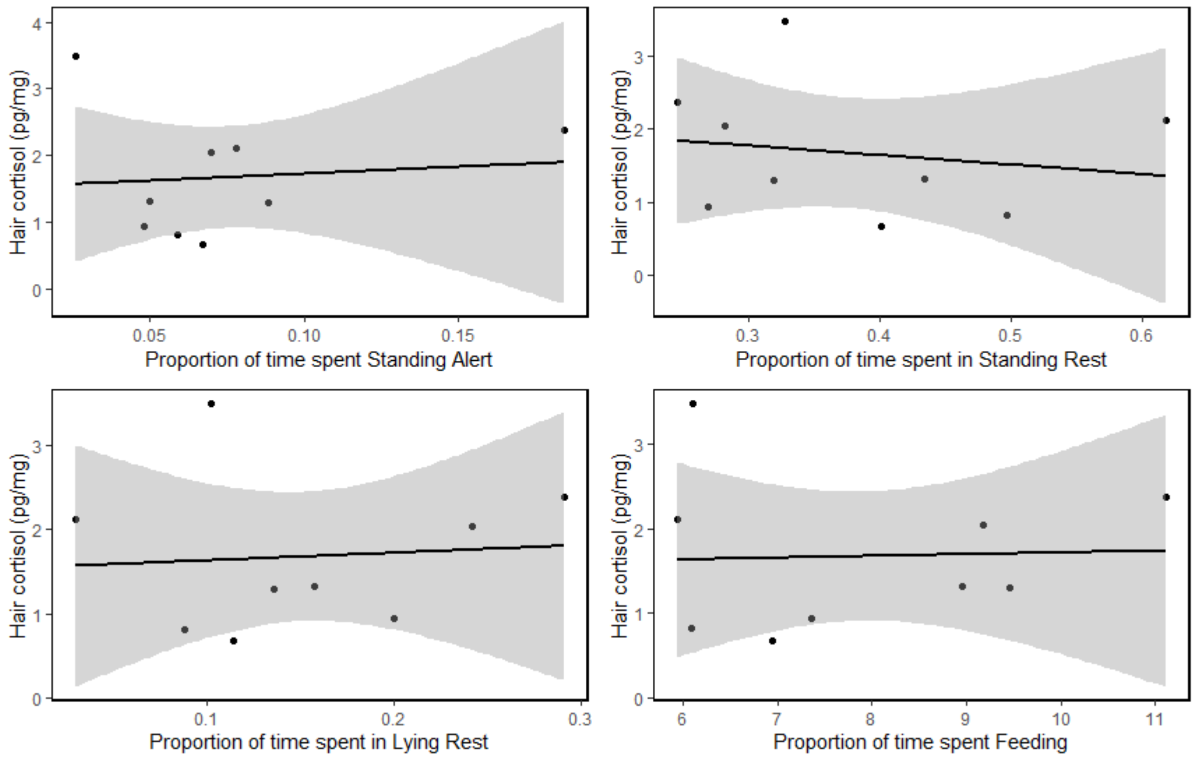


Figure 4.5: Estimated fitted means for hair cortisol concentrations against proportion of time standing alert, standing resting, lying rest and feeding with 95% confidence intervals.

Horses showed some synchrony of lying behaviour, with immediate neighbours to the left or right or directly across lying down at the same time in 29 out of 136 lying bouts over a five night period. Lying bouts occurred within 15 minutes of each other in 19 of these cases (Appendix VII)

4.4 Discussion

This study investigated the relationship between behavioural measures of rest in horses and long-term physiological measures of stress, in this case, hair cortisol concentrations. We found no significant relationship between hair cortisol concentrations and night-time behaviour of horses. Additionally, there does not seem to be strong support for the presence of synchronous behaviour in individually stabled horses, in agreement of the findings of Martin et al. (2010). Horses in this study only showed synchronous lying behaviour with an immediate neighbour to the left right or across from their stall in 21% of the lying bouts and only 13% of lying bouts occurred within 15 minutes of each other.

Average lying times for horses in this study were 14.5 ± 7.9% which, if using feral horse lying times as a benchmark, fall within the upper range of what would be considered normal at between 2.7-15.5% over a 24 hour period (Auer et al., 2021). Although this study only covers the time period between 7pm and 7am, previous work on this particular group of horses established that they rarely, if ever, adopt a recumbent position during the daytime (Chapter 3). This is not the case for any other behaviour recorded in this study, including standing rest, as these frequently occur during the daytime period.

There was no significant relationship between age and hair cortisol, which is consistent with the findings of other studies on adult horses (Jolivald et al., 2023; Sauveroche et al., 2020) and strengthens the argument that age may not need to be controlled for in future hair cortisol investigations (Jolivald et al., 2023). Similarly, no significant relationship between horse height and cortisol was found. Chung et al. (2018) found horses in smaller stables (3.6 x 3.6m) spent significantly less time in recumbency compared to horses in larger stables, likely due to the lack of space for the horse to feel comfortable lying down. While it is likely large horses would be reluctant to lie down in a stable they did not perceive as large enough, that does not appear to be a factor in this case.

Although this study was observational and did not induce any form of stress, the spontaneous collapse of H1 suggests this horse entered REM sleep while standing resulting in muscle atonia causing the collapse (Williams et al., 2008b; Wöhr et al., 2016). A full veterinary check-up was unable to find any physical reason for collapse and the horse appeared to stop collapsing when turned out shortly after this study. This, in conjunction with 7 out 20 nights observed where no recumbent behaviour was recorded, suggests this horse was suffering from sleep deprivation (Fuchs et al., 2016; Wöhr et al., 2016). Interestingly, this horse did not appear to show altered cortisol levels with 2.12 pg/mg, only slightly above the average for this group of horses which was 1.68 ± 0.91 pg/mg and the

4th highest in the group after the outlier was removed. This individual did show increased levels of standing rest which could be an indication of NREM sleep compensation for loss of REM sleep (Burla et al., 2017). The extent to which standing NREM sleep can act as a compensatory mechanism for reduced REM is not clear (Greening and McBride, 2022). However, as this individual was still achieving recumbency most nights, this may allow the individual to cope with reduced recumbency and minimise HPA axis activation.

Another explanation as to why this particular horse showed no signs of disrupted cortisol measures could be the difficulty associated with interpreting cortisol results. While many studies have reported a mild increase in cortisol in relation to sleep deprivation some have shown no change or even reductions in cortisol (Meerlo et al., 2008). Interpretation of cortisol results as a measure of psychological health can be problematic as it is involved in a number of bodily processes and can be subject to blunting over time (Steiger, 2002). The type and duration of the stressor can have a significantly impact both the sleep-wake cycle and the HPA axis activation (Lo Martire et al., 2020). Additionally, the individual differences in sleep requirement is genetically controlled (Banks et al., 2005) with high inter-individual variation (Mezick et al., 2009) which may mean this individual simply required less sleep that conspecifics. However, this is unlikely due to the spontaneous collapse demonstrated in this case (Wöhr et al., 2016).

Horse 9 showed the highest cortisol level (3.48 pg/mg) in this group of horses. This horse also spent the most amount of time standing alert (18.33%), the most amount of time laterally recumbent (11.11%), and the least amount of time in standing rest (24.67%). Activation of the HPA axis occurs to maintain metabolic homeostasis in response to an energy flux. This result supports that finding as horse 9 appeared to be a particularly active individual with the most time alert, which encompassed most waking behaviours apart from feeding and rolling. The increased time this horse spent in lateral recumbency may indicate a need for increased recovery time as a result of increased activity. While the HPA axis is often utilised to cope with a stressor, psychological distress is not always a factor and cortisol levels can be increased as a result of arousal irrespective of affective state (Hall et al., 2018; Mormède et al., 2007). Therefore, it is difficult to determine if the higher cortisol for horse 9 was caused by distress resulting in higher activity levels or if higher activity levels caused higher cortisol concentrations – thus providing a good example of the difficulties of interpreting cortisol as a measure of stress. No significant relationship was found between time standing alert and cortisol level in the present study, however further work utilising a bigger sample size is required to investigate this further. Chaplin and Gretgrix, (2010) found level of activity to be a more sensitive measure of stress when compared to lying behaviour of horses introduced to a new environment. They suggest that total sleep time may not be an accurate measure of welfare in horses the way it is with other large mammals such as cows (Rushen et al., 2012), due to the large inter-individual differences (Chaplin and Gretgrix, 2010). The results found here also point to large inter-individual differences in both time spent resting and cortisol concentrations. Both cortisol and rest are highly sensitive to a number of external and internal processes (Chaplin and Gretgrix, 2010; Ghassemi Nejad et al., 2022). Selective breeding for different roles has led to high variability in how individual horses respond to stress (Ijichi et al., 2013b; Lloyd et al., 2008). Additionally huge variation in how horses are handled and managed throughout their life is likely to play a role cortisol reactivity (Sauer et al., 2019).

Lying behaviour has always been assumed to be a good indicator of stress in horses, because as a prey species the adoption of a recumbent position makes them vulnerable to predation, so only adopting this position when the horse feels safe makes sense (Lima et al., 2005; Littlejohn and Munro, 1972). However, it is becoming increasingly clear that the relationship between sleep and stress is far more complex than originally assumed. Some studies have found an increase in lying behaviour in response to a stressful event in young horses (Heleski et al., 2002; Visser et al., 2008) and some health conditions (Oliveira et al., 2022). In humans, the type of stressor and length of time the subject is exposed to it, in addition to the individual's capacity to cope with stressful situations, all influence the effect stress has on the sleep-wake cycle (Lo Martire et al., 2020). Studies in rodents have shown that the type of stressor can dictate the effect on the sleep cycle. Exposure to chronic stressors did not result in disrupted sleep for the entire time the animal was exposed. Immobilisation was the only stressor to show continued impacts on sleep patterns with decreased sleep over the full four days of the study (Papale et al., 2005). Forced swimming altered sleep patterns for the first two days (Papale et al., 2005) and footshock only impacted sleep patterns for the first day of the four days exposed (Jean Kant et al., 1995). This is likely due to the differences in predictability and controllability of each stressor (Lo Martire et al., 2020). In humans, abnormally high levels of REM sleep are associated with stress and depression (Cai, 2016; Greening and McBride, 2022).

4.4.1 Conclusion

In this study, we did not find any significant relationship between resting in horses and hair cortisol levels, and these results suggest that there may not be a simple cause and effect relationship between hair cortisol and total sleep time. However, this study does highlight the importance of long-term monitoring of sleep patterns as the identification of a collapse episode in one horse was only captured on night 24 of the observation period. This individual lay down in the majority of the nights observed and only failed to lie down in 7 out of 20 nights which could easily have been missed if fewer nights had been observed. This supports the suggestion that normal, healthy horses should lie down every night as this was the only individual to have nights where no recumbency occurred. There are a number of limitations to the use of hair cortisol as a measure of welfare as it fails to distinguish between physical and mental stress, and therefore may not be appropriate for identifying sleep related stress. It may be that hair cortisol is not a sensitive enough measure to detect lack of sleep. At least for now, observation of behaviour is still the most reliable measure of sleep health.

Chapter 5: General Discussion

The importance of a good night's rest has long been recognised in humans as essential for good physical and mental health. Even mild sleep deprivation can lead to poor emotional processing (Rasch and Born, 2013), increased response to pain (Roehrs et al., 2006) and decreased time to exhaustion (Meerlo, et al. 2008). There is significant overlap between the physiological systems that control the stress response and those that control the sleep wake cycle, resulting in a bi-directional relationship between sleep and stress activation, making sleep a good potential candidate for monitoring welfare state (Lo Martire et al., 2020). In the case of horses, the symptoms of sleep deprivation not only impact the animals themselves, but also that of the human riders and handlers who may be at risk of injury when riding or handling an animal experiencing exhaustion and a reduced welfare state.

It is unclear if the wider population of domestic horses are having their sleep monitored or if horse owners feel sleep is a care priority. Recent efforts to study horse sleep in more detail have revealed that abnormal sleep patterns may go unrecognised or be misdiagnosed (Fuchs et al., 2016). Lack of sleep monitoring so signs of sleep deprivation are missed or mislabelling sleep deprived horses as "narcoleptic" rather than investigating potential causes of abnormal sleep, is likely to prolong suffering and may be causing a major welfare issue, the extent of which is unknown. The horses identified as collapsing due to extreme sleep deprivation in the study by Fuchs et al. (2016) may represent only the tip of the iceberg of domestic horses that are experiencing abnormal sleep. There is an urgent need to identify how horse owners are able to monitor sleep and if so, how this information can be incorporated into equine care guidelines.

If owners are going to monitor sleep there is a need to recognise what features of sleep need to be monitored and identify the early signs that something is wrong. Sleep in humans can be influenced by a variety of internal and external factors and small alterations in sleep may be a normal response to the individual situation. For example, altered sleep in response to changes in the seasons or level of physical activity may be a normal response for the body to maintain homeostasis (Axelsson and Vyazovskiy, 2015). There is a need to identify what variation in sleep pattern can be considered normal in order to establish when a horse falls outside that range.

Sleep in horses is yet to be linked to a physiological marker of stress such as cortisol. Both sleep and circadian rhythms are physiologically linked to stress levels through the HPA axis (Lo Martire et al., 2020). However, the complex relationship between cortisol, psychological stress and sleep may limit the usefulness of this measure. Additionally, horses display synchrony of behaviour in free living environments, and it has been suggested that increased synchrony of behaviour is related to improved welfare (Duranton and Gaunet, 2016; Muhammad et al., 2022). Whilst synchrony of lying behaviour has been recorded in domestic horses kept in social groups in a field (Martin et al., 2010), there are yet to be investigations into if synchrony of lying behaviour persists in horses housed next to each other in individual stables. In light of these gaps in the literature, below are how this thesis has contributed to furthering our understanding of horse rest patterns.

5.1 Owner attitudes to sleep as a care priority and sleep monitoring methods

The aim of Chapter 2 was to determine if horse owners and caretakers understood the importance of sleep as a holistic part of horse care. While most respondents recognised that sleep was important to horse welfare, very few actively monitored rest. Owners with equine-specific qualifications were more likely to recognise the importance of sleep as a management priority, however, there is currently no guidance on the best way to monitor sleep and the respondents employed a variety of methods. The ability to monitor sleep patterns accurately is hindered by the nocturnal sleep behaviours of horses, making strategies such as direct observation difficult. Looking for evidence of rest in the bedding or on the horse's coat or rug is likely the best, low cost/time input method of monitoring horse rest on a daily basis and could be easily incorporated into equine care education.

However, this method does not give the observer information about how often or how long the horse has been recumbent. In Chapter 2, I found that owners of horses over 25 years old and more likely to suffer from advanced osteoarthritis were reported to lay down less frequently than all other age groups in the summer. This is consistent with the finding from Oliveira et al., (2022), that horses with mild osteoarthritis spend more time lying down, but as the disease progresses horses spend less time lying down. Technology should be considered to monitor this, particularly for high-risk individuals such as aged horses. The findings in this chapter suggest that both awareness of the need to monitor rest and strategies for monitoring rest behaviour in horses are needed. In order to develop an effective education program for horse owners, we must first identify what aspects of rest behaviour are the best candidate to be used as an indicator of welfare.

5.2 Factors that influence horse rest patterns

In Chapter 3 I explored the different parameters that are likely to influence inter- and intra-individual variation of rest patterns in horses. The internal factors that were most likely to influence time spent resting was time spent feeding, with individuals who spend more time feeding spending less time resting. Auer et al., (2021) identified both time spent feeding and time spent resting as particularly important when comparing time budgets of domestic horses to that of free-living individuals. The link between these two important biological processes is probably not surprising, as the circadian rhythm dictates the timing of both and changes to one is likely to influence the timing of the other (Murphy, 2010). Although there has been much focus on time spent feeding as a measure of welfare (Aristizabal et al., 2014; Sartori et al., 2017), these results suggest that time spent resting may be of equal importance.

Intra-individual variation in time spent resting may be a good metric to use to identify stress in an individual and should be used as a metric in future welfare studies. Horses with a higher stress sensitivity, indicated by scoring highly for neuroticism in a personality questionnaire, were more likely to show more variation in sleep patterns than less stress sensitive counterparts. Repeatability of behaviour in a daily routine can be an element of welfare assessments (Auer et al., 2021). Repeatability of rest patterns is no exception. The results from this chapter suggest that if using rest as a measure of welfare, multiple nights must be monitored to determine the intra-individual variation of both total time in recumbency and number of lying bouts. Variation in rest behaviour within individuals should be considered as a welfare assessment benchmark for Quality-of-Life assessment protocols. While some features of rest patterns were identified from short term monitoring, it was determined that 4-6 nights of consecutive recording was not long enough to get a clear view of emerging trends. Further investigation is required to reach any conclusions about interindividual differences in resting patterns.

5.3 Hair cortisol as a measure of stress and relationship with rest patterns

The results from Chapter 4 suggest that hair cortisol is not a reliable proxy measure for abnormal rest patterns but may act as an indicator for time spent alert. The complex nature of sleep and the HPA axis may mean that these measures do not display a simple cause and effect relationship. Interestingly the horse that had the highest hair cortisol also spent the most time standing alert and the most time in lateral recumbency. It may be that hair cortisol is more appropriate as a measure of stress that results in increased vigilance and is less sensitive to the low but consistent HPA axis stimulation that occurs as a results of sleep deprivation (Meerlo et al., 2002).

This chapter further supports the importance of monitoring multiple nights. The incidental discovery that one horse showed at least one episode of collapse presumed to be caused by REM sleep deprivation makes an interesting case study for the early signs of sleep deprivation. This was the same individual that showed substantial inter-individual variation in sleep pattern in chapter 3, suggesting that variation in rest pattern between nights is likely an indicator of abnormal sleep pattern. This is supported in the human literature where nightly variability in individual sleep duration is associated with psychosocial and physiological stress (Mezick et al., 2009). Future work could examine fine scale cortisol secretion by looking at a night-by-night cortisol and rest pattern relationship using faecal cortisol to see if cortisol levels increase and decrease on nights where less or more sleep is observed.

A preliminary look at synchrony of behaviour in horses stabled next to each other was carried out but no clear pattern of synchrony was observed. Although synchrony of behaviour is believed to be a measure of good welfare, it's unclear if the lack of synchrony in stabled horses is necessarily a measure of poor welfare, or if the lack of a clear sight line in individual stables simply means they are less aware of what others around them are doing.

5.4 Recommendations

Monitoring horse sleep is an important aspect of welfare. Despite this, few horse owners are aware that they should be monitoring horse sleep, and where rest is being monitored, there is lack of agreement over how this should be done. The results of this thesis suggest that it is normal for a horse to lie down every night. As a minimum horse owners should be looking for signs that their horse has achieved recumbency at least once each night by looking for signs in the bedding/ground or dirt/bedding attached to the horse or the horse's rug. This should be taught as a matter of routine in all horse management and care education. Additionally, high risk individuals such as aged horses, horses with medical conditions that may predispose them to pain when lying down, horses that have high stress sensitivity or those that have moved to a new environment such as a new yard, should have rest patterns monitored more closely. This can be done using an accelerometer device, video observation using scan samples every 30 minutes or, where funds allow, an AI system. While hair cortisol does not appear to be an appropriate measure of sleep deprivation in horses, future research should explore more fine scale measures of cortisol, such as from faecal samples to monitor how daily cortisol is affected by variations in night-by-night rest patterns. Overall, more work needs to be done on defining the upper and lower boundaries of what is considered normal rest in horses and how it is impacted by factors such as temperature and exercise. However, the present work indicates that assessment of rest patterns could be added to welfare assessments as an animal-based measure. Within-individual variation in sleeping patterns over consecutive nights appears to be a good indicator that a horse is experiencing physical or psychological stress.

In terms of how sleep can be used in scientific research, when horses are required to be moved to a new environment sleep could be used as an indicator of habituation to that environment before the study begins. Often studies pick an arbitrary length of time for habituation, usually 1-2 weeks, but some horses may take significantly longer to habituate leading to unreliable results. A baseline measure of rest behaviour before the move could be used as a benchmark for true habituation before the experimental procedures begins.

5.4.1 Future work

Having identified variability in rest pattern as a potential indicator of psychological distress in horses, the next step of this work would be to identify how long it takes for horses to establish a routine rest pattern after moving to a new environment. As previously mentioned, horses are routinely moved to a new environment for several reasons, either short or long term. Constant movement could be a significant contributor to chronic stress and may be an under-explored source of poor welfare in horses. It is important that we investigate the effect this change has on individual horses, particularly those that score highly for neuroticism who are likely to have a greater magnitude stress response and take longer to recover after a move. This work may be of particular interest to riders who attend competitions that require overnight housing of the horse, as poor sleep before a competition may contribute to poor performance, safety and welfare. Loss of sleep and sleep deprivation of horses that are travelling to compete in high-risk competitions, such as cross-country, could be a significant factor to consider for the safety of both horse and rider. Additionally, more work needs to be done on why some individuals appear to increase time in recumbency in response to stress. A better understanding of the relationship between age and type of stressor can allow for an upper-bound of what is considered a normal level of recumbency to allow for welfare assessment.

An epidemiological study using a cluster analysis as used in Chapter 3 on a large sample size could identify risk factors to different sleep types and identify if these sleep types are due to normal variation in sleep strategy or if they are driven by physical and psychological distress. This is where the use of technology such as accelerometers would be useful. Having identified key features of rest behaviour to monitor in this thesis, that is both frequency and variability of lying rest patterns, future work can focus on recording these variables in a larger group of individuals using less labour-intensive methods of data collection.

Although this work identified only a weak link between exercise and rest pattern, exercise intensity was relatively low in the horses sampled relative to horses in competition training such as racehorses. The clear link between rest and physical exertion in humans strongly suggests that intense exercise is likely to influence rest patterns in subsequent nights in horses.

Finally, as previously mentioned, horse owners and carers need to be better informed about the importance of rest when monitoring horse health and welfare. There are now commercially available tools to monitor rest patterns. However, simple steps such as looking for signs that their horse has lain down each night could allow health and welfare issues to be identified early and would be straight forward to introduce to basic horse care training courses.

5.4.2 Conclusions

This body of work goes some way to assessing rest pattern in horses as a potential measure of welfare in QoL assessments alongside other established measures. Although many recognise the importance of rest in horses and ensuring an appropriate environment in which horses can rest, particularly those with equine-related qualifications, practical measures to monitor rest are lacking. In particular, geriatric horses would benefit from monitoring of rest patterns to ensure good welfare and if necessary, as an additional tool when making end of life decisions. A horse that is no longer able to get down or up and is showing signs of collapse and despite all efforts made to facilitate lying behaviour through management and health care such as pain relief, is likely suffering prolonged sleep deprivation and quality of life should be seriously considered by the treating vet and horse carer. Variation in rest behaviour over successive nights and/or skipping nights where no recumbency behaviour occurs, are likely good early indicators that a horse is experiencing physical or psychological stress. Although rest behaviour cannot be used to determine the type or source of the stressor, this information can be used for further investigation into the physical and psychological health of the horse and should be included in welfare assessments whenever practical to do so.

References

Albar, W.F., Russell, E.W., Koren, G., Rieder, M.J., van Umm, S.H., 2013. Human hair cortisol analysis: comparison of the internationally-reported ELISA methods. Clin Invest Med 36, E312-316. https://doi.org/10.25011/cim.v36i6.20629

Allen, M.S., Magee, C.A., Vella, S.A., 2016. Personality, hedonic balance and the quality and quantity of sleep in adulthood. Psychology & Health 31, 1091–1107. https://doi.org/10.1080/08870446.2016.1178745

Anafi, R.C., Kayser, M.S., Raizen, D.M., 2019. Exploring phylogeny to find the function of sleep. Nat Rev Neurosci 20, 109–116. https://doi.org/10.1038/s41583-018-0098-9

Arfuso, F., Giannetto, C., Giudice, E., Fazio, F., Panzera, M., Piccione, G., 2021. Peripheral Modulators of the Central Fatigue Development and Their Relationship with Athletic Performance in Jumper Horses. Animals 11, 743. https://doi.org/10.3390/ani11030743

Aristizabal, F., Nieto, J., Yamout, S., Snyder, J., 2014. The effect of a hay grid feeder on feed consumption and measurement of the gastric pH using an intragastric electrode device in horses: A preliminary report. Equine Veterinary Journal 46, 484–487. https://doi.org/10.1111/evj.12175

Asher, L., Collins, L.M., 2012. Assessing synchrony in groups: Are you measuring what you think you are measuring? Applied Animal Behaviour Science, Special Issue: Living In Large Groups 138, 162–169. https://doi.org/10.1016/j.applanim.2012.02.004

Auer, U., Kelemen, Z., Engl, V., Jenner, F., 2021. Activity Time Budgets—A Potential Tool to Monitor Equine Welfare? Animals 11, 850. https://doi.org/10.3390/ani11030850

Axelsson, J., Vyazovskiy, V.V., 2015. Banking Sleep and Biological Sleep Need. Sleep 38, 1843–1845. https://doi.org/10.5665/sleep.5222

Balbo, M., Leproult, R., Van Cauter, E., 2010. Impact of Sleep and Its Disturbances on Hypothalamo-Pituitary-Adrenal Axis Activity. International Journal of Endocrinology 2010, e759234. https://doi.org/10.1155/2010/759234

Balzani, A., Hanlon, A., 2020. Factors that Influence Farmers' Views on Farm Animal Welfare: A Semi-Systematic Review and Thematic Analysis. Animals 10, 1524. https://doi.org/10.3390/ani10091524

Banks, S., Dorrian, J., Basner, M., Dinges, D.H., 2005. Sleep Deprivation, in: Principles and Practice of Sleep Medicine. pp. 49–55.

Bao, J., Xie, Q., 2022. Artificial intelligence in animal farming: A systematic literature review. Journal of Cleaner Production 331, 129956. https://doi.org/10.1016/j.jclepro.2021.129956

Barnard, S., Calderara, S., Pistocchi, S., Cucchiara, R., Podaliri-Vulpiani, M., Messori, S., Ferri, N., 2016. Quick, Accurate, Smart: 3D Computer Vision Technology Helps Assessing Confined Animals' Behaviour. PLOS ONE 11, e0158748. https://doi.org/10.1371/journal.pone.0158748

Bartolomé, E., Cockram, M.S., 2016. Potential Effects of Stress on the Performance of Sport Horses. Journal of Equine Veterinary Science 40, 84–93. https://doi.org/10.1016/j.jevs.2016.01.016

Bei, B., Wiley, J.F., Trinder, J., Manber, R., 2016. Beyond the mean: A systematic review on the correlates of daily intraindividual variability of sleep/wake patterns. Sleep Medicine Reviews 28, 108–124. https://doi.org/10.1016/j.smrv.2015.06.003

Belling, T.H.J., 1990. Sleep patterns in the horse. Equine practice (USA).

Bertone, J.J., Furr, M., Reed, S., 2015. Sleep and Sleep Disorders in Horses, in: Equine Neurology. John Wiley & Sons, Inc, pp. 123–129.

Blumberg, M.S., Lesku, J.A., Libourel, P.-A., Schmidt, M.H., Rattenborg, N.C., 2020. What Is REM Sleep? Current Biology 30, R38–R49. https://doi.org/10.1016/j.cub.2019.11.045

Bohák, Zs., Szabó, F., Beckers, J.-F., Melo de Sousa, N., Kutasi, O., Nagy, K., Szenci, O., 2013. Monitoring the circadian rhythm of serum and salivary cortisol concentrations in the horse. Domestic Animal Endocrinology 45, 38–42. https://doi.org/10.1016/j.domaniend.2013.04.001

Bonk, S., Burfeind, O., Suthar, V.S., Heuwieser, W., 2013. Technical note: Evaluation of data loggers for measuring lying behavior in dairy calves. Journal of Dairy Science 96, 3265–3271. https://doi.org/10.3168/jds.2012-6003

Boy, V., Duncan, P., 1979. Time-Budgets of Camargue Horses I. Developmental Changes in the Time-Budgets of Foals. Behaviour 71, 187–201. https://doi.org/10.1163/156853979X00160

Boyd, L.E., Carbonaro, D.A., Houpt, K.A., 1988. The 24-hour time budget of Przewalski horses. Applied Animal Behaviour Science, Behavior of Przewalski horses 21, 5–17. https://doi.org/10.1016/0168-1591(88)90098-6

Breedlove, N.V.W.S.M., 2013. Biological Psychology: An Introduction to Behavioral, Cognitive, and Clinical Neuroscience, 7th ed. Sinauer Associates Inc., Sunderland, USA.

British Horse Society (BHS), 2022. Horse Stables: Safety, Design & Size | The British Horse Society. URL https://www.bhs.org.uk/horse-care-and-welfare/health-care-management/stable-safety/ (accessed 6.10.23).

Brown, 2012. Can sleep deprivation studies explain why human adults sleep? Current Opinion in Pulmonary Medicine 18, 541–545. https://doi.org/10.1097/MCP.0b013e3283596740

Brown, A.E., Twigg-Flesner, A., 2018. Are we over-rugging? A preliminary research article into how rugging affects clipped, stabled horses using the Orscana© rug-monitoring device.

Brupbacher, G., Gerger, H., Zander-Schellenberg, T., Straus, D., Porschke, H., Gerber, M., von Känel, R., Schmidt-Trucksäss, A., 2021. The effects of exercise on sleep in unipolar depression: A systematic review and network meta-analysis. Sleep Medicine Reviews 59, 101452. https://doi.org/10.1016/j.smrv.2021.101452

Brylewski, J., Wiggs, L., 1999. Sleep problems and daytime challenging behaviour in a community-based sample of adults with intellectual disability. Journal of Intellectual Disability Research 43, 504–512. https://doi.org/10.1046/j.1365-2788.1999.00234.x

Burkhardt, J., 1947. Transition from anoestrus in the mare and the effects of artificial lighting. The Journal of Agricultural Science 37, 64–68. https://doi.org/10.1017/S0021859600013083

Burla, J.-B., Rufener, C., Bachmann, I., Gygax, L., Patt, A., Hillmann, E., 2017. Space allowance of the littered area affects lying behavior in group-housed horses. Front. Vet. Sci. 4. https://doi.org/10.3389

Burnett, T.A., Madureira, A.M.L., Silper, B.F., Nadalin, A., Tahmasbi, A., Veira, D.M., Cerri, R.L.A., 2014. Short communication: Factors affecting hair cortisol concentrations in lactating dairy cows. Journal of Dairy Science 97, 7685–7690. https://doi.org/10.3168/jds.2014-8444

Byström, A., Clayton, H. m., Hernlund, E., Rhodin, M., Egenvall, A., 2020. Equestrian and biomechanical perspectives on laterality in the horse. Comparative Exercise Physiology 16, 35–45. https://doi.org/10.3920/CEP190022

Caanitz, H., O'Leary, L., Houpt, K., Petersson, K., Hintz, H., 1991. Effect of exercise on equine behavior. Applied Animal Behaviour Science 31, 1–12. https://doi.org/10.1016/0168-1591(91)90148-Q

Cai, Z.-J., 2016. Two types of REM sleep: The atonic and brain REM sleep. Sleep and Hypnosis - International Journal. https://doi.org/10.5350/Sleep.Hypn.2016.18.0113

Cangar, Ö., Leroy, T., Guarino, M., Vranken, E., Fallon, R., Lenehan, J., Mee, J., Berckmans, D., 2008. Automatic real-time monitoring of locomotion and posture behaviour of pregnant cows prior to calving using online image analysis. Computers and Electronics in Agriculture, Smart Sensors in precision livestock farming 64, 53–60. https://doi.org/10.1016/j.compag.2008.05.014

Capellini, Nunn, C.L., McNamara, P., Preston, B.T., Barton, R.A., 2008b. Energetic constraints, not predation, influence the evolution of sleep patterning in mammals. Functional Ecology 22, 847–853. https://doi.org/10.1111/j.1365-2435.2008.01449.x

Carroll, D., Ginty, A.T., Whittaker, A.C., Lovallo, W.R., de Rooij, S.R., 2017. The behavioural, cognitive, and neural corollaries of blunted cardiovascular and cortisol reactions to acute psychological stress. Neuroscience & Biobehavioral Reviews 77, 74–86. https://doi.org/10.1016/j.neubiorev.2017.02.025

Carskadon, M., Dement, W., 2005. Normal Human Sleep: An Overview, in: Principles and Practice of Sleep Medicine. pp. 15–24.

Chaplin, S.J., Gretgrix, L., 2010. Effect of housing conditions on activity and lying behaviour of horses. animal 4, 792–795. https://doi.org/10.1017/S1751731109991704

Chung, E.L.T., Khairuddin, N.H., Azizan, T.R.P.T., Adamu, L., 2018. Sleeping patterns of horses in selected local horse stables in Malaysia. Journal of Veterinary Behavior 26, 1–4. https://doi.org/10.1016/j.jveb.2018.03.014

Cipolli, C., 1995. Sleep, Dreams and memory: an overview. Journal of Sleep Research 4, 2–9. https://doi.org/10.1111/j.1365-2869.1995.tb00143.x

Clarke, N., Fraser, D., 2016. Automated monitoring of resting in dogs. Applied Animal Behaviour Science 174, 99–102. https://doi.org/10.1016/j.applanim.2015.11.019

Clothier, J., Small, A., Hinch, G., Barwick, J., Brown, W.Y., 2019. Using Movement Sensors to Assess Lying Time in Horses With and Without Angular Limb Deformities. Journal of Equine Veterinary Science 75, 55–59. https://doi.org/10.1016/j.jevs.2019.01.011

Code of welfare: Dairy Cattle, 2019. . New Zealand Ministry for Primary Indistries, Wellington, New Zealand.

Cook, N.B., Bennett, T.B., Nordlund, K.V., 2004. Effect of Free Stall Surface on Daily Activity Patterns in Dairy Cows with Relevance to Lameness Prevalence. Journal of Dairy Science 87, 2912–2922. https://doi.org/10.3168/jds.S0022-0302(04)73422-0

Correa, M.G., Rodrigues e Silva, C.F., Dias, L.A., da Silva Rocha Junior, S., Thomes, F.R., Alberto do Lago, L., de Mattos Carvalho, A., Faleiros, R.R., 2020. Welfare benefits after the implementation of slow-feeder hay bags for stabled horses. Journal of Veterinary Behavior 38, 61–66. https://doi.org/10.1016/j.jveb.2020.05.010

Cousillas, H., Oger, M., Rochais, C., Pettoello, C., Ménoret, M., Henry, S., Hausberger, M., 2017. An Ambulatory Electroencephalography System for Freely Moving Horses: An Innovating Approach. Front Vet Sci 4. https://doi.org/10.3389/fvets.2017.00057

Crowell-Davis, S.L., 1986. Developmental Behavior. Veterinary Clinics of North America: Equine Practice, Behavior 2, 573–590. https://doi.org/10.1016/S0749-0739(17)30707-1

Crowell-Davis, S.L., Houpt, K.A., Kane, L., 1987. Play development in Welsh pony (Equus caballus) foals. Applied Animal Behaviour Science 18, 119–131. https://doi.org/10.1016/0168-1591(87)90186-9

Daigle, C.L., Siegford, J.M., 2014. When continuous observations just won't do: Developing accurate and efficient sampling strategies for the laying hen. Behavioural Processes 103, 58–66. https://doi.org/10.1016/j.beproc.2013.11.002

Dallaire, A., 1986. Rest behavior. The Veterinary clinics of North America. Equine practice 2, 591–607.

Dallaire, A., Ruckebusch, Y., 1974a. Sleep patterns in the pony with observations on partial perceptual deprivation. Physiology & Behavior 12, 789–796. https://doi.org/10.1016/0031-9384(74)90015-8

Dallaire, A., Ruckebusch, Y., 1974b. Sleep and Wakefulness in the Housed Pony Under Different Dietary Conditions. Can J Comp Med 38, 65–71.

Datta, S., 2010. Cellular and chemical neuroscience of mammalian sleep. Sleep Medicine 11, 431–440. https://doi.org/10.1016/j.sleep.2010.02.002

Davenport, M.D., Lutz, C.K., Tiefenbacher, S., Novak, M.A., Meyer, J.S., 2008. A Rhesus Monkey Model of Self-Injury: Effects of Relocation Stress on Behavior and Neuroendocrine Function. Biological Psychiatry, Genes, Brain Development, and Disorders of Childhood Onset 63, 990–996. https://doi.org/10.1016/j.biopsych.2007.10.025

Davenport, M.D., Tiefenbacher, S., Lutz, C.K., Novak, M.A., Meyer, J.S., 2006. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. Gen Comp Endocrinol 147, 255–261. https://doi.org/10.1016/j.ygcen.2006.01.005

Dawkins, M.S., 2008. The Science of Animal Suffering. Ethology 114, 937–945. https://doi.org/10.1111/j.1439-0310.2008.01557.x

Della Costa, E., Dai, F., Lebelt, D., Scholz, P., Barbieri, S., Canali, E., Zanella, A.J., Minero, M., 2016. Welfare assessment of horses: the AWIN approach. Animal Welfare 25, 481–488. https://doi.org/10.7120/09627286.25.4.481

Dillon, H.R., Lichstein, K.L., Dautovich, N.D., Taylor, D.J., Riedel, B.W., Bush, A.J., 2015. Variability in Self-Reported Normal Sleep Across the Adult Age Span. The Journals of Gerontology: Series B 70, 46–56. https://doi.org/10.1093/geronb/gbu035

Dippel, S., Dolezal, M., Brenninkmeyer, C., Brinkmann, J., March, S., Knierim, U., Winckler, C., 2009. Risk factors for lameness in freestall-housed dairy cows across two breeds, farming systems, and countries. Journal of Dairy Science 92, 5476–5486. https://doi.org/10.3168/jds.2009-2288

Doufas, A.G., 2017. Chapter 133 - Pain and Sleep A2 - Kryger, Meir, in: Roth, T., Dement, W.C. (Eds.), Principles and Practice of Sleep Medicine (Sixth Edition). Elsevier, pp. 1313-1322.e5.

DuBois, C., Zakrajsek, E., Haley, D.B., Merkies, K., 2015. Validation of triaxial accelerometers to measure the lying behaviour of adult domestic horses. animal 9, 110–114. https://doi.org/10.1017/S175173111400247X

Duncan, P., 1985. Time-Budgets of Camargue Horses III. Environmental Influences. Behaviour 92, 188–208.

Duncan, P., 1980. Time-Budgets of Camargue Horses Ii. Time-Budgets of Adult Horses and Weaned Sub-Adults. Behaviour 72, 26–48. https://doi.org/10.1163/156853980X00023

Duran, M.C., Janz, D.M., Waldner, C.L., Campbell, J.R., Marques, F.J., 2017. Hair Cortisol Concentration as a Stress Biomarker in Horses: Associations With Body Location and Surgical Castration. Journal of Equine Veterinary Science 55, 27–33. https://doi.org/10.1016/j.jevs.2017.03.220

Duranton, C., Gaunet, F., 2016. Behavioural synchronization from an ethological perspective: Overview of its adaptive value. Adaptive Behavior 24, 181–191. https://doi.org/10.1177/1059712316644966

El Mlili, N., Ahabrach, H., Cauli, O., 2021. Hair Cortisol Concentration as a Biomarker of Sleep Quality and Related Disorders. Life 11, 81. https://doi.org/10.3390/life11020081

Elder, G.J., Wetherell, M.A., Barclay, N.L., Ellis, J.G., 2014. The cortisol awakening response – Applications and implications for sleep medicine. Sleep Medicine Reviews 18, 215–224. https://doi.org/10.1016/j.smrv.2013.05.001

Ellis, A.D., Redgate, S., Zinchenko, S., Owen, H., Barfoot, C., Harris, P., 2015. The effect of presenting forage in multi-layered haynets and at multiple sites on night time budgets of stabled horses. Applied Animal Behaviour Science 171, 108–116. https://doi.org/10.1016/j.applanim.2015.08.012

Fenner, K., Dashper, K., Wilkins, C., Serpell, J., McLean, A., Wilson, B., McGreevy, P., 2020. Building Bridges between Theory and Practice: How Citizen Science Can Bring Equine Researchers and Practitioners Together. Animals 10, 1644. https://doi.org/10.3390/ani10091644

Feuser, A.-K., Gesell-May, S., Müller, T., May, A., 2022. Artificial Intelligence for Lameness Detection in Horses—A Preliminary Study. Animals 12, 2804. https://doi.org/10.3390/ani12202804

Fleming, K., Hessel, E.F., Van den Weghe, H.F.A., 2008. Generation of Airborne Particles from Different Bedding Materials Used for Horse Keeping. Journal of Equine Veterinary Science 28, 408–418. https://doi.org/10.1016/j.jevs.2008.05.004

Fogarty, E.S., Swain, D.L., Cronin, G., Trotter, M., 2018. Autonomous on-animal sensors in sheep research: A systematic review. Computers and Electronics in Agriculture 150, 245–256. https://doi.org/10.1016/j.compag.2018.04.017

Fraiwan, M.A., Abutarbush, S.M., 2020. Using Artificial Intelligence to Predict Survivability Likelihood and Need for Surgery in Horses Presented With Acute Abdomen (Colic). Journal of Equine Veterinary Science 90, 102973. https://doi.org/10.1016/j.jevs.2020.102973

Fraser, A.F., 1992. The behaviour of the horse. The behaviour of the horse.

Fraser, L., 2012. The Horse’s Manifesto: What do we want?? Friends, Forage and Freedom! Part 1 of 3. [WWW Document]. International Association of Animal Behaviour Consultants. URL https://iaabc.org/horse/the-horses-manifesto-what-do-we-want-friends-forage-and-freedom-part-1-of-3 (accessed 7.23.22).

Friedman, H.S., Kern, M.L., 2014. Personality, Well-Being, and Health. Annual Review of Psychology 65, 719–742. https://doi.org/10.1146/annurev-psych-010213-115123

Fuchs, C., Kiefner, C., Reese, S., Erhard, M., Wöhr, A.-C., 2016. Narcolepsy: Do Adult Horses Really Suffer from a Neurological Disorder or Rather from a Recumbent Sleep Deprivation/Rapid Eye Movement (REM)-Sleep Deficiency? Equine Vet J 48, 9–9. https://doi.org/10.1111/evj.09_12612

Fullagar, H., HK, Skorski, S., Duffield, R., Hammes, D., Coutts, A., 2015. Sleep and Athletic Performance: The Effects of Sleep Loss on Exercise Performance, and Physiological and Cognitive Responses to Exercise - ProQuest [WWW Document]. URL https://search.proquest.com/docview/1666285540/fulltext/A6EAB5B22E4B1DPQ/1?accountid=14693 (accessed 7.21.20).

Genzel, L., Spoormaker, V.I., Konrad, B.N., Dresler, M., 2015. The role of rapid eye movement sleep for amygdala-related memory processing. Neurobiology of Learning and Memory, REM Sleep and Memory 122, 110–121. https://doi.org/10.1016/j.nlm.2015.01.008

Ghassemi Nejad, J., Ghaffari, M.H., Ataallahi, M., Jo, J.-H., Lee, H.-G., 2022. Stress Concepts and Applications in Various Matrices with a Focus on Hair Cortisol and Analytical Methods. Animals 12, 3096. https://doi.org/10.3390/ani12223096

Golem, D.L., Martin-Biggers, J.T., Koenings, M.M., Davis, K.F., Byrd-Bredbenner, C., 2014. An Integrative Review of Sleep for Nutrition Professionals. Advances in Nutrition 5, 742–759. https://doi.org/10.3945/an.114.006809

Gonfalone, A., Jha, S., 2015. The influence of gravity on REM sleep. Open Access Animal Physiology 65. https://doi.org/10.2147/OAAP.S80731

Goodwin, D., 2007. Horse Behaviour: Evolution, Domestication and Feralisation, in: Waran, N. (Ed.), The Welfare of Horses, Animal Welfare. Springer Netherlands, Dordrecht, pp. 1–18. https://doi.org/10.1007/978-0-306-48215-1_1

Gravett, N., Bhagwandin, A., Sutcliffe, R., Landen, K., Chase, M.J., Lyamin, O.I., Siegel, J.M., Manger, P.R., 2017. Inactivity/sleep in two wild free-roaming African elephant matriarchs – Does large body size make elephants the shortest mammalian sleepers? PLOS ONE 12, e0171903. https://doi.org/10.1371/journal.pone.0171903

Greening, L., Downing, J., 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, 105229. https://doi.org/10.1016/j.applanim.2021.105229

Greening, L., McBride, S., 2022. A Review of Equine Sleep: Implications for Equine Welfare. Frontiers in Veterinary Science 9.

Greening, L., Pullen, A., Tame, S., Bufton, C.-A., 2020. The welfare implications of owner perceptions and knowledge of equine sleep: UFAW Virtual Animal Welfare Conference.

Greening, L., Shenton, V., Wilcockson, K., Swanson, J., 2013. Investigating duration of nocturnal ingestive and sleep behaviors of horses bedded on straw versus shavings. Journal of Veterinary Behavior: Clinical Applications and Research 8, 82–86. https://doi.org/10.1016/j.jveb.2012.05.003

Grossman, G.H., Mistlberger, R.E., Antle, M.C., Ehlen, J.C., Glass, J.D., 2000. Sleep deprivation stimulates serotonin release in the suprachiasmatic nucleus. NeuroReport 11, 1929.

Haghayegh, S., Khoshnevis, S., Smolensky, M.H., Diller, K.R., Castriotta, R.J., 2019. Accuracy of Wristband Fitbit Models in Assessing Sleep: Systematic Review and Meta-Analysis. Journal of Medical Internet Research 21, e16273. https://doi.org/10.2196/16273

Hall, C., Goodwin, D., Heleski, C., Randle, H., Waran, N., 2008. Is There Evidence of Learned Helplessness in Horses? Journal of Applied Animal Welfare Science 11, 249–266. https://doi.org/10.1080/10888700802101130

Hall, C., Randle, H., Pearson, G., Preshaw, L., Waran, N., 2018. Assessing equine emotional state. Applied Animal Behaviour Science 205, 183–193. https://doi.org/10.1016/j.applanim.2018.03.006

Halson, S.L., 2014. Sleep in Elite Athletes and Nutritional Interventions to Enhance Sleep. Sports Med 44, 13–23. https://doi.org/10.1007/s40279-014-0147-0

Harewood, E.J., McGowan, C.M., 2005. Behavioral and physiological responses to stabling in naive horses. Journal of Equine Veterinary Science 25, 164–170. https://doi.org/10.1016/j.jevs.2005.03.008

Hartmann, E., Søndergaard, E., Keeling, L.J., 2012. Keeping horses in groups: A review. Applied Animal Behaviour Science 136, 77–87. https://doi.org/10.1016/j.applanim.2011.10.004

Hauglund, N.L., Pavan, C., Nedergaard, M., 2020. Cleaning the sleeping brain – the potential restorative function of the glymphatic system. Current Opinion in Physiology, Physiology of sleep 15, 1–6. https://doi.org/10.1016/j.cophys.2019.10.020

Heleski, C.R., Shelle, A.C., Nielsen, B.D., Zanella, A.J., 2002. Influence of housing on weanling horse behavior and subsequent welfare. Applied Animal Behaviour Science, Equine Behavior 78, 291–302. https://doi.org/10.1016/S0168-1591(02)00108-9

Högberg, N., Höglund, J., Carlsson, A., Saint-Jeveint, M., Lidfors, L., 2020. Validation of accelerometers to automatically record postures and number of steps in growing lambs. Applied Animal Behaviour Science 229, 105014. https://doi.org/10.1016/j.applanim.2020.105014

Holdgate, M.R., Meehan, C.L., Hogan, J.N., Miller, L.J., Rushen, J., de Passillé, A.M., Soltis, J., Andrews, J., Shepherdson, D.J., 2016. Recumbence Behavior in Zoo Elephants: Determination of Patterns and Frequency of Recumbent Rest and Associated Environmental and Social Factors. PLoS ONE 11, 1–19. https://doi.org/10.1371/journal.pone.0153301

Houpt, K., 1980. The characteristics of Equine Sleep. Equine practice (USA) 2, 8–17.

Houpt, K., Houpt, T.R., Johnson, J.L., Erb, H.N., Yeon, S.C., 2001. The Effect of Exercise Deprivation on the Behaviour and Physiology of Straight Stall Confined Pregnant Mares. Animal Welfare 10, 257–267. https://doi.org/10.1017/S0962728600024039

Ijichi, C., Collins, L.M., Creighton, E., Elwood, R.W., 2013a. Harnessing the power of personality assessment: subjective assessment predicts behaviour in horses. Behavioural Processes 96, 47–52. https://doi.org/10.1016/j.beproc.2013.02.017

Ijichi, C.L., Collins, L.M., Elwood, R.W., 2013b. Evidence for the role of personality in stereotypy predisposition. Animal Behaviour 85, 1145–1151. https://doi.org/10.1016/j.anbehav.2013.03.033

Ijichi, C., Collins, L.M., Elwood, R.W., 2014. Pain expression is linked to personality in horses. Applied Animal Behaviour Science 152, 38–43. https://doi.org/10.1016/j.applanim.2013.12.007

Jean Kant, G., Pastel, R.H., Bauman, R.A., Meininger, G.R., Maughan, K.R., Robinson, T.N., Wright, W.L., Covington, P.S., 1995. Effects of chronic stress on sleep in rats. Physiology & Behavior 57, 359–365. https://doi.org/10.1016/0031-9384(94)00241-V

Jolivald, A., Ijichi, C., Hall, C., Yarnell, K., 2023. The mane factor: Compliance is associated with increased hair cortisol in the horse. Applied Animal Behaviour Science 258, 105819. https://doi.org/10.1016/j.applanim.2022.105819

Keeling, L.J., Winckler, C., Hintze, S., Forkman, B., 2021. Towards a Positive Welfare Protocol for Cattle: A Critical Review of Indicators and Suggestion of How We Might Proceed. Frontiers in Animal Science 2.

Keiper, R.R., Keenan, M.A., 1980. Nocturnal Activity Patterns of Feral Ponies. Journal of Mammalogy 61, 116–118. https://doi.org/10.2307/1379963

Kelemen, Z., Grimm, H., Long, M., Auer, U., Jenner, F., 2021. Recumbency as an Equine Welfare Indicator in Geriatric Horses and Horses with Chronic Orthopaedic Disease. Animals (Basel) 11, 3189. https://doi.org/10.3390/ani11113189

Kirischuk, S., Sinning, A., Blanquie, O., Yang, J.-W., Luhmann, H.J., Kilb, W., 2017. Modulation of Neocortical Development by Early Neuronal Activity: Physiology and Pathophysiology. Frontiers in Cellular Neuroscience 11.

Kjellberg, L., Sassner, H., Yngvesson, J., 2022. Horses' resting behaviour in shelters of varying size compared with single boxes. Applied Animal Behaviour Science 254, 105715. https://doi.org/10.1016/j.applanim.2022.105715

Knoop, M.S., de Groot, E.R., Dudink, J., 2021. Current ideas about the roles of rapid eye movement and non–rapid eye movement sleep in brain development. Acta Paediatrica 110, 36–44. https://doi.org/10.1111/apa.15485

Koo, T.K., Li, M.Y., 2016. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med 15, 155–163. https://doi.org/10.1016/j.jcm.2016.02.012

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience & Biobehavioral Reviews 23, 925–935. https://doi.org/10.1016/S0149-7634(99)00026-3

Kredlow, M.A., Capozzoli, M.C., Hearon, B.A., Calkins, A.W., Otto, M.W., 2015. The effects of physical activity on sleep: a meta-analytic review. J Behav Med 38, 427–449. https://doi.org/10.1007/s10865-015-9617-6

Kroshko, T., Kapronczai, L., Cattet, M.R.L., Macbeth, B.J., Stenhouse, G.B., Obbard, M.E., Janz, D.M., 2017. Comparison of methanol and isopropanol as wash solvents for determination of hair cortisol concentration in grizzly bears and polar bears. MethodsX 4, 68–75. https://doi.org/10.1016/j.mex.2017.01.004

Kull, J.A., Proudfoot, K.L., Pighetti, G.M., Bewley, J.M., O'Hara, B.F., Donohue, K.D., Krawczel, P.D., 2019. Effects of acute lying and sleep deprivation on the behavior of lactating dairy cows. PLOS ONE 14, e0212823. https://doi.org/10.1371/journal.pone.0212823

Langford, F., Cockram, M., 2010. Is sleep in animals affected by prior waking experiences? Animal Welfare 19, 215–222.

Lecorps, B., Weary, D.M., von Keyserlingk, M.A.G., 2021. Captivity-Induced Depression in Animals. Trends in Cognitive Sciences 25, 539–541. https://doi.org/10.1016/j.tics.2021.03.010

Ledgerwood, D.N., Winckler, C., Tucker, C.B., 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. Journal of Dairy Science 93, 5129–5139. https://doi.org/10.3168/jds.2009-2945

Lerner, I., Lupkin, S.M., Tsai, A., Khawaja, A., Gluck, M.A., 2021. Sleep to remember, sleep to forget: Rapid eye movement sleep can have inverse effects on recall and generalization of fear memories. Neurobiology of Learning and Memory 180, 107413. https://doi.org/10.1016/j.nlm.2021.107413

Lesimple, C., 2020. Indicators of Horse Welfare: State-of-the-Art. Animals 10, 294. https://doi.org/10.3390/ani10020294

Lesku, J.A., Roth II, T.C., Amlaner, C.J., Lima, S.L., Stamps, A.E.J.A., Losos, E.J.B., 2006. A Phylogenetic Analysis of Sleep Architecture in Mammals: The Integration of Anatomy, Physiology, and Ecology. The American Naturalist 168, 441–453. https://doi.org/10.1086/506973

Lima, S.L., Rattenborg, N.C., Lesku, J.A., Amlaner, C.J., 2005. Sleeping under the risk of predation. Animal Behaviour 70, 723–736. https://doi.org/10.1016/j.anbehav.2005.01.008

Littlejohn, A., Munro, R., 1972. Equine recumbency. Veterinary Record 90, 83–85. https://doi.org/10.1136/vr.90.4.83

Lloyd, A.S., Martin, J.E., Bornett-Gauci, H.L.I., Wilkinson, R.G., 2008. Horse personality: Variation between breeds. Applied Animal Behaviour Science 112, 369–383. https://doi.org/10.1016/j.applanim.2007.08.010

Lo Martire, V., Caruso, D., Palagini, L., Zoccoli, G., Bastianini, S., 2020. Stress & sleep: A relationship lasting a lifetime. Neurosci Biobehav Rev 117, 65–77. https://doi.org/10.1016/j.neubiorev.2019.08.024

Lo Martire, V., Caruso, D., Palagini, L., Zoccoli, G., Bastianini, S., 2019. Stress & sleep: A relationship lasting a lifetime. Neuroscience & Biobehavioral Reviews. https://doi.org/10.1016/j.neubiorev.2019.08.024

Ludvikova, E., Nishino, S., Sakai, N., Jahn, P., 2012. Familial narcolepsy in the Lipizzaner horse: a report of three fillies born to the same sire. Vet Q 32, 99–102. https://doi.org/10.1080/01652176.2012.714089

Lunn, D.P., Cuddon, P.A., Shaftoe, S., Archer, R.M., 1993. Familial occurrence of narcolepsy in Miniature Horses. Equine Veterinary Journal 25, 483–487. https://doi.org/10.1111/j.2042-3306.1993.tb02998.x

Mangold, S.A., Das, J., 2023. Neuroanatomy, Reticular Formation, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Martin, A.-M., Elliott, J.A., Duffy, P., Blake, C.M., Attia, S.B., Katz, L.M., Browne, J.A., Gath, V., McGivney, B.A., Hill, E.W., Murphy, B.A., 2010. Circadian regulation of locomotor activity and skeletal muscle gene expression in the horse. Journal of Applied Physiology 109, 1328–1336. https://doi.org/10.1152/japplphysiol.01327.2009

Martin, B.J., Chen, H.-I., 1984. Sleep loss and the sympathoadrenal response to exercise. Medicine & Science in Sports & Exercise 16, 56–59.

Martin, J.L., Hakim, A.D., 2011. Wrist Actigraphy. Chest 139, 1514–1527. https://doi.org/10.1378/chest.10-1872

Mata, F., 2021. A Framework for Using Epidemiology in Animal Welfare Science. Journal of Applied Animal Welfare Science 0, 1–13. https://doi.org/10.1080/10888705.2021.1981902

Matthews, S.G., Miller, A.L., PlÖtz, T., Kyriazakis, I., 2017. Automated tracking to measure behavioural changes in pigs for health and welfare monitoring. Sci Rep 7, 17582. https://doi.org/10.1038/s41598-017-17451-6

McGreevy, P., Yeates, J., 2018. Horses (Equus caballus), in: Companion Animal Care and Welfare. John Wiley & Sons, Ltd, pp. 266–292. https://doi.org/10.1002/9781119333708.ch13

Mcgreevy, P.D., Cripps, P.J., French, N.P., Green, L.E., Nicol, C.J., 1995. Management factors associated with stereotypic and redirected behaviour in the Thoroughbred horse. Equine Veterinary Journal 27, 86–91. https://doi.org/10.1111/j.2042-3306.1995.tb03041.x

Meerlo, P., Koehl, M., van der Borght, K., Turek, F.W., 2002. Sleep Restriction Alters the Hypothalamic-Pituitary-Adrenal Response to Stress. Journal of Neuroendocrinology 14, 397–402. https://doi.org/10.1046/j.0007-1331.2002.00790.x

Meerlo, P., Sgoifo, A., Suchecki, D., 2008. Restricted and disrupted sleep: Effects on autonomic function, neuroendocrine stress systems and stress responsivity. Sleep Medicine Reviews 12, 197–210. https://doi.org/10.1016/j.smrv.2007.07.007

Metz, J.H.M., 1985. The reaction of cows to a short-term deprivation of lying. Applied Animal Behaviour Science 13, 301–307. https://doi.org/10.1016/0168-1591(85)90010-3

Meyer, J.S., Novak, M.A., Hamel, A., Rosenberg, K., 2014. Extraction and analysis of cortisol from human and monkey hair [WWW Document]. URL https://pubmed.ncbi.nlm.nih.gov/24513702/ (accessed 7.26.22).

Mezick, E.J., Matthews, K.A., Hall, M., Kamarck, T.W., Buysse, D.J., Owens, J.F., Reis, S.E., 2009. Intra-individual variability in sleep duration and fragmentation: Associations with stress. Psychoneuroendocrinology 34, 1346–1354. https://doi.org/10.1016/j.psyneuen.2009.04.005

Mills, D.S., Eckley, S., Cooper, J.J., 2000. Thoroughbred bedding preferences, associated behaviour differences and their implications for equine welfare. Animal Science 70, 95–106.

Minkel, J., Banks, S., Htaik, O., Moreta, M., Jones, C., McGlinchey, E., Simpson, N., Dinges, D., 2012. Sleep deprivation and stressors: Evidence for elevated negative affect in response to mild stressors when sleep deprived [WWW Document]. URL https://search.proquest.com/docview/920364391?accountid=14693 (accessed 8.3.17).

Montoliu, T., Pulopulos, M.M., Puig-Pérez, S., Hidalgo, V., Salvador, A., 2022. Mediation of perceived stress and cortisol in the association between neuroticism and global cognition in older adults: A longitudinal study. Stress and Health 38, 290–303. https://doi.org/10.1002/smi.3088

Mormède, P., Andanson, S., Aupérin, B., Beerda, B., Guémené, D., Malmkvist, J., Manteca, X., Manteuffel, G., Prunet, P., van Reenen, C.G., Richard, S., Veissier, I., 2007. Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. Physiology & Behavior, Stress and Welfare in Farm Animals 92, 317–339. https://doi.org/10.1016/j.physbeh.2006.12.003

Muhammad, M., Stokes, J.E., Manning, L., 2022. Positive Aspects of Welfare in Sheep: Current Debates and Future Opportunities. Animals 12, 3265. https://doi.org/10.3390/ani12233265

Munk, R., Jensen, R.B., Palme, R., Munksgaard, L., Christensen, J.W., 2017. An exploratory study of competition scores and salivary cortisol concentrations in Warmblood horses. Domestic Animal Endocrinology 61, 108–116. https://doi.org/10.1016/j.domaniend.2017.06.007

Murase, H., Matsui, A., Endo, Y., Sato, F., Hada, T., 2018a. Changes of lying behavior in Thoroughbred foals influenced by age, pasturing time, and weather conditions. J Equine Sci 29, 61–66. https://doi.org/10.1294/jes.29.61

Murase, H., Matsui, A., Endo, Y., Sato, F., Hada, T., 2018b. Changes of lying behavior in Thoroughbred foals influenced by age, pasturing time, and weather conditions. Journal of Equine Science 29, 61–66. https://doi.org/10.1294/jes.29.61

Murphy, B.A., 2019. Circadian and Circannual Regulation in the Horse: Internal Timing in an Elite Athlete. Journal of Equine Veterinary Science 76, 14–24. https://doi.org/10.1016/j.jevs.2019.02.026

Murphy, B.A., 2010. Chronobiology and the horse: Recent revelations and future directions. The Veterinary Journal 185, 105–114. https://doi.org/10.1016/j.tvjl.2009.04.013

Nicol, C.J., Davidson, H.P.D., Harris, P.A., Waters, A.J., Wilson, A.D., 2002. Study of crib-biting and gastric inflammation and ulceration in young horses. Veterinary Record 151, 658–662. https://doi.org/10.1136/vr.151.22.658

Ninomiya, S., Aoyama, M., Ujiie, Y., Kusunose, R., Kuwano, A., 2008. Effects of Bedding Material on the Lying Behavior in Stabled Horses. Journal of Equine Science 19, 53–56. https://doi.org/10.1294/jes.19.53

Nunes, L., Ampatzidis, Y., Costa, L., Wallau, M., 2021. Horse foraging behavior detection using sound recognition techniques and artificial intelligence. Computers and Electronics in Agriculture 183, 106080. https://doi.org/10.1016/j.compag.2021.106080

Okinda, Cedric, Nyalala, I., Korohou, T., Okinda, Celestine, Wang, J., Achieng, T., Wamalwa, P., Mang, T., Shen, M., 2020. A review on computer vision systems in monitoring of poultry: A welfare perspective. Artificial Intelligence in Agriculture 4, 184–208. https://doi.org/10.1016/j.aiia.2020.09.002

Oliveira, T., Santos, A., Silva, J., Trindade, P., Yamada, A., Jaramillo, F., Silva, L., Baccarin, R., 2022. Hospitalisation and Disease Severity Alter the Resting Pattern of Horses. Journal of Equine Veterinary Science 110, 103832. https://doi.org/10.1016/j.jevs.2021.103832

Pan, L., Lian, Z., Lan, L., 2012. Investigation of sleep quality under different temperatures based on subjective and physiological measurements. HVAC&R Research 18, 1030–1043. https://doi.org/10.1080/10789669.2012.667037

Papale, L.A., Andersen, M.L., Antunes, I.B., Alvarenga, T.A.F., Tufik, S., 2005. Sleep pattern in rats under different stress modalities. Brain Research 1060, 47–54. https://doi.org/10.1016/j.brainres.2005.08.021

Patel, A.K., Reddy, V., Shumway, K.R., Araujo, J.F., 2023. Physiology, Sleep Stages, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Pawluski, J., Jego, P., Henry, S., Bruchet, A., Palme, R., Coste, C., Hausberger, M., 2017. Low plasma cortisol and fecal cortisol metabolite measures as indicators of compromised welfare in domestic horses (Equus caballus). PLOS ONE 12, e0182257. https://doi.org/10.1371/journal.pone.0182257

Pawlyk, A.C., Morrison, A.R., Ross, R.J., Brennan, F.X., 2008. Stress-induced changes in sleep in rodents: Models and mechanisms. Neuroscience & Biobehavioral Reviews 32, 99–117. https://doi.org/10.1016/j.neubiorev.2007.06.001

Pedersen, G.R., Søndergaard, E., Ladewig, J., 2004. The influence of bedding on the time horses spend recumbent. Journal of Equine Veterinary Science 24, 153–158. https://doi.org/10.1016/j.jevs.2004.03.013

Pickering, P., Hockenhull, J., 2020. Optimising the Efficacy of Equine Welfare Communications: Do Equine Stakeholders Differ in Their Information-Seeking Behaviour and Communication Preferences? Animals 10, 21. https://doi.org/10.3390/ani10010021

Pierard, M., Hall, C., König von Borstel, U., Averis, A., Hawson, L., McLean, A., Nevison, C., Visser, K., McGreevy, P., 2015. Evolving protocols for research in equitation science. Journal of Veterinary Behavior: Clinical Applications and Research 10, 255–266. https://doi.org/10.1016/j.jveb.2015.01.006

Pilcher, J.J., Huffcutt, A.I., 1996. Effects of Sleep Deprivation on Performance: A Meta-Analysis. Sleep 19, 318–326. https://doi.org/10.1093/sleep/19.4.318

Prinz, P.N., Peskind, E.R., Vitaliano, P.P., Raskind, M.A., Eisdorfer, C., Zemcuznikov, N., Gerber, C.J., 1982. Changes in the sleep and waking EEGs of nondemented and demented elderly subjects. J Am Geriatr Soc 30, 86–93. https://doi.org/10.1111/j.1532-5415.1982.tb01279.x

Pritchett, L.C., Ulibarri, C., Roberts, M.C., Schneider, R.K., Sellon, D.C., 2003. Identification of potential physiological and behavioral indicators of postoperative pain in horses after exploratory celiotomy for colic. Applied Animal Behaviour Science 80, 31–43. https://doi.org/10.1016/S0168-1591(02)00205-8

R Core Team, 2020. R: A Language and environment for statistical computing.

R Core Team, 2014. R: A language and environment for statistical computing. R foudation for Statistical Computing, Vienna, Austria.URL https://www.R-project.org/.

Raabymagle, P., Ladewig, J., 2006. Lying behavior in horses in relation to box size. Journal of Equine Veterinary Science 26, 11–17. https://doi.org/10.1016/j.jevs.2005.11.015

Rasch, B., Born, J., 2013. About Sleep's Role in Memory. Physiological Reviews 93, 681–766. https://doi.org/10.1152/physrev.00032.2012

Raspa, F., Tarantola, M., Bergero, D., Nery, J., Visconti, A., Mastrazzo, C.M., Cavallini, D., Valvassori, E., Valle, E., 2020. Time-Budget of Horses Reared for Meat Production: Influence of Stocking Density on Behavioural Activities and Subsequent Welfare. Animals 10, 1334. https://doi.org/10.3390/ani10081334

Rattenborg, N.C., de la Iglesia, H.O., Kempenaers, B., Lesku, J.A., Meerlo, P., Scriba, M.F., 2017. Sleep research goes wild: new methods and approaches to investigate the ecology, evolution and functions of sleep. Philosophical Transactions of the Royal Society B: Biological Sciences 372, 20160251. https://doi.org/10.1098/rstb.2016.0251

Raw, Z., Rodrigues, J.B., Rickards, K., Ryding, J., Norris, S.L., Judge, A., Kubasiewicz, L.M., Watson, T.L., Little, H., Hart, B., Sullivan, R., Garrett, C., Burden, F.A., 2020. Equid Assessment, Research and Scoping (EARS): The Development and Implementation of a New Equid Welfare Assessment and Monitoring Tool. Animals 10, 297. https://doi.org/10.3390/ani10020297

Rechtschaffen, A., Bergmann, B.M., 2002. Sleep Deprivation in the Rat: An Update of the 1989 Paper. Sleep 25, 18–24. https://doi.org/10.1093/sleep/25.1.18

Ree, M. van der, Wijnberg, I., 2012. A review on epilepsy in the horse and the potential of Ambulatory EEG as a diagnostic tool. Veterinary Quarterly 32, 159–167. https://doi.org/10.1080/01652176.2012.744496

Richardson, K., Murray, J.-A.M.D., 2016. Fiber for Performance Horses: A Review. Journal of Equine Veterinary Science 46, 31–39. https://doi.org/10.1016/j.jevs.2016.02.234

Roebuck, A., Monasterio, V., Gederi, E., Osipov, M., Behar, J., Malhotra, A., Penzel, T., Clifford, G.D., 2013. A review of signals used in sleep analysis*. Physiol. Meas. 35, R1. https://doi.org/10.1088/0967-3334/35/1/R1

Roehrs, T., Hyde, M., Blaisdell, B., Greenwald, M., Roth, T., 2006. Sleep Loss and REM Sleep Loss are Hyperalgesic. Sleep 29, 145–151.

Rojas Vega, S., Hollmann, W., Strüder, H.K., 2012. Influences of Exercise and Training on the Circulating Concentration of Prolactin in Humans. Journal of Neuroendocrinology 24, 395–402. https://doi.org/10.1111/j.1365-2826.2011.02266.x

Ruckebusch, Y., 1972. The relevance of drowsiness in the circadian cycle of farm animals. Animal Behaviour 20, 637–643. https://doi.org/10.1016/S0003-3472(72)80136-2

Ruckebusch, Y., Barbey, P., Guillemot, P., 1970. Stages of sleep in the horse (Equus caballus). Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales 164, 658–665.

Rue, H., Riebler, A., Sørbye, S.H., Illian, J.B., Simpson, D.P., Lindgren, F.K., 2017. Bayesian Computing with INLA: A Review. Annual Review of Statistics and Its Application 4, 395–421. https://doi.org/10.1146/annurev-statistics-060116-054045

Rushen, J., Chapinal, N., de Passillé, A., 2012. Automated monitoring of behavioural-based animal welfare indicators. Animal Welfare 21, 339–350. https://doi.org/10.7120/09627286.21.3.339

Rushen, J., Weary, D.M., Smid, V., Girard, C., Hall, M., 2009. Code of practice for the care and handling of dairy cattle: Review of scientific research on priority issues. National Farm Animal Care Council:Lacombe, AB, Canada 63–65.

Russell, E., Koren, G., Rieder, M., Van Uum, S., 2012. Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. Psychoneuroendocrinology 37, 589–601. https://doi.org/10.1016/j.psyneuen.2011.09.009

Samuels, C., 2008. Sleep, Recovery, and Performance: The New Frontier in High-Performance Athletics. Neurologic Clinics, Sports Neurology 26, 169–180. https://doi.org/10.1016/j.ncl.2007.11.012

Sanei, S., Chambers, J. a., Sanei, S., Chambers, J. a., 2007. Introduction to EEG, in: EEG Signal Processing. John Wiley & Sons Ltd, pp. 1–34.

Sartori, C., Guzzo, N., Normando, S., Bailoni, L., Mantovani, R., 2017. Evaluation of behaviour in stabled draught horse foals fed diets with two protein levels. Animal 11, 147–155. https://doi.org/10.1017/S1751731116001221

Sauer, F.J., Hermann, M., Ramseyer, A., Burger, D., Riemer, S., Gerber, V., 2019. Effects of breed, management and personality on cortisol reactivity in sport horses. PLOS ONE 14, e0221794. https://doi.org/10.1371/journal.pone.0221794

Sauveroche, M., Henriksson, J., Theodorsson, E., Svensson Holm, A.-C., Roth, L.S.V., 2020. Hair cortisol in horses (Equus caballus) in relation to management regimes, personality, and breed. Journal of Veterinary Behavior 37, 1–7. https://doi.org/10.1016/j.jveb.2019.12.002

Schiffmann, C., Hoby, S., Wenker, C., Hård T., Scholz, R., Clauss, M., Hatt, J.M., 2018. When elephants fall asleep: A literature review on elephant rest with case studies on elephant falling bouts, and practical solutions for zoo elephants. Zoo Biology 0. https://doi.org/10.1002/zoo.21406

Schlupp, A., Anielski, P., Thieme, D., Müller, R.K., Meyer, H., Ellendorff, F., 2004. The beta-agonist clenbuterol in mane and tail hair of horses. Equine Vet J 36, 118–122. https://doi.org/10.2746/0425164044868620

Scrucca, L., Fop, M., Murphy, B.T., Raftery, A.E., 2016. mclust 5: Clustering, Classification and Density Estimation Using Gaussian Finite Mixture Models - PMC [WWW Document]. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5096736/ (accessed 3.3.24).

Siegel, J.M., 2001. The REM Sleep-Memory Consolidation Hypothesis. Science 294, 1058–1063.

Siegel, J.M., 2005a. Clues to the functions of mammalian sleep. Nature 437, 1264–1271. https://doi.org/10.1038/nature04285

Siegel, J.M., 2005b. Functional Implications of Sleep Development. PLOS Biol 3, e178. https://doi.org/10.1371/journal.pbio.0030178

Sinovich, M., Hewetson, M., Lombardi, M.F., Issard, P., Archer, D.C., 2021. Hospital-Based Validation of a Commercially Available Remote Activity Tracker for Determining Behaviour Changes, Including Colic, in Horses. Equine Veterinary Education 33, 22–22. https://doi.org/10.1111/eve.35_13534

Smith, C., 1995. Sleep states and memory processes. Behavioural Brain Research, The Function of Sleep 69, 137–145. https://doi.org/10.1016/0166-4328(95)00024-N

Squibb, K., Griffin, K., Favier, R., Ijichi, C., 2018. Poker Face: Discrepancies in behaviour and affective states in horses during stressful handling procedures. Applied Animal Behaviour Science 202, 34–38. https://doi.org/10.1016/j.applanim.2018.02.003

Steiger, A., 2002. Sleep and the hypothalamo–pituitary–adrenocortical system. Sleep Medicine Reviews 6, 125–138. https://doi.org/10.1053/smrv.2001.0159

Stephan, Y., Sutin, A.R., Bayard, S., Križan, Z., Terracciano, A., 2018. Personality and sleep quality: Evidence from four prospective studies. Health Psychol 37, 271–281. https://doi.org/10.1037/hea0000577

Steptoe, A., O'Donnell, K., Marmot, M., Wardle, J., 2008. Positive affect, psychological well-being, and good sleep. Journal of Psychosomatic Research 64, 409–415. https://doi.org/10.1016/j.jpsychores.2007.11.008

Stomp, M., d'Ingeo, S., Henry, S., Cousillas, H., Hausberger, M., 2021. Brain activity reflects (chronic) welfare state: Evidence from individual electroencephalography profiles in an animal model. Applied Animal Behaviour Science 236, 105271. https://doi.org/10.1016/j.applanim.2021.105271

St-Onge, M.-P., Mikic, A., Pietrolungo, C.E., 2016. Effects of Diet on Sleep Quality. Advances in Nutrition 7, 938–949. https://doi.org/10.3945/an.116.012336

Temesi, J., Arnal, P.J., Davranche, K., Bonnefoy, R., Levy, P., Verges, S., Millet, G.Y., 2013. Does Central Fatigue Explain Reduced Cycling after Complete Sleep Deprivation? Medicine & Science in Sports & Exercise 45, 2243–2253. https://doi.org/10.1249/MSS.0b013e31829ce379

The Swiss Federal Council Animal Welfare Ordinance (No. 455.1), 2008.

Tobler, I., 1995. Is sleep fundamentally different between mammalian species? Behavioural Brain Research, The Function of Sleep 69, 35–41. https://doi.org/10.1016/0166-4328(95)00025-O

Tortonese, D.J., Preedy, D.F., Hesketh, S.A., Webb, H.N., Wilkinson, E.S., Allen, W.R., Fuller, C.J., Townsend, J., Short, R.V., 2011. Experimental Jetlag Disrupts Circadian Clock Genes But Improves Performance in Racehorses After Light-Dependent Rapid Resetting of Neuroendocrine Systems and the Rest-Activity Cycle. Journal of Neuroendocrinology 23, 1263–1272. https://doi.org/10.1111/j.1365-2826.2011.02222.x

Tucker, C.B., Jensen, M.B., de Passillé, A.M., Hänninen, L., Rushen, J., 2021. Invited review: Lying time and the welfare of dairy cows. Journal of Dairy Science 104, 20–46. https://doi.org/10.3168/jds.2019-18074

Uchida, S., Soya, S., Saito, Y.C., Hirano, A., Koga, K., Tsuda, M., Abe, M., Sakimura, K., Sakurai, T., 2021. A Discrete Glycinergic Neuronal Population in the Ventromedial Medulla That Induces Muscle Atonia during REM Sleep and Cataplexy in Mice. J. Neurosci. 41, 1582–1596. https://doi.org/10.1523/JNEUROSCI.0688-20.2020

Velluti, R., 1997. Interactions between sleep and sensory physiology. Journal of Sleep Research 6, 61–77. https://doi.org/10.1046/j.1365-2869.1997.00031.x

Visser, E.K., Ellis, A.D., Van Reenen, C.G., 2008. The effect of two different housing conditions on the welfare of young horses stabled for the first time. Applied Animal Behaviour Science 114, 521–533. https://doi.org/10.1016/j.applanim.2008.03.003

Volkow, N.D., Tomasi, D., Wang, G.-J., Telang, F., Fowler, J.S., Logan, J., Benveniste, H., Kim, R., Thanos, P.K., Ferré, S., 2012. Evidence That Sleep Deprivation Downregulates Dopamine D2R in Ventral Striatum in the Human Brain. J. Neurosci. 32, 6711–6717. https://doi.org/10.1523/JNEUROSCI.0045-12.2012

von Borell, E., Langbein, J., Després, G., Hansen, S., Leterrier, C., Marchant-Forde, J., Marchant-Forde, R., Minero, M., Mohr, E., Prunier, A., Valance, D., Veissier, I., 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals — A review. Physiology & Behavior, Stress and Welfare in Farm Animals 92, 293–316. https://doi.org/10.1016/j.physbeh.2007.01.007

Walker, M.P., 2010. Sleep, memory and emotion, in: Kerkhof, G.A., Dongen, H.P.A. van (Eds.), Progress in Brain Research. Elsevier, pp. 49–68. https://doi.org/10.1016/B978-0-444-53702-7.00004-X

Walsh, C.M., Prendergast, R.L., Sheridan, J.T., Murphy, B.A., 2013. Blue light from light-emitting diodes directed at a single eye elicits a dose-dependent suppression of melatonin in horses. The Veterinary Journal 196, 231–235. https://doi.org/10.1016/j.tvjl.2012.09.003

Werhahn, H., Hessel, E.F., Van den Weghe, H.F.A., 2012. Competition Horses Housed in Single Stalls (II): Effects of Free Exercise on the Behavior in the Stable, the Behavior during Training, and the Degree of Stress. Journal of Equine Veterinary Science 32, 22–31. https://doi.org/10.1016/j.jevs.2011.06.009

Whitham, J., Miller, L., 2016. Using technology to monitor and improve zoo animal welfare. Animal Welfare 25, 395–409. https://doi.org/10.7120/09627286.25.4.395

Whitham, J.C., Wielebnowski, N., 2013. New directions for zoo animal welfare science. Applied Animal Behaviour Science, Welfare of Zoo Animals 147, 247–260. https://doi.org/10.1016/j.applanim.2013.02.004

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer.

Williams, D. c., Aleman, M., Holliday, T. a., Fletcher, D. j., Tharp, B., Kass, P. h., Steffey, E. p., LeCouteur, R. a., 2008. Qualitative and Quantitative Characteristics of the Electroencephalogram in Normal Horses during Spontaneous Drowsiness and Sleep. Journal of Veterinary Internal Medicine 22, 630–638. https://doi.org/10.1111/j.1939-1676.2008.0096.x

Wöhr, A.-C., Kalus, M., Fuchs, C., Erhard, M., 2016. Equine Sleep Behaviour and Physiology Based on Polysomnographic Examinations. Equine Vet J 48, 9–9. https://doi.org/10.1111/evj.08_12612

Wu, D., Wang, Y., Han, M., Song, L., Shang, Y., Zhang, X., Song, H., 2021. Using a CNN-LSTM for basic behaviors detection of a single dairy cow in a complex environment. Computers and Electronics in Agriculture 182, 106016. https://doi.org/10.1016/j.compag.2021.106016

Wurtz, K., Camerlink, I., D'Eath, R.B., Fernández, A.P., Norton, T., Steibel, J., Siegford, J., 2019. Recording behaviour of indoor-housed farm animals automatically using machine vision technology: A systematic review. PLOS ONE 14, e0226669. https://doi.org/10.1371/journal.pone.0226669

Yamazaki, R., Toda, H., Libourel, P.-A., Hayashi, Y., Vogt, K.E., Sakurai, T., 2020. Evolutionary Origin of Distinct NREM and REM Sleep. Front Psychol 11, 567618. https://doi.org/10.3389/fpsyg.2020.567618

Yarnell, K., Hall, C., Royle, C., Walker, S.L., 2015. Domesticated horses differ in their behavioural and physiological responses to isolated and group housing. Physiology & Behavior 143, 51–57. https://doi.org/10.1016/j.physbeh.2015.02.040

Zepelin, H., Rechtschaffen, A., 1974. Mammalian sleep, longevity, and energy metabolism. Brain, Behavior and Evolution 10, 425–470. https://doi.org/10.1159/000124330

Zepelin, H., Siegel, J.M., Tobler, I., 2005. Mammalian Sleep, in: Principles and Practice of Sleep Medicine. pp. 91–100.

Zhang, Y., Shen, J., Zhou, Z., Sang, L., Zhuang, X., Chu, M., Tian, T., Xiao, J., Lian, Y., 2020. Relationships among shift work, hair cortisol concentration and sleep disorders: a cross-sectional study in China. BMJ Open 10, e038786. https://doi.org/10.1136/bmjopen-2020-038786

Appendix I

Questionnaire distributed to horse owners about horse sleep as a care priority, knowledge and management of horse rest patterns.

Horse care survey

Page 1-Privacy and consent

This survey forms part of the studies required to complete my PhD project at Nottingham Trent University.

- The following questionnaire has been designed to be filled out by people who are the primary carer for at least 1 horse or pony.
- Questions relate to carer perceptions of the importance of different aspects of horse care.
- Data collected may be used for other research activities, including subsequent publication.
- All individual response information will be stored on a password-protected system and will only be accessible to the research team. No personal information will be passed on to third parties.
- This survey should take no longer than 10 minutes for a single horse and an additional 5 minutes for each horse thereafter.
- All participants must be 18 or over.
- Your responses are anonymous and will only be used once any identifying features are removed.
- You can choose to stop and withdraw from the study at any time during the survey by closing the browser.

By submitting your data at the end of this questionnaire you assent to their use, unless withdrawn. If you wish to withdraw after completing the survey, you can do so by e-mailing the study lead with your unique participant number (that you will receive after completing the survey) by the 01/03/2020. Any contributions made to the project will be destroyed and data removed by that date. After this period, data will be anonymised in preparation for analyses, therefore responses cannot be individually identified and so cannot be withdrawn.

Compliance with the Research Data Management Policy

Nottingham Trent University is committed to respecting the ethical code of conducts of the United Kingdom Research Councils. Thus, in accordance with procedures for transparency and scientific verification, the University will conserve all information and data collected during your survey in line with the University Policy and RCUK Common Principles on Data Policy (http://www.rcuk.ac.uk/research/datapolicy/) and the relevant legislative frameworks. The final data will be retained in accordance with the Retention Policy. All data will be anonymised and made available to be re-used in this form where appropriate and under appropriate safeguards.

For more information about this study then please contact the study lead Kym Griffin:

Kym.griffin@ntu.ac.uk

+44 (0)115 848 5204

Q1. I declare my consent:

- ☐ I have read and understand the above information

- ☐ I consent to the information I provide to be used for the purposes of research, including potential publication
- ☐ I confirm I am 18 years or older

Page 2 – Carer information

Q2. In which country do you live?

- o UK
- o Ireland
- o Austria
- o Belgium
- o Denmark
- o Estonia
- o Finland
- o France
- o Germany
- o Italy
- o Lithuania
- o Netherlands
- o Poland
- o Portugal
- o Spain
- o Sweden
- o Other: *single line free text*

Q3. Gender

- o Male
- o Female
- o Prefer not to say
- o Other: *single line free text*

Q4. What age category are you?

- o 18-24
- o 25-34
- o 35-44
- o 45-54
- o 55-64
- o >65

Q5. Approximately how many years have you cared for horses?

- o 1 year or less
- o 2-5 years
- o 6-10 years
- o 11-15 years
- o 16-20 years

- >20 years

Q6. Do you have any formal equine related qualifications (please select all that apply)?

- No formal equine related training
- Training method specific formal training (e.g. Monty Roberts Training, Parelli Natural Horsemanship etc.)
- British Horse Society Qualification (or equivalent)
- Pony Club Training
- Equine related National Vocational Qualification
- Equine related undergraduate degree
- Equine related postgraduate degree
- Other: *Multiple line free text*

Q7. What discipline/s would you describe yourself as belonging to (please select all that apply)?

- I don't ride
- Leisure rider
- All round/riding club activities
- Eventing
- Showjumping
- Dressage
- Western
- Polo
- Driving
- Endurance
- Other: *Multiple lines free text*

Q8. Do you currently compete in any discipline?

- Yes
- No -> *Q9.*

Q8a. What discipline/s:

Multiple lines free text

Q9. Do you consider yourself an equine professional where you derive the majority or all your income in a horse care and/or riding role?

- Yes
- No

Q10. How many horses are in your care?

- 1
- 2
- 3

- 4
- 5 or more

Page 3 – Horse information

Please fill out the following part of the survey with details of one horse only. There is an opportunity to complete this section for up to 5 horses later in the survey if you wish. Please fill in the survey in order of horse name that comes first alphabetically.

Q11. Horse's name:

Single line free text

Q12. Horse's approximate age:

- 1 year or less
- 2-4
- 5-9
- 10-14
- 15-19
- 20-24
- 25 years or more

Q13. Horse breed/s (where a horse is a cross of multiple breeds please select multiple boxes):

- ☐ Arabian
- ☐ Appaloosa
- ☐ Cob type
- ☐ Connemara
- ☐ Clydesdale
- ☐ Friesian
- ☐ Haflinger
- ☐ Iberian (including Andalusian, Lusitano, Lippizzaner etc.)
- ☐ Irish draught
- ☐ Shetland
- ☐ Shire
- ☐ Standardbred
- ☐ Sport horse type
- ☐ Thoroughbred
- ☐ Warmblood
- ☐ Welsh pony
- ☐ Welsh cob
- ☐ Quarter horse
- ☐ Other*: Single line free text*

Q14. Horse's approximate height:

- less than or equal to 9hh (91.5 cm)
- 9.1 - 12hh (92 - 122 cm)
- 12.1 - 13hh (123 - 132 cm)
- 13.1 - 14hh (133 - 142 cm)
- 14.1 - 15hh (143 - 152 cm)
- 15.1 - 16hh (153 - 162 cm)
- 16.1 - 17hh (163 - 172 cm)
- 17.1 - 18hh (173 - 182 cm)
- 18hh (183 cm) or taller

Q15. Has this horse previously or currently been diagnosed with an ongoing medical condition (please select all that apply)?

- ☐ No known medical condition
- ☐ Arthritis
- ☐ Laminitis
- ☐ Neck or back pain
- ☐ Recurring colic
- ☐ Stomach ulcers
- ☐ Ligament or tendon damage
- ☐ Rhabdomyolysis (tying-up)
- ☐ Equine Metabolic Syndrome
- ☐ PPID
- ☐ Other: *Single line free text*

Q16. Indicate how important you consider each aspect of care for this horse:

	Important	Somewhat important	Neither important or unimportant	Somewhat unimportant	Unimportant	Unsure
Forage feed (grass, hay, haylage)						
Exercise (either ridden or from the ground)						
Turnout						
Dry place to lie down						
Regular grooming						
Rugging in cold weather						
Social contact						

Q16a. Further comments if required: *Multiple lines free text*

Q17. Typically, how many months of the year would this horse spend stabled for either part or all of the day?

- Never or the occasional day -> *Q18.*
- 1-2 months
- 3-4 months
- 5-6 months
- 6-7 months
- 8-9 months
- 10-12 months

Q17a. Approximately how many hours in the day is this horse in the stable?

- 0-2 hours -> *Q18.*
- 3-5 hours
- 6-10 hours
- 11-15 hours
- 16-20 hours
- 21 hours or more
- Other: *Single line free text*

Q17b. When stabled, does this horse experience social contact with other horses:

- Part contact (can touch other horses over a wall or fence)
- In sight (can see but not touch other horses)
- No contact (cannot see or touch other horses)
- Other: *Multiple lines free text*

Q18. When not stabled, does this horse experience social contact with other horses:

- Full contact (in a field or barn together)
- Part contact (can touch other horses over a wall or fence)
- In sight (can see but not touch other horses)
- No contact (cannot see or touch other horses)
- Other: *Multiple lines free text*

Q19. Does this horse have access to (please select all that apply)?

- Grass all year
- Grass part of the year
- Hay all year
- Hay part of the year
- Haylage all year
- Haylage part of the year
- This horse doesn't have access to forage

Q20. On an average day, approximately when is the earliest 'first check' made on this horse by any person?

Time question: _ _:_ _

Q21. On an average day, approximately when is the latest 'last check' made on this horse by any person?

Time question: _ _:_ _

Q22. Do you ever observe this horse at night, either directly via CCTV or other methods?

- Yes, frequently
- Yes, occasionally
- No, never

Q23. What is most important for this horse? Rank the following in order of importance, where 1 = most important and 4 = least important. Each item must be given a unique rank based on what you feel you could or could not compromise on for this horse.

	1 (most important)	2	3	4 (least important)
Exercise (either ridden or from the ground)				
Regular grooming				
A dry place to lie down				
Rugging in cold weather				

Q24. On average in the summer months, how many occasions do you see your horse lying down to rest over the entirety of the summer (not including rolling)?

- Never or very rarely
- 1-2 times
- 3-5 times
- 6-8 times
- More than 8 times
- I don't know

Q25. On average in the winter months, how many occasions do you see this horse lying down to rest over the entirety of the winter (not including rolling)?

- Never or very rarely

- 1-2 times
- 3-5 times
- 6-8 times
- More than 8 times
- I don't know

Q26. Do you monitor this horse's rest pattern? If so, how?

Multiple line free text

Q27. Any further comments relating to the care of this horse?

Multiple line free text

Q28. Would you like to fill in this survey for another horse?

- Yes -> *Page 4 Horse 2 information (This is a copy of page 3 and can be completed for up to 5 horses)*
- No

Page 8 – Contact details

Q100. If you would like to be updated on the results of this study, then please enter your email in the space below and you will be added to the mailing list:

Single line free text

Page 9 - Final Page

Thank you for your time, your answers will go towards our understanding of what elements of horse care people find important :)

If you have any further questions, then please don't hesitate to contact me at:

Kym Griffin

kym.griffin@ntu.ac.uk

+44 (0)115 848 5204

(On this page participants will receive their randomly generated code to withdraw their data later if they wish)

Appendix II

Individual details of demographics and management of horses used in Chapter 3 to monitor rest patterns over consecutive nights.

Horse	Sex	Age	Height (cm)	Weight (kg)	Feed	% time lying on each side	Additional notes
H1	G	14	164	604	Morning (7:15am) - 1kg *pasture cubes Night (5:00pm) – 1kg **chaff and 1kg pasture cubes	Left: 25% Right: 75%	
H2	M	10	172	652	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes	Left: 77.27% Right: 22.73%	
H3	G	11	164	620	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes Supplement – 7g Cortaflex once a day	Left: 100% Right: 0%	Diagnosed in 2012 with ossification of the lateral cartilage and a fracture at its base in the right hind limb. Was treated with 6 months rest and full rehabilitation
H4	G	13	162	626	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes	Left: 58.82% Right: 41.18%	
H5	G	14	164	668	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 2kg pasture cubes	Left: 50% Right: 50%	
H6	G	11	162	610	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes	Left: 27.59% Right: 72.41%	
H7	G	18	154	512	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes	Left: 84.62% Right: 15.38%	
H8	G	25	154	592	Morning (7:15am) - 1kg pasture cubes	Left: 71.43%	

					Night (5:00pm) – 1kg chaff and 2kg pasture cubes Supplement – 7g Cortaflex once a day	Right: 28.57%	
H9	M	18	162	550	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 2kg pasture cubes Supplement – 7g Cortaflex once a day	Left: 75% Right: 25%	
H10	G	15	154	564	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes Supplement – 7g Cortaflex once a day	Left: 37.04% Right: 62.96%	Hay fed is slow feeder resulting in longer feeding times
H11	G	17	174	644	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 2kg pasture cubes Supplement – 14g Cortaflex once a day	Left: 50% Right: 50%	Very mild weaving
H12	M	21	154	512	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 2kg pasture cubes Supplement – 7g Cortaflex once a day	Left: 35.71% Right: 64.29%	Chronic windsucker
H13	G	13	165	624	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes	Left: 37.04% Right: 62.96%	
H14	G	14	164	618	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes Supplement – 7g Cortaflex once a day	Left: 41.67% Right: 58.33%	

* Dodson and Horrell Pasture Cubes
**Ossi Original Chaff

Appendix III

Frequency count of recumbent bouts per horse and nights they occurred. 'NA' represents no recording on that night due to technical problems and 'Out' represents no recording as the horse was turned out on that night. One horse was moved to a different stable after 5 nights recording represented by 'Moved'. Shaded areas represent weekends when horses were not ridden but were either turned out from 10am-3pm or for the entire weekend.

Horse	Night 1	Night 2	Night 3	Night 4	Night 5	Night 6	Night 7
H01	NA	3	1	3	0	2	3
H02	5	4	4	3	3	5	5
H03	3	4	3	3	Out	Out	Out
H04	4	5	6	5	Out	Out	Out
H05	2	2	2	2	2	0	2
H06	3	4	5	3	5	4	5
H07	NA	2	2	3	2	2	2
H08	NA	3	1	0	1	1	1
H09	3	3	3	3	3	2	2
H10	3	3	6	4	4	3	4
H11	3	3	4	2	3	3	4
H12	NA	3	5	3	Out	Out	3
H13	4	4	4	4	3	4	4
H14	2	4	2	2	2	Moved	Moved

Appendix IV

Personality questionnaire completed by two members of yard staff for each horse

Equine Personality Test

Horse name: ________________________

The questionnaire asks about your observations of your horse's behaviour and its interaction with you, other people and other horses. **It is important that you answer the questions as truthfully as possible** (warts and all!).

Below are pairs of words joined by a line. Please put a cross on the line at the point that best describes this horse. For example a fairly spirited rather than a steady horse might be scored as:

Spirited |-----------X---| Steady

Easy-going |---| Intolerant

Argumentative |---| Well-mannered

Anxious |---| Confident

Obedient	|---|	Wayward
Sluggish	|---|	Forward-going
Willing	|---|	Stubborn
Placid	|---|	Active
Gentle	|---|	Rough
Adventurous	|---|	Habitual
Excitable	|---|	Laid-back
Nervous	|---|	Calm
Spirited	|---|	Steady
Relaxed	|---|	Tense
Quiet	|---|	Restless
Friendly	|---|	Standoffish

Please circle the number that indicates how often you have seen this horse behave in the following manner:

	Never		Some-times		Always
1. When it has the opportunity, how often does this horse initiate interaction with you?	1	2	3	4	5
2. When it has the opportunity, how often does this horse initiate interaction with other people?	1	2	3	4	5
3. When it has the opportunity, how often does this horse initiate interaction with other horses?	1	2	3	4	5

4. Does this horse ever show affection towards other horses? 1 2 3 4 5

For the following questions please circle the number that best describes to you this horse's behaviour with regard to the following traits:

	Not at all		Moderately		Very much
5. In general how fearful is this horse around other horses?	1	2	3	4	5
6. Generally how energetic would you say this horse is?	1	2	3	4	5
7. Generally how dependable would you say this horse is?	1	2	3	4	5

Appendix V

Individual demographics and management of horses used in Chapter 4 for long term sleep and cortisol study.

Horse	Nights monitored	Sex	Age	Height (cm)	Weight (kg)	Feed	Nights without lying down	Additional notes
*H1	20	G	16	164	604	Morning (7:15am) - 1kg *pasture cubes Night (5:00pm) – 1kg **chaff and 1kg pasture cubes	7/20	
*H2	21	M	12	172	652	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes	0	
*H3	21	M	23	154	512	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and	0	Chronic windsucker

						2kg pasture cubes Supplement – 7g Cortaflex once a day		
*H4	28	G	15	165	624	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes	0	Very mild weaver
*H5	20	G	16	164	668	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 2kg pasture cubes	0	
H6	28	G	8	162	502	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes	0	
H7	20	G	14	152	444	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes Supplement – 7g Cortaflex once a day	0	
H8	28	M	13	178	666	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 2kg pasture cubes Supplement – 7g Cortaflex once a day	0	
H9	18	M	12	155	564	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and	0	

						2kg pasture cubes Supplement – 7g Cortaflex once a day		
*H10	20	G	17	154	564	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes Supplement – 7g Cortaflex once a day	0	
H11	28	G	13	165	592	Morning (7:15am) - 1kg pasture cubes Night (5:00pm) – 1kg chaff and 1kg pasture cubes Supplement – 7g Cortaflex once a day	0	

*Denotes horse who participated in studies reported in chapter 3.

Appendix VI

Summary of nights recorded for each horse for long term behavioural analysis of rest patterns, nights represent the 1-28 February 2020. Dark squares indicate a non-recording night as horse was not in their stable at that time. The difference in routine is due to individual horse management as some horses are turned out more often than others. Nights 17-23 were midterm break when some horses were turned out for the full week.

		Night recorded																											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Horse	H1																												
	H2																												
	H3																												
	H4																												
	H5																												
	H6																												
	H7																												
	H8																												
	H9																												
	H10																												

Appendix VII

Synchrony of lying behaviour in each stable block on nights 9 to 13 where grey cells represent when a lying bout has occurred. Orange highlighted cells show where two horses either immediately beside or across from one another were lying at the same time. Yellow highlights when lying bouts were initiated within 15 minutes of each other and numbers in highlighted cells denotes exact time of lying bout initiation. Double lines denote the stable corridor.

	Day 9 - Stable block 1																								
Stable	**18:00**	**18:30**	**19:00**	**19:30**	**20:00**	**20:30**	**21:00**	**21:30**	**22:00**	**22:30**	**23:00**	**23:30**	**00:00**	**00:30**	**01:00**	**01:30**	**02:00**	**02:30**	**03:00**	**03:30**	**04:00**	**04:30**	**05:00**	**05:30**	**06:00**
S1																							04:32		
S2																			02:53				04:46		
S3																									
S4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S5																									
S6																									
S7																									
S8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S9																			02:43				04:52		
	Day 9 - stable block 2																								
S12									21:40						00:38					02:52					
S13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S17																									
S18																									
S19									21:24						00:37					03:16					

Day 10 - Stable block 1

Stable	18:00	18:30	19:00	19:30	20:00	20:30	21:00	21:30	22:00	22:30	23:00	23:30	00:00	00:30	01:00	01:30	02:00	02:30	03:00	03:30	04:00	04:30	05:00	05:30	06:00
S1																		02:22							
S2																		02:01							
S3																									
S4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S5																									
S6																									
S7																									
S8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S9																									
	Day 10 - Stable block 2																								
S12																									
S13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S17																							04:33		
S18																							04:33		
S19																									

	Day 11 - Stable block 1																								
Stable	**18:00**	**18:30**	**19:00**	**19:30**	**20:00**	**20:30**	**21:00**	**21:30**	**22:00**	**22:30**	**23:00**	**23:30**	**00:00**	**00:30**	**01:00**	**01:30**	**02:00**	**02:30**	**03:00**	**03:30**	**04:00**	**04:30**	**05:00**	**05:30**	**06:00**
S1													23:41						02:37						
S2													23:38						02:48						
S3																			02:45						
S4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S5																									
S6																									
S7																									
S8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S9																			02:42						
	Day 11 - Stable block 2																								
S12																				03:22					
S13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S17																									
S18											22:58			00:20											
S19											22:48			00:21						03:25					

	Day 12 - stable block 1																								
Stable	**18:00**	**18:30**	**19:00**	**19:30**	**20:00**	**20:30**	**21:00**	**21:30**	**22:00**	**22:30**	**23:00**	**23:30**	**00:00**	**00:30**	**01:00**	**01:30**	**02:00**	**02:30**	**03:00**	**03:30**	**04:00**	**04:30**	**05:00**	**05:30**	**06:00**
S1														23:59											
S2														00:26											05:37
S3																									
S4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S5																									
S6																									
S7																									
S8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S9																									05:55
	Day 12 - Stable block 2																								
S12															00:53										
S13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S17															00:46										
S18															00:41										
S19															00:48										

	Day 13 - stable block 1																								
Stable	**18:00**	**18:30**	**19:00**	**19:30**	**20:00**	**20:30**	**21:00**	**21:30**	**22:00**	**22:30**	**23:00**	**23:30**	**00:00**	**00:30**	**01:00**	**01:30**	**02:00**	**02:30**	**03:00**	**03:30**	**04:00**	**04:30**	**05:00**	**05:30**	**06:00**
S1												23:11													
S2											22:50	22:50													
S3											22:51														
S4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S5																					03:22	03:22			
S6																					03:53	03:53			
S7																						04:09			
S8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S9												22:38													
	Day 13 - Stable block 2																								
S12																									
S13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S17												23:08				01:10							04:41		
S18												23:21				01:19							04:33		
S19																									

Appendix VIII

Appendix VIII and Appendix IX present other work carried out as part of the PhD but not reported in the main body of the thesis for various reasons.

Chapter 3

Fine scale behaviour methods: The decision was made to use continuous video observations for the study in Chapter 3 in an attempt to identify fine scale behavioural signs that may provide a more accurate indicator of sleep state. Specifically, these included facial movements including the eyes ears and muzzle. Despite attempts to place multiple cameras at a lower angle to capture this information, the videos were not close enough to capture more fine scale behaviours. Although, I did find at night eyelid position was easier to see due to the glowing eyes caused by infrared light, the videos were not of good enough quality to be sure about these fine scale movements, hence I decided to focus on body posture which also has the advantage of being easier for untrained observers to identify.

Heart rate: in conjunction with fine scale behavioural measures, I also attempted to use Polar Equine Heart Rate Monitors (HRM) to identify cardiovascular changes associated with different stages of sleep (irregular heart rate). I found HRM always stopped working after 4 hours of being fitted (they were fitted around 5pm each day). The HRM are required to be wet in order to use electrical conduction to detect the heart rate; 2 hours was the amount of time it took for the horse's body temperature to dry out the HRM. The use of conductive gel lengthened the time before drying out to 4 hours but ultimately it was decided the Polar Equine was not going to be appropriate for this purpose and an ECG would be required.

Electroencephalogram (EEG), electrooculogram (EoG), electrocardiogram (ECG): To validate the behavioural measures, I obtained the short-term loan of a portable EEG device. I attempted on five occasions to use this on a horse overnight. After every attempt, most if not all the electrodes had detached by the morning. When reviewing the cameras, the horse made no attempt to rub their face and I could only conclude that the glue used did not last the whole night. When conversing with a colleague who was successful in obtaining overnight readings, I believe this was due to the presence of horsehair under the electrodes. The horse I was using had a clipped face, but I did not obtain permission to shave the area of electrode placement to the skin. I believe this caused issues with the electrodes remaining in place for the duration of the night.

Accelerometers: During data collection for Chapter 3 all horses were fitted with accelerometers for the duration of their observation period. The accelerometers used only gave the raw data. While an initial pilot study showed good accuracy in terms of identifying standing, sternal and lateral recumbency when located on the front foreleg (as opposed to a hind leg or the head (figure 1) I struggled with the analysis part of this project. I attempted to collaborate with someone with a computer engineering background to develop an algorithm for the accelerometers but ultimately was unable to find anyone with the time. In the end I felt video observations would be better and used scan samples for the long-term observations to save time. The accelerometer data and video observations are still available and could be utilised in future studies.

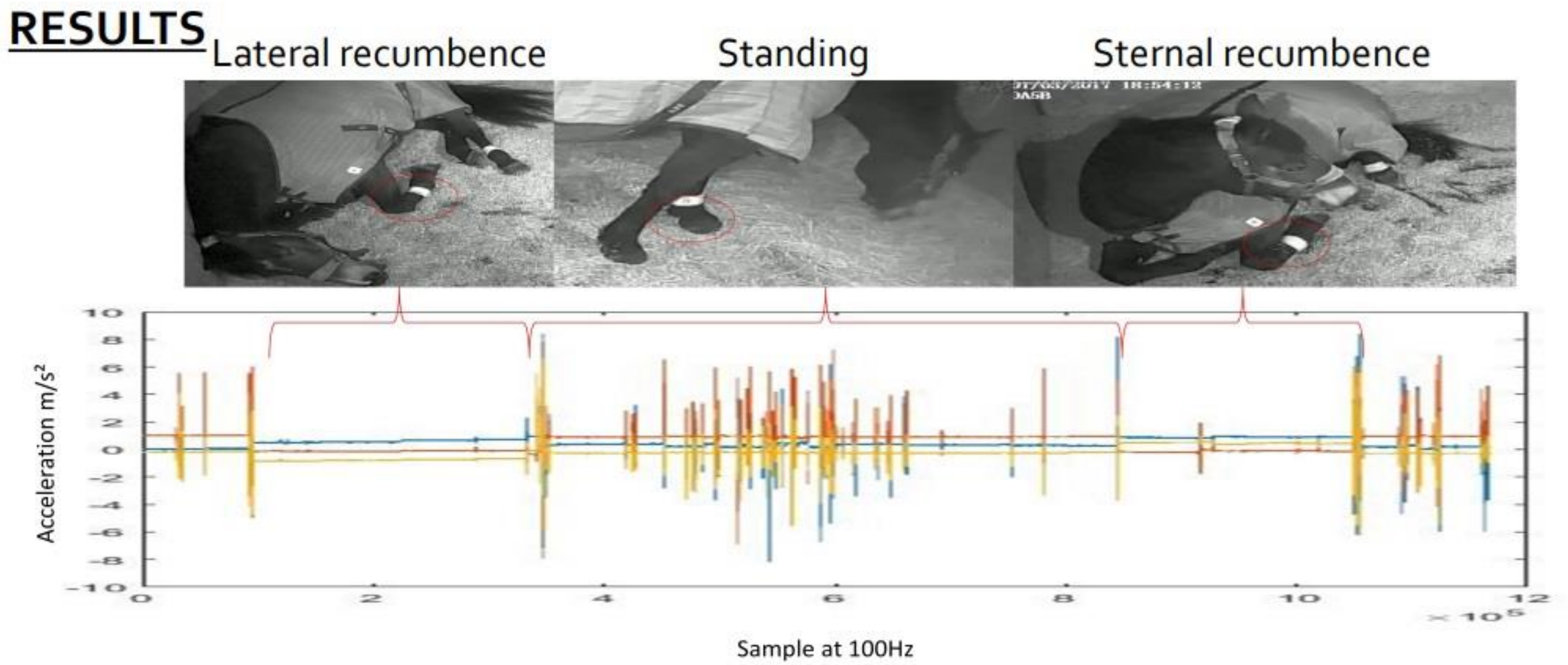
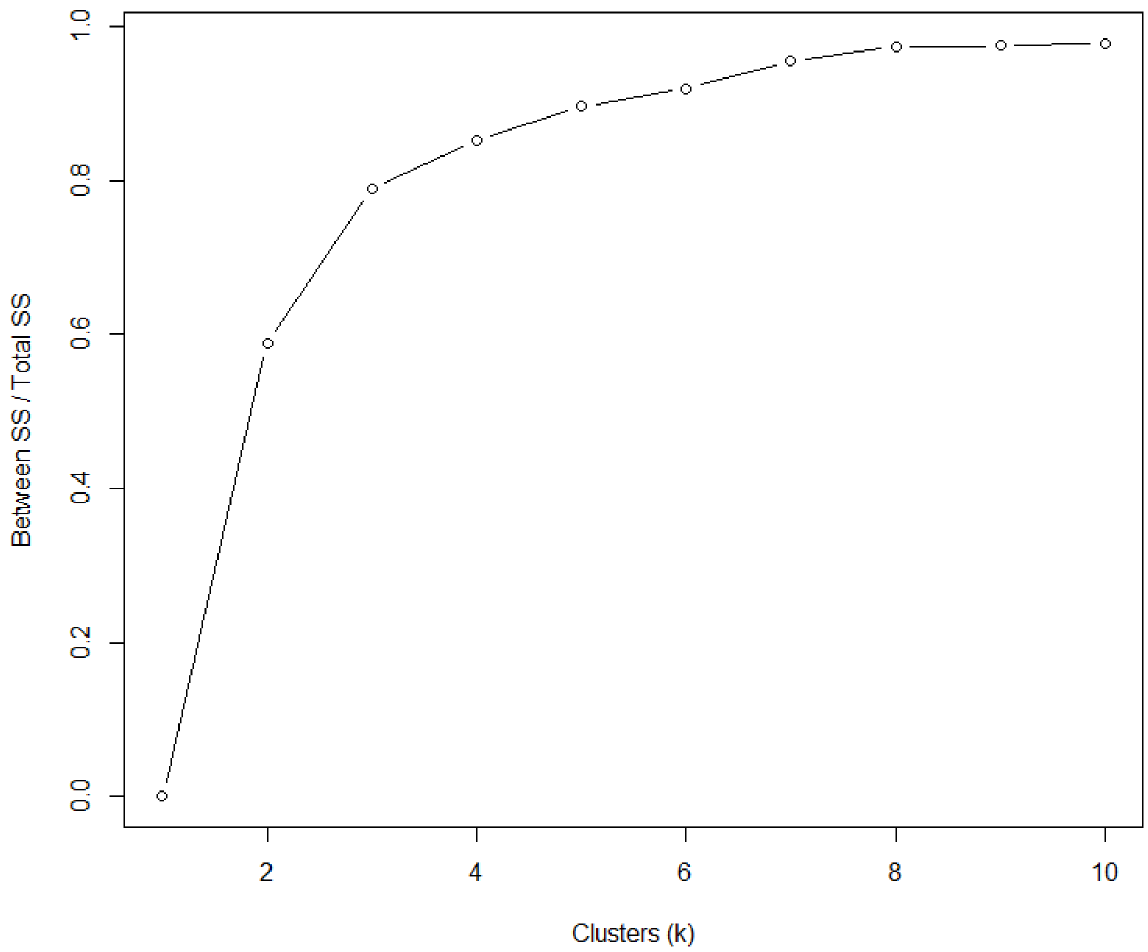


Figure 1: Accelerometer reading for standing, lateral and sternal recumbency when located on the front foreleg.

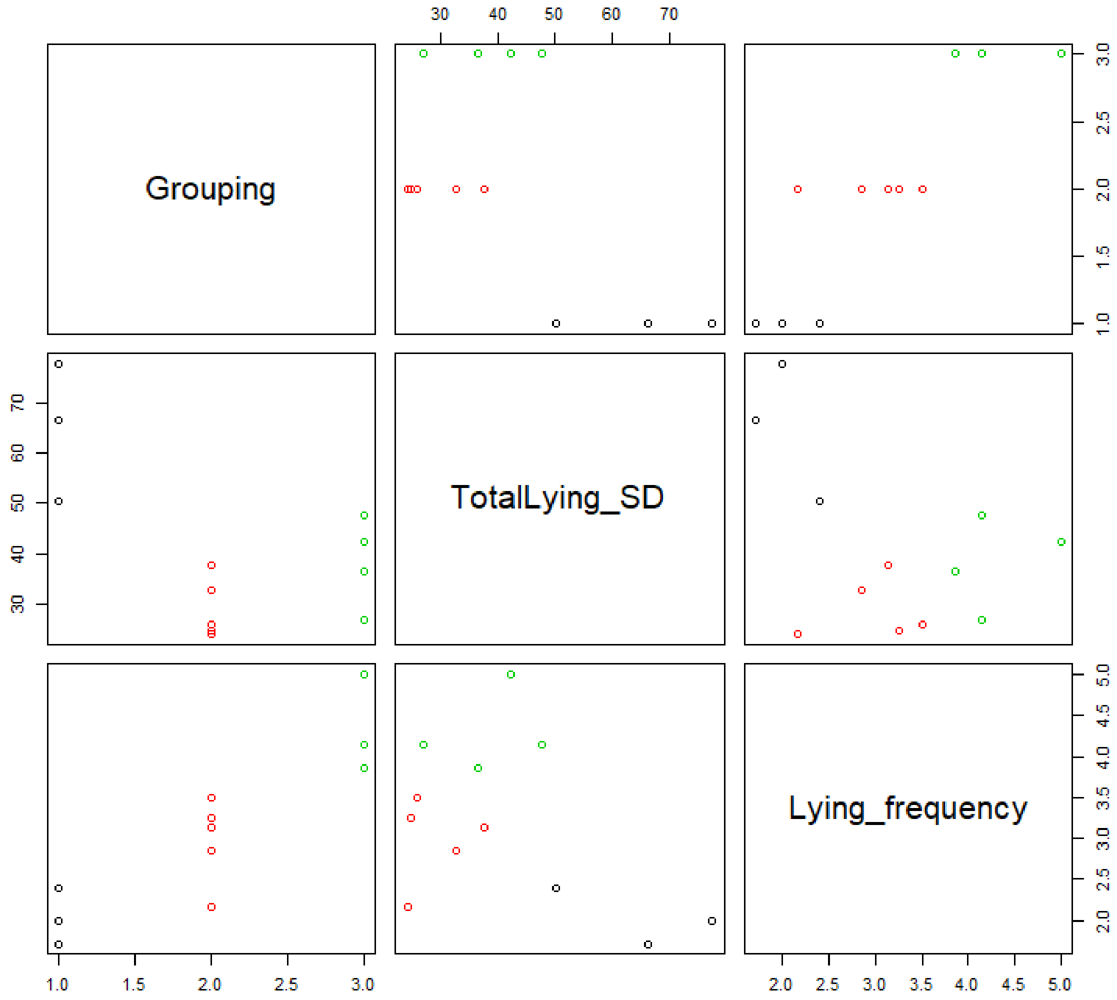
<u>Additional cluster analysis results:</u> Variability of rest patterns represented as the SD of the total time spent lying down and the mean frequency of lying over all nights recorded for each horse. I compared the results of three types of clustering analysis to compare results: K-means Clustering, Hierarchical clustering (using complete linkage method) and model-based cluster (using mclust package in R). Ultimately, I didn't feel my sample size was big enough for the results to be reliable and therefore decided to exclude from the main body of the thesis. However, pilot results are as follows:

K-means clustering



This graph is interpreted by looking at where there is the sharpest 'shoulder' as the point where the best number of groups is located. With mine it isn't very clear, but I would say three groups.

Three groups



This shows three groups:

Group 1 (black): Low lying frequency but high variability in time spent resting each night.

Group 2 (Red): Low frequency of lying but low variability in time spent resting.

Group 3 (Green): High frequency and low variability in time spent resting.

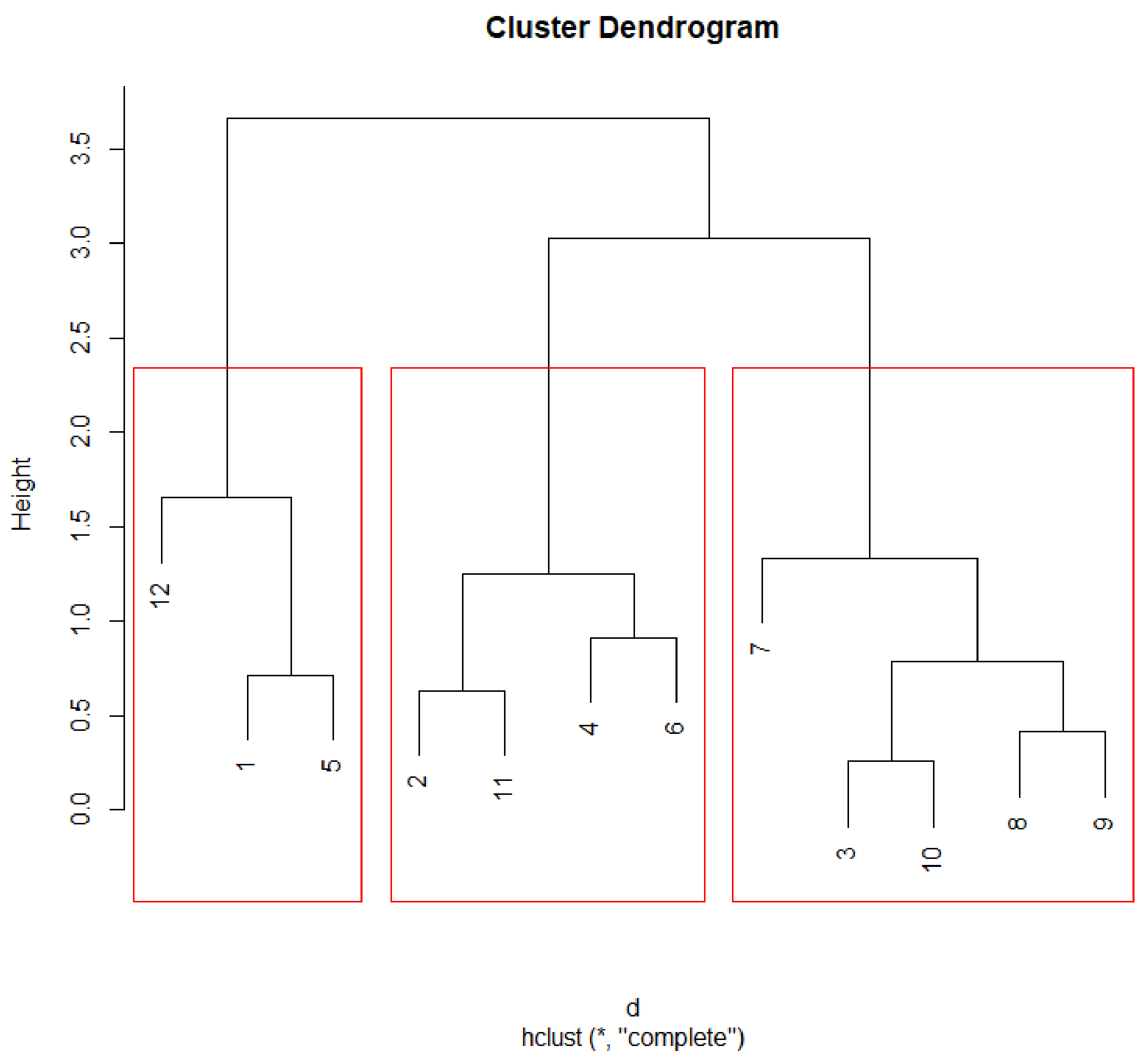
Stats of results:

```
K-means clustering with 3 clusters of sizes 3, 4, 5

Cluster means:

      TotSD        LyF

1  1.3903245 -1.1423302

2 -0.1596250  1.1038626

3 -0.7064947 -0.1976919

Clustering vector:

 [1] 1 2 3 2 1 2 3 3 3 3 2 1

Within cluster sum of squares by cluster:

[1] 1.536847 1.552847 1.529489

 (between_SS / total_SS =  79.0 %)


Available components:
```

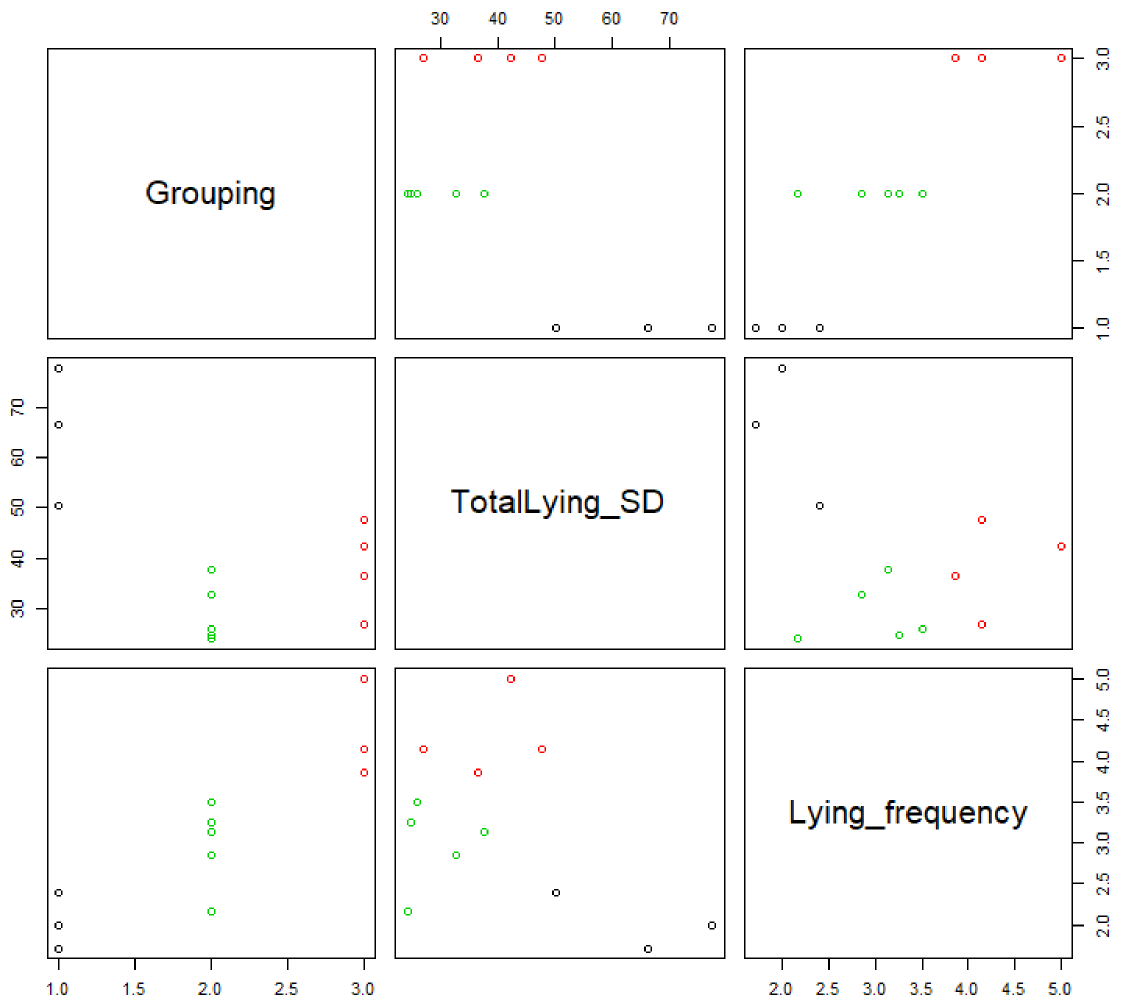
```
[1] "cluster"      "centers"      "totss"        "withinss"     "tot.withinss" "betweenss"
[7] "size"         "iter"         "ifault"
```

Main take away is the 79% of the variability in the data can be accounted for by the assigned cluster membership.

Hierarchical clustering



The hierarchical cluster seemed to agree with the K-means clustering results in that the most obvious split seemed to be into three groups, but this is subjective.

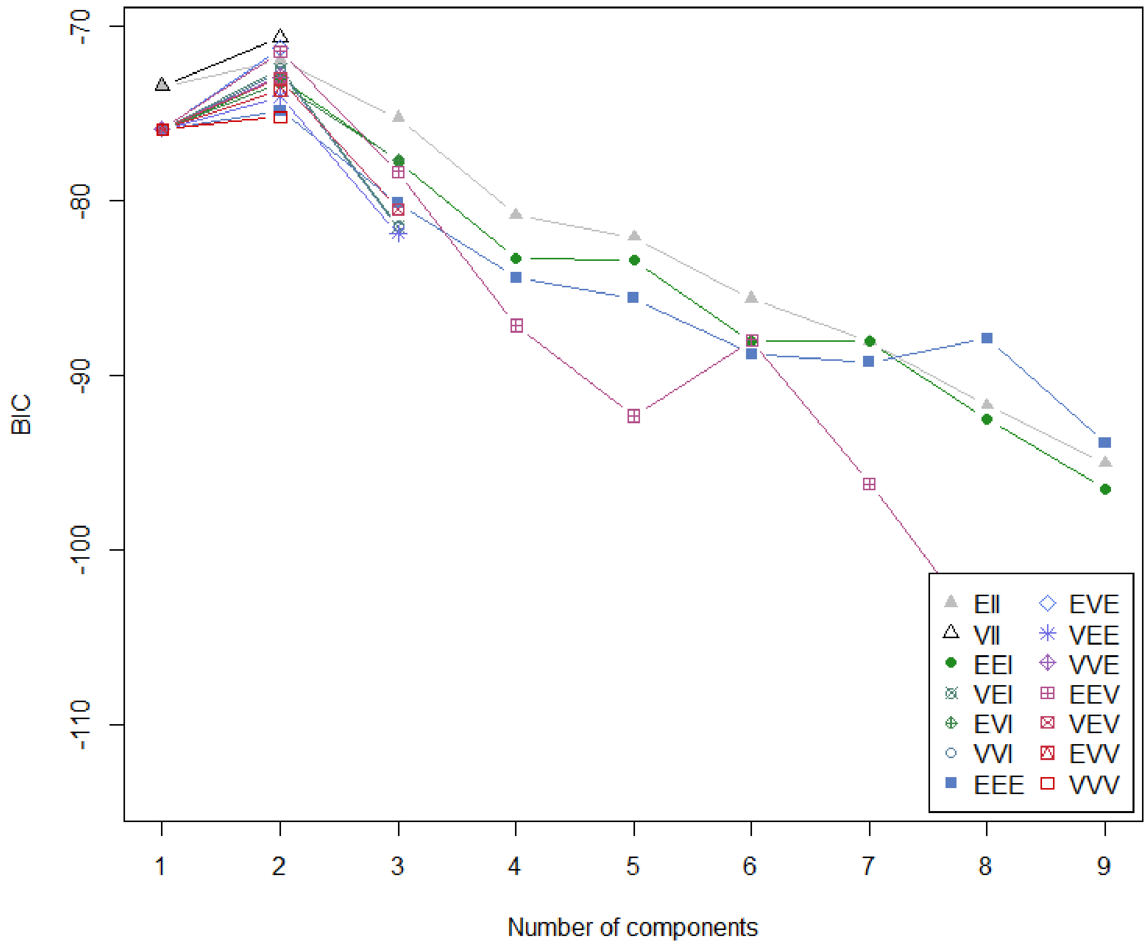


Hierarchical clustering shows same three groups showing the same individuals in each group.

Model based clustering

Model based clustering is less subjective and more sophisticated but is more rigid in its results so less room for fine tuning.

Modelling was completed using the R-package called mclust which is a Finite Gaussian mixture modelling fitted via Expectation-Maximization (EM) algorithm for model-based clustering, classification and density estimation, including Bayesian regularization and dimension reduction.



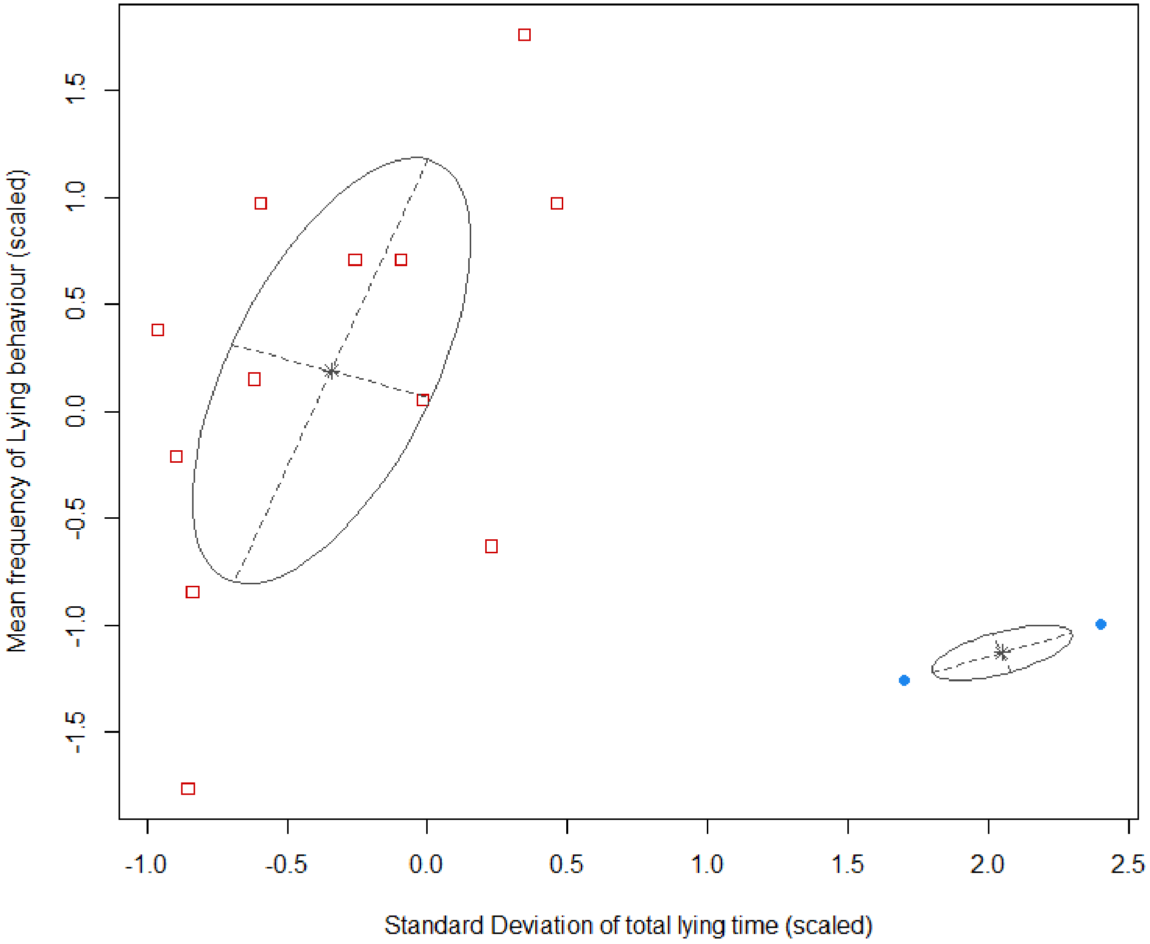
Bayesian Information Criterion (BIC) Model selection shows all models prefer a 2 cluster outcome VII, 2 shows best model fit.

```
Best BIC values:
```

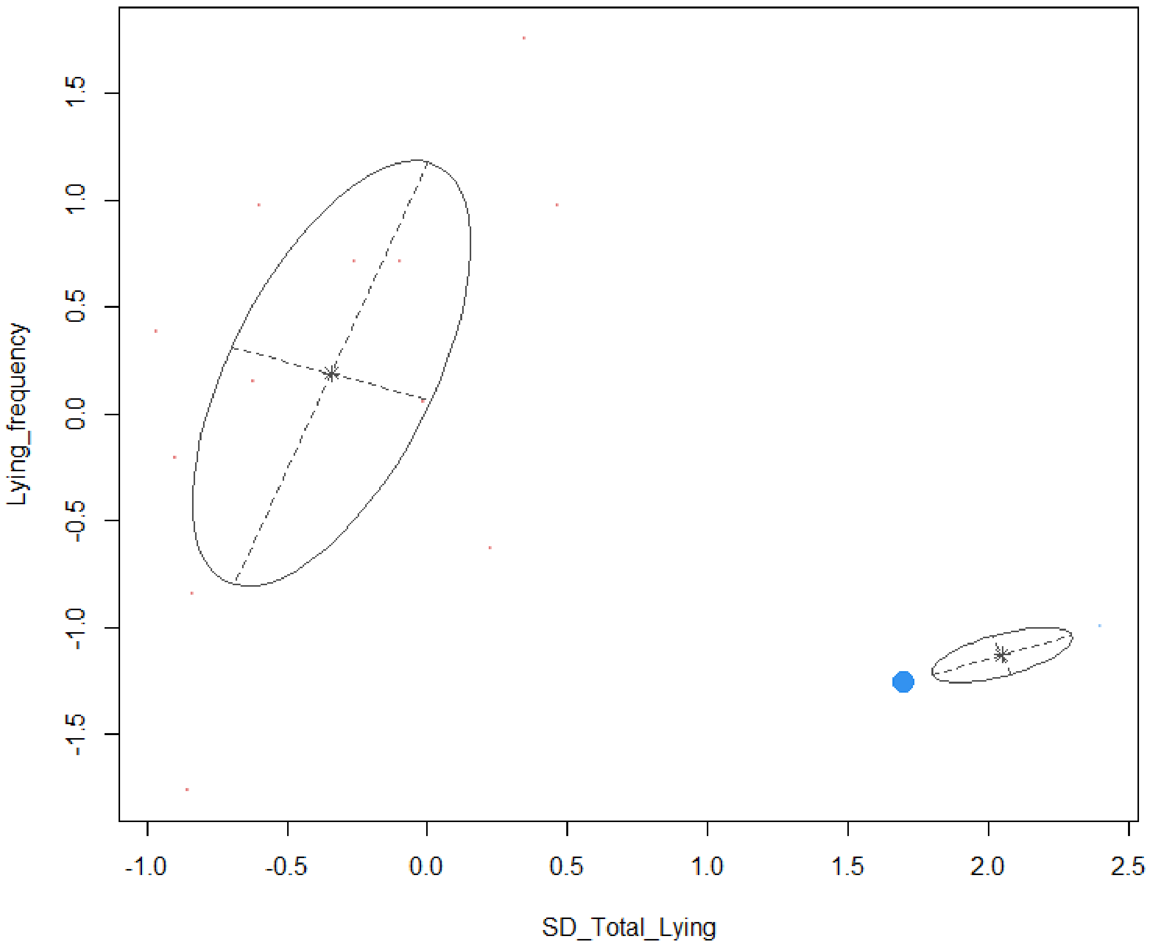
```
             VEV,2       VEV,5       EEE,2
BIC      -80.28916 -80.4395380 -82.550208
BIC diff   0.00000  -0.1503734  -2.261043
```

```
----------------------------------------------------
Gaussian finite mixture model fitted by EM algorithm
----------------------------------------------------

Mclust VEV (ellipsoidal, equal shape) model with 2 components:

 log-likelihood  n df       BIC       ICL
       -26.9493 14 10 -80.28916 -80.28916

Clustering table:
 1  2
 2 12

Mixing probabilities:
        1         2
0.1428571 0.8571429

Means:
           [,1]       [,2]
TotSD  2.048633 -0.3414388
LyF   -1.128589  0.1880982

Variances:
[,,1]
           TotSD        LyF
TotSD 0.06284834 0.02008013
LyF   0.02008013 0.01658967
[,,2]
          TotSD       LyF
TotSD 0.2454958 0.2972401
LyF   0.2972401 0.9931965
```

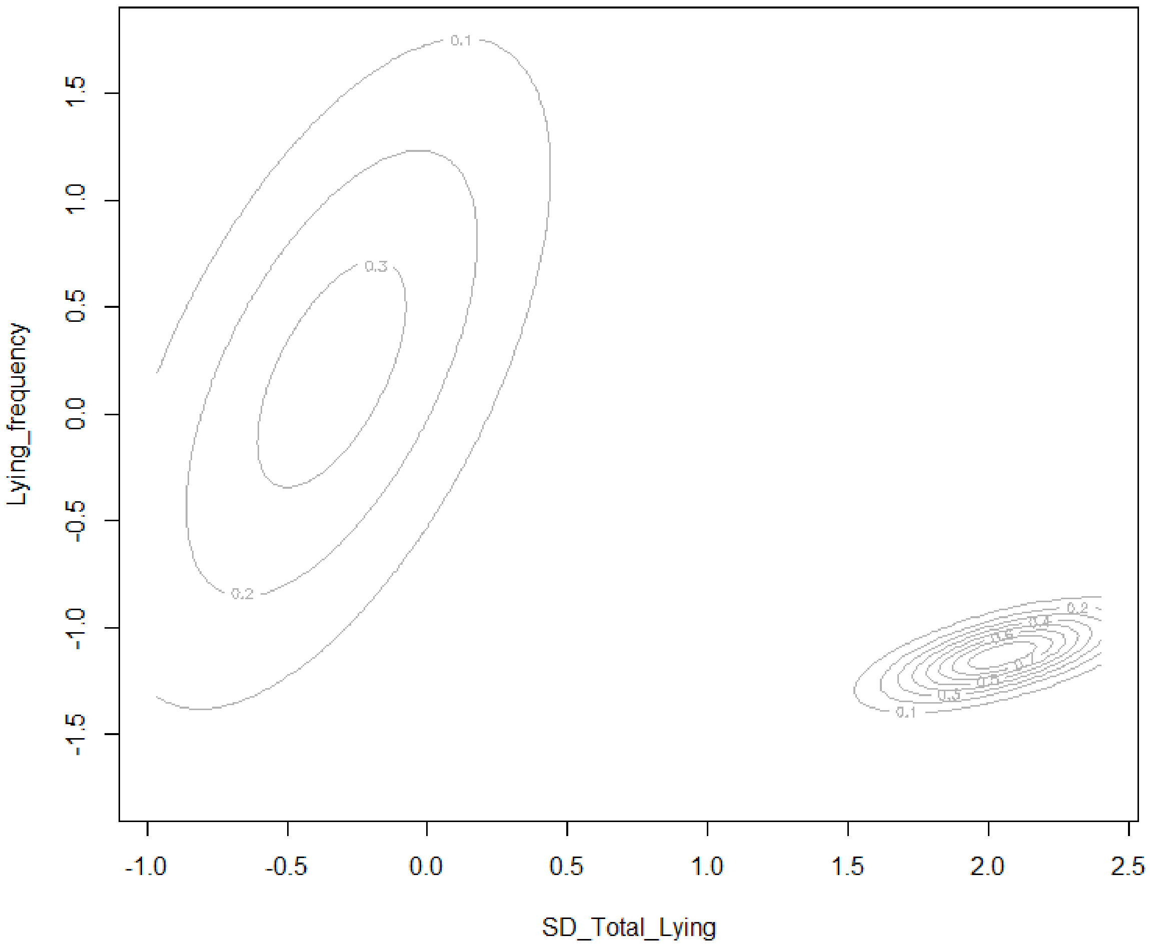
```
Best ICL values:
             VEV,2       VEV,5       EEE,2
ICL      -80.28916 -80.5786207 -82.550242
ICL diff   0.00000  -0.2894561  -2.261078
```



Two clear clusters but has classified one of the individuals between the 2 clusters as belonging to a different group compared to the K-mean and Hierarchical clusters analysis. So now there are only 2 individuals in one cluster instead of 3.



One of the points from the second cluster is marked as uncertain as to what group it belongs to. This is a symptom of my small sample size.



Cluster density shows one cluster is denser than the other, probably due to the small number of members in that cluster.

Summary of cluster analysis results:

The K-mean cluster and hierarchical cluster analysis agreed with each other that there are three groups while the model-based analysis could only determine two groups. I feel that the three groups is more correct. It makes sense that there would be individuals who would fall into three groups.

Group 1 (black): Low lying frequency but high variability in time spent resting each night.

Group 2 (Red): Low frequency of lying but low variability in time spent resting.

Group 3 (Green): High frequency and low variability in time spent resting.

Appendix IX

Chapter 4

Racehorse sleep patterns pre- and post-race: In an attempt to understand horse sleep in high performance situations, I began a project looking at sleep patterns of racehorses in the week leading up to a race and the week post-race. For this I had videos installed in the horse's stables and took faecal samples to analyse nightly cortisol. I hypothesised that the horses would detect the change in routine the day before a race as the horses would be washed, lorry packed etc. and would show elevated levels of cortisol in anticipation of Raceday which would cause alterations to their sleep pattern. However, 2 weeks into starting this project the Covid pandemic hit, and I was no longer allowed to conduct field research. Summaries of scan behavioural observations and faecal cortisol results of the 2 horses I did collect data for are shown below.

Horse 1: 6-year-old mare, 16hh and had resided at the yard for 4 years. She was an experienced racehorse (45 starts) and her race occurred on day 9 of 15 days of observation. This horse was fed 6kg haylage, 4.8kg nuts, 1.2kg alfalfa/day and was bedded on straw. Personality score indicated she scored high for neuroticism.

Horse 2: 3-year-old, 15hh filly who had been at the yard for 18 months. This was her first race and it occurred on day 8 of 16 days of observation. This horse was fed 3kg haylage and 1.5kg of Alfalfa a day and was bedded on straw. Personality score indicated she scored low for neuroticism.

Table 1: Horse 1 interval scan sample behaviour scored over 15 nights between 6 pm and 6 am at 30-minute intervals. Numbers denote percentage of observations horse was engaged in each behaviour. Ethogram was the same as in Chapter 4. Greyed column indicates day the horses raced. NA indicates the nights the camera malfunction resulted in a loss of data for nights 2-6.

Behaviour	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15
Not visible	8	NA	NA	NA	NA	NA	8	12	20	24	12	20	12	24	12
Standing alert	8	NA	NA	NA	NA	NA	36	16	16	24	52	40	20	28	32
Feeding	36	NA	NA	NA	NA	NA	24	16	16	4	24	8	28	20	8
Standing rest	24	NA	NA	NA	NA	NA	20	32	0	32	0	16	24	0	32
Sternally recumbent	20	NA	NA	NA	NA	NA	4	24	0	16	12	8	16	12	16
Laterally recumbent	4	NA	NA	NA	NA	NA	8	0	0	0	0	8	0	12	0
Rolling	0	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0
Not in stable	0	NA	NA	NA	NA	NA	0	0	48	0	0	0	0	4	0

Table 2: Horse 2 interval scan sample behaviour scored over 16 nights between 6 pm and 6 am at 30-minute intervals. Numbers denote percentage of observations horse was engaged in each behaviour. Ethogram was the same as in Chapter 4. Greyed column indicates day the horses raced.

Behaviour	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16
Not visible	8	20	4	8	4	8	16	20	8	4	0	0	4	8	4	0
Standing alert	16	12	16	16	0	4	4	4	16	12	8	0	24	12	12	4
Feeding	28	24	24	40	28	48	28	24	32	20	28	40	32	40	52	32
Standing rest	24	16	8	16	40	16	20	24	32	40	40	28	16	16	4	40
Sternally recumbent	16	16	20	16	12	12	16	8	8	24	20	12	12	8	20	12
Laterally recumbent	8	4	16	4	16	12	16	20	4	0	4	20	12	16	8	12
Rolling	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Not in stable	0	8	8	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3: Layout of 96 well microtiter plates used for cortisol EIA assays of prepared horse faecal sample (n=2) over 16 days for horse 1 and 13 days for horse 2. Colours denote six standards (green), Zeros or blanks (blue), Non-Specific Binding wells (yellow) control low (light orange), Control high (dark orange), 16 samples in duplicate (grey) for horse 1 (light grey), 12 samples in duplicate for horse 2 (dark grey), 6 variance samples (pink) and 6 empty wells (white).

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	3.00 Std	3.00 Std	Ctrl-L	Ctrl-L	SMP-P7	SMP-P7	SMP-P15	SMP-P15	SMP-D7	SMP-D7	SMP-V1	SMP-V1
B	1.00 Std	1.00 Std	Ctrl-H	Ctrl-H	SMP-P8	SMP-P8	SMP-P16	SMP-P16	SMP-D8	SMP-D8	SMP-V2	SMP-V2
C	0.333 Std	0.333 Std	SMP-P1	SMP-P1	SMP-P9	SMP-P9	SMP-D1	SMP-D1	SMP-D9	SMP-D9	SMP-V3	SMP-V3
D	0.111 Std	0.111 Std	SMP-P2	SMP-P2	SMP-P10	SMP-P10	SMP-D2	SMP-D2	SMP-D10	SMP-D10	SMP-V4	SMP-V4
E	0.037 Std	0.037 Std	SMP-P3	SMP-P3	SMP-P11	SMP-P11	SMP-D3	SMP-D3	SMP-D11	SMP-D11	SMP-V5	SMP-V5

F	0.012 Std	0.012 Std	SMP-P4	SMP-P4	SMP-P12	SMP-P12	SMP-D4	SMP-D4	SMP-D12	SMP-D12	SMP-V6	SMP-V6
G	Zero	Zero	SMP-P5	SMP-P5	SMP-P13	SMP-P13	SMP-D5	SMP-D5	SMP-D13	SMP-D13	EMPTY	EMPTY
H	*NSB	*NSB	SMP-P6	SMP-P6	SMP-P14	SMP-P14	SMP-D6	SMP-D6	EMPTY	EMPTY	EMPTY	EMPTY

Table 4: Faecal cortisol results, highlighted numbers represent samples that are outside the range of the curve fit and/or the range of the standards.

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	0.1371	0.2026	1.1153	1.2911	0.1365	0.1272	0.2378	0.212	0.1737	0.1509	0.1601	0.1289
B	0.2567	0.3781	0.3684	0.4051	0.1666	0.126	0.1609	0.1492	0.1399	0.1357	0.213	0.207
C	0.4407	0.5525	0.1535	0.1365	0.1511	0.1453	0.1493	0.1655	0.1913	0.1707	0.2563	0.1805
D	0.6619	0.9732	0.1279	0.1066	0.1709	0.1884	0.1794	0.1545	0.1377	0.1276	0.335	0.2421
E	1.2898	1.4573	0.2387	0.1255	0.1744	0.1936	0.1583	0.1665	0.1451	0.1554	0.3464	0.275
F	0.9647	1.4981	0.3675	0.1389	0.1753	0.1702	0.1313	0.1262	0.165	0.1628	0.3905	0.3838
G	1.0204	1.5672	0.459	0.1321	0.1977	0.1884	0.1554	0.1655	0.1719	0.3235	1.3116	1.4885
H	0.0573	0.0578	0.1466	0.1269	0.1898	0.1634	0.1006	0.2118	2.1361	2.0132	0.8974	1.305

High number of samples that show they are outside the range of curve fit/standard (table 4) indicate that the faecal results were not reliable. Behaviour results (table 1 and 2) show no clear pattern leading into, or after a race in terms of rest behaviour.

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