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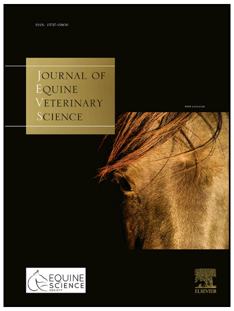

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A preliminary study investigating the influence of auditory stimulation on the occurrence of nocturnal equine sleep related behaviour in stabled horses

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Abstract

The physical environment is known to influence nocturnal behavioural time budgets of the stabled horse, but less evidence exists to suggest how this might be affected by including additional sensory stimuli. This study aimed to establish the impact of novel auditory stimuli on the frequency of equine sleep-related behaviour. Seven horses stabled for 24 hours per day on the same yard receiving the same daily management routine were observed from 2030 to 0630 over nine nights. Frequency of nocturnal behaviour were recorded using focal intermittent sampling against a predetermined ethogram and an infrared CCTV camera system. Data were recorded under the following conditions: without music for two nights (phase A1), five nights exposure to music (Beethovens' 9th Symphony) played at an average of 62.3 decibels (phases B1 [nights 3-4] & B2 [nights 6-7]), and two further non-consecutive nights (phase A2) when music was no longer played. General Linear Model was used to determine differences in the frequency of parametric behavioural data with a significantly higher occurrence of 'ingestion' ($F[3,18]=7.910$, $P=0.001$) during phases in B compared with A, and a significant decrease in the occurrence of 'other' behaviour ($F[3,18]=10.25$, $P=0.000$) comparing phase A1 with all other phases. The Wilcoxon Signed Rank highlighted significant differences in the frequency of 'lateral recumbency' between specific phases ($P<0.05$). The addition of music appears to have a significant effect on the equine nocturnal time budget that might be beneficial from an equine sleep perspective.

Keywords: equine, nocturnal, sleep-related behavior, music, enrichment

1.0 Introduction

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

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

2.0 Methods & Materials

2.1 Subjects

Seven horses (5 geldings, 2 mares; native mixed breeds; age range 6-16 years; height range 14hh-15.3hh, none displaying stereotypic behaviours) used within riding lessons for College and University students at Hartpury Equestrian Centre were observed whilst in their usual stable (3.6m x 3.6m). The floor surface of the stable was half exposed concrete, and half concrete covered by rubber matting with a shavings bed (approx. 5cm thickness with banks approx. 30cm high), which were cleaned as required throughout the day. Stables were located within a barn and organised into two rows that faced each other, separated by a walkway through the middle. Stable half doors opened into the walkway and with metal bars enclosing the top half of the front of the stable. Horses were prevented from sensory communication with neighbours as the three remaining walls of the stable were solid. All observations took place between the 30th January and the 20th February 2018 (mean regional temperature 4.5°c) and horses were rugged individually and in accordance with this. Horses were maintained in their usual routine involving 24 hour stabling due to the time of year which limited grazing opportunities, with ridden exercise for two to three non-consecutive hours per day (using indoor and outdoor arenas). The daily routine involved provision of individualized forage rations at 07:00 and hard feeds at 08:00 repeated at 18:00, with *ad lib* access to forage and water throughout the day, a final forage ration at 19:30, and lights out at 20:00.

2.2 Methods

Similar to Wells and Irwin (2008), this study employed an ABA design. Horses were observed first under control conditions with no music playing; Phase A1 = two consecutive nights (N1&2), followed by the experimental condition with music playing; Phase B = five consecutive nights (N3 to 7), and finally a control condition with no music playing; Phase A2 = two non-consecutive nights (N8&9). Horse behaviours were observed between 20:30 and 06:30 using night vision cameras (Sony Super HAD Bullet CCTV Camera, 650 TV Line Infrared Night vision Hikvision 4 Channel H.263 960H USB DVR, & SpyCamera digital wireless CCTV infrared cameras Model: DIGIRC1003 with Spy Camera Portable LCD Model: DIG03SCR) mounted above the stable. Beethoven's 9th Symphony [18, 20] was played in its entirety and on loop, at an average of 62.3 decibels between 20:30 and 01:30 to comply with the wishes of the horse's caregivers, using an iPod and speaker (apple iPod and Anker mini boom speaker). Frequency of behaviour was recorded using continuous intermittent focal sampling every 2 minutes and a pre-determined ethogram (table 1). Ethical approval was granted by Hartpury Ethics Committee.

Table 1. Ethogram of nocturnal behaviour observed (adapted from [11, 22])

Behavioural label	**Definition for the purpose of the study**
Ingestion (hay, bed, water)	Muzzle is lowered to ground/ within bucket; lips grasp hay/ bedding; masticating, prehending or swallowing food/ water
Lateral recumbency	Recumbent, either lateral thoracic area parallel to and in contact with the ground, head immobile and in contact with the ground, legs extended
Sternal recumbency	Recumbent, with sternum in contact with the ground, legs folded beneath the body, no or limited ear movement
Standing	Immobile displaying either of the following; no or limited ear movement, relaxed tail, eyes closed or half shut, head close to level with the withers or lower
Head over door	Head out of view of the camera i.e. over the stable door
Other	Any behaviour other than those listed above including stand alert, locomotion, excretion.

2.3 Data analysis

Behavioural data are presented as frequency of occurrence (sampled every two minutes over 600 minutes per night of observation) and arithmetic means are presented $\pm$ standard deviation. Parametric and non-parametric repeated measures statistical analyses were used to determine differences in behavioural frequency between phases. Phase B was divided into two sub-phases to observe specifically the initial versus longer term effects of music exposure for the purpose of analysis. Thus, difference in total frequency of behavioural display for each horse were compared between four phases; phase A1 = Nights 1&2 (no music), phase B1 = Nights 3&4 (first two nights with music), phase B2 = Nights 6&7 (last two nights with music), phase A2 = Nights 8&9 (no music). All behavioural frequency data were tested to meet the assumptions of parametric testing. For the behaviours that met parametric assumptions, the General Linear Model was used to determine whether the frequency of behaviours was equal across the four phases ($P<0.05$) Differences in behavioural frequency between phases for lateral recumbency were determined using Wilcoxon

Signed Rank (significant at P<0.05) as data were found to be non-parametric. All statistical tests were conducted using IBM SPSS version 24.

3.0 Results

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

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

	Phase A1	**Phase B1**	**Phase B2**	**Phase A2**
Lateral recumbency (REM sleep)	14.3 (7.4)	22.3 (11.4) a	10.9 (6.2) a, b	20.0 (10.6) b
Sternal recumbency (NREM sleep)	115.4 (24.8)	119.7 (26.5)	119.4 (30.6)	124.1 (26.5)
Standing (NREM sleep)	156.1 (49.1)	132.6 (32.3)	126.6 (38.3)	165.1 (37.9)
Ingestion	152.7 (10.0) a, b	188.6 (12.8) a, c	187.9 (15.0) b, d	144.7 (15.4) c, d
Head over door	137.9 (56.0)	130.6 (51.9)	136.9 (60.6)	131.0 (55.4)
Other	20.6 (3.0) a, b, c	10.6 (1.5) a	7.9 (0.9) b	12.3 (2.4) c

(Means in a row with different letters differ significantly)

The frequency of lateral recumbency was significantly higher in phase B1 compared with B2 (Z=-2.197, P=0.028), and higher in phase A2 compared with B2 (Z=-1.997, P=0.046). No significant differences (were detected for sternal recumbency (F[3, 18]=0.093; P=0.963) and standing behaviour (F[3, 18]=1.882; P=0.169) between different phases. A significant change in the frequency of ingestion (F[3,18]=7.910, P=0.001), specifically between phase A1 and B1 (P=0.038), A1 and B2 (P=0.019), B1 and A2 (P=0.02) and B2 and A2 (P=0.004) was apparent. The occurrence of 'other' behaviours was also significantly higher (F[3,18]=10.25, P=0.000) in phase A1 compared with B1 (P=0.008), B2 (P=0.007) and A2 (P=0.017). Finally, no significant difference (F[3, 18]=0.157; P=0.924) for head over door behaviour was detected between different phases.

4.0 Discussion

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

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

Conclusion

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

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Highlights

- The addition of music to the nocturnal environment of the stabled horse had a significant effect on behaviour
- Ingestion significantly increased during the phases when music was played compared with control phases
- The occurrence of behaviours other than head over door, standing, ingestion and recumbency decreased during the phases when music was played, especially compared to the control phase (pre-music exposure)
- Exposure to music also significantly affected the occurrence of lateral recumbency, although the increase observed at the start of music exposure was not mirrored towards the end of exposure

Ethical Statement

Authorship of this paper is limited to those who made a significant contribution. Please see CRediT statement below:

N Hartman: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – original draft

L Greening: Conceptualization, Formal analysis, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing

The authors confirm that they have written entirely original works with appropriate citations included.

This publication is not a multiplication of manuscripts appearing elsewhere.

There are no potential conflicts of interest associated with this research.

The study design was risk assessed in a way to ensure the safety of the animals and humans involved in data collection.

The study was purely observational and thus no changes were made to routines/ feed rations/ exercise schedules for the purposes of this study.

Conflict of Interest

The authors confirm there are no conflicts of interest linked to the data or the publication of the submitted article.

CRediT Author Statement

Conceptualization – N Hartman and L Greening

Data curation – N Hartman

Formal analysis – L Greening

Funding acquisition – N/A

Investigation – N Hartman

Methodology - N Hartman and L Greening

Project administration – N Hartman

Resources – L Greening

Software - N Hartman and L Greening

Supervision – L Greening

Validation – L Greening

Roles/Writing – original draft – N Hartman and L Greening

Writing – review & editing – L Greening