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Sleep in dairy cows recorded with a non-invasive EEG technique

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A B S T R A C T

Sufficient sleep time is important for both an adequate metabolic system and the immune function. Sleep in animals is often estimated by behavioural observations, or recorded on restrained animals with invasive electroencephalogram (EEG) techniques, which might affect sleep patterns. Earlier studies on sleep in cows showed that they sleep about 4 h per day and drowse almost twice the time. The aim of this study was to record and differentiate between vigilance states in dairy cows using a non-invasive EEG method.

Brain activity (electroencephalography, EEG), eye movements (electrooculography, EOG) and muscle activity (electromyography, EMG) were recorded for 6 h per animal using surface-attached electrodes to measure different vigilance states. Behaviour registrations from direct observations were combined with the EEG data in order to confirm the identification of different vigilance states from the EEG, EOG and EMG recordings. 8 dry dairy cows, lactation number 1–8 and age 3–11 years, of the Swedish Red breed from the research herd at Kungsängen Research Centre, Uppsala, Sweden, were used in the study.

The EEG recordings showed that non-rapid eye movement (NREM) sleep displayed low frequency waves, sometimes with slow wave activity. Rapid eye movement (REM) sleep and alert wakefulness shared similar features of desynchronised waves with varying frequency and could be differentiated by reduced neck muscle activity during REM sleep.

The main conclusion from this study is that it is possible to distinguish different vigilance states in dairy cows using surface-attached EEG electrodes.

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1. Introduction

All mammals studied to date display sleep, although the classic definition of sleep as a homeostatically regulated and rapidly reversible state of immobility along with greatly reduced sensory responsiveness does not fit all species (Zepelin et al., 2005; Siegel, 2005). The true function of sleep is still being discussed, but sleep undoubtedly affects the endocrine and metabolic systems and the immune function (Rechtsaffen et al., 1983; Bergmann et al., 1989; Everson, 1995; Datta and MacLean, 2007).

Different states of sleep and wakefulness can commonly be distinguished through different brain wave patterns (EEG; electroencephalography) (see Staunton (2005) for review) and for mammalian species, patterns share similar overall characteristics, even though actual frequency and amplitude might differ between species (Zepelin et al., 2005). These vigilance states can be divided into alert awake, drowsiness, non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. For nonhuman mammals the term NREM sleep is used synonymously with slow wave sleep (Zepelin et al., 2005). The EEG signal

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from an alert individual is characterised by a desynchronised pattern of mixed frequency with a predominance of fast activity. In the awake state the muscle activity measured by electromyography (EMG) on the neck muscle is pronounced and might change due to movement (Rechtschaffen and Kales, 1968; Ruckebusch, 1972). The same desynchronised EEG pattern is apparent in the EEG signal from REM sleep and different body parts can be seen twitching. A reduction in amplitude in EMG recordings due to relaxation of the neck muscles has been reported along with rapid eye movements recorded with the electrophysiology (EOG) (Rechtschaffen and Kales, 1968; Zepelin et al., 2005). The typical EEG pattern for NREM sleep is characterised by delta waves; slow wave activity with low frequency (2-5 Hz) and high amplitude. The EMG signals are often reduced in amplitude and may be as low as for REM sleep (Rechtschaffen and Kales, 1968). The pattern for drowsing is less regular than during NREM sleep and shows a mix of high and low frequency waves (Rechtschaffen and Kales, 1968; Ruckebusch, 1972). Muscle activity in drowsing is reduced compared to the awake state but might increase for short periods due to re-positioning of the body (Ruckebusch, 1972).

Sleep spindles, which consists of short sequences with high frequency waves, has been described throughout NREM sleep in mammals (Zepelin et al., 2005), but also during drowsing in sheep and ruminating in cows (Ruckebusch, 1972). Another feature in EEG sleep data is K-complexes, which consist of a sharp negative wave followed by a sharp positive wave. K-complexes are present in NREM (stage 2) sleep in humans (Rechtschaffen and Kales, 1968) and have been reported in NREM sleep in ponies (Hale and Huggins, 1980). K-complexes are also often connected with transition between vigilance states (Rechtschaffen and Kales, 1968).

EEG, EMG and EOG signals from ruminating animals are disturbed by the high muscle activity from rhythmic chewing during ruminating. This is apparent as rhythmic high amplitude noise in all these signals (Hänninen et al., 2008). This pattern has also been observed when animals are eating (De Moura Filho et al., 1983), but is then not as rhythmic as during ruminating.

Little is known about sleep in cattle. Like most mammals, adult cattle display polyphasic sleep, e.g. they sleep in intervals during a 24 h period. According to Ruckebusch (1972), total sleep time in cows is rather short, about 3 h per day of NREM sleep and 45 min of REM sleep. However, cows also display an intermediate state that is not well described in cattle but commonly referred to as drowsiness, which occupies twice the time of NREM sleep (Ruckebusch, 1972). Drowsiness in humans is a sleep preparing transitional state which a person passes when going from wake to sleep and is not, in a normal subject, a lasting state. The significance and a clear description of drowsing in cattle is not known.

Recording techniques for measuring brain activity in mammals have long been mainly invasive, involving subcutaneous needles or implantation of electrodes in the brain of restrained animals. Earlier studies in adult cows (Ruckebusch, 1972) have shown the specific sleep patterns but have all been invasive, with drilled holes in the skull to place the electrodes directly on the brain. Even though subcutaneous needles are minimally invasive compared to electrode implantation the technique might be difficult to use with longer recording intervals, e.g. 24 h, in freely moving cows. However, a non-invasive EEG method for measuring sleep that recently has been evaluated on freely moving calves, using surface-attached electrodes for recording, showed that it is possible to distinguish between different vigilance states with this type of technique (Hänninen et al., 2008). However, adult cows have thicker sculls than calves which may cause problems in recording EEG signals from surface attached electrodes. Also, sleep characteristics may differ between young and adult animals. The aim of the present study was to measure vigilance states in freely moving adult dairy cows with a non-invasive method with surface-attached electrodes.

2. Methods

The experiment was approved by the Uppsala local ethics committee (Ref. C75/10, Uppsala, Sweden). 8 dry dairy cows of the Swedish Red breed from the research herd at the Kungsängen Research Centre, Uppsala, Sweden, were used in the study. The cows were 5.5 ± 2.7 years old (range 3–11) in lactation number 3.2 ± 2.5 (range 1–8). During recording sessions cows were kept in individual pens (3 × 3 m) and recordings lasted 5 h (between 9 pm and midnight and between 1 am and 3 am). The pens had a feeding trough for concentrate and silage, and a pressure valve water bowl, and bedding was replaced daily. The cows had ad libitum access to water and silage during recording sessions. Ventilation was mechanic and lighting was manually turned on at 7 am and off at 4.30 pm, with a dim night-light provided.

2.1. Equipment, electrophysiological recordings and behaviour registrations

The cows were fitted with an udder holder and a textile halter a minimum of 10 h before the recording started and were shaved at the electrode attachment sites to ensure sufficient conduction. A minimum of 3 h prior to recording, the shaved patches were cleaned with alcohol and adhesive electrodes (Unilect, Unomedical Ltd., Birkerød, Denmark) were secured to the skin with cyanoacrylate (i.e. super glue). The electrodes (Ø 3 cm) were positioned as described by Hänninen et al. (2008) (Fig. 1); four EEG electrodes were placed vertically two by two in lines drawn from the middle of the eye bulb up to the cranial part of the horn base in a square on the flat area of the cow’s forehead. The reference electrode was placed in the middle of the square and the ground electrode was placed caudally of the horn base. The electrodes were sometimes rubbed off against the interior by the cow. This did not interfere with the scoring since each electrode was part of a set of four. Electrodes for measuring eye movements were positioned above and below one eye, and two electrodes for measuring neck muscle activity were fixed symmetrically on each side of the neck on cervical part of the trapezius muscle. All electrodes were connected to a mobile recording device that weighed 0.3 kg and measured 12 cm × 8 cm × 3 cm (Embla Titanium,
in order to confirm the identification of different vigilance states from the EEG, EOG and EMG recordings, e.g. when the EEG showed REM sleep the cow was also observed to be lying with head in REM position. When EEG showed drowsing or NREM sleep the cow was observed quietly lying with the head in NREM position.

2.2. Data analysis

The data comprised in total 45 h 13 min of polygraphic data recorded from the 8 cows. Of this, 43 h 34 min was readable. The majority of the unreadable data were due to poor connection of the ground electrode, as the data became readable after the ground electrode was changed. Unreadable data per cow averaged 12 ± 24 min, with a range of 0–70.5 min per cow.

The EEG, EMG and EOG data were analysed visually using digital sleep recording, monitoring and analysing software (RemLogic 2.0.1, Embla Systems, Broomfield, USA) and scored for sleep states. Before scoring, the signals were filtered using the following individual thresholds: EEG 0.3–30 Hz; EMG > 10 Hz and EOG 0.15–15 Hz. The signals were displayed and analysed in 30 s intervals and given a fixed scale: 50 μV for EEG and EMG and 500 μV for EOG. From each recording session, one good quality trace of the EEG and EMG, and both EOG traces were selected for visual analysis. We used the monopolar connections, and measured the electrical potential between reference and each EEG. Power spectrum (Welch, 1967) was calculated using fast Fourier transform (FF) with sample size of 2048 points and 50% overlap and was displayed in the frequencies 10–30 Hz and the power 0–1 μV²/Hz (Fig. 2).

In order to calculate the duration of each continuous vigilance state, 30 s intervals with the same defined states were added together and presented as mean ± standard deviation. When an interval in the EEG data was interrupted but not finished with a 30 s interval of a different vigilance state, this interrupting vigilance state was ignored. This procedure was used because the sleep intervals were sometimes interrupted by short bouts of locomotion. 1 cow did not show REM sleep during the recording and was therefore excluded from the calculation of REM sleep bout duration. Total sleep time per recording was not calculated, since only part of the day was recorded and this part might not have been representative of a 24 h period.

The vigilance states (awake, NREM sleep and REM sleep) were scored according to the Rechtschaffen and Kales (1968) definition for human sleep. K-complexes and sleep spindles in the data were noted but not scored, as they were not considered crucial for sleep scoring in dairy cows. Since the EEG signal amplitude varies between and within species, exact values are not presented (Zepelin et al., 2005). REM sleep was scored when the EEG data displayed a desynchronised pattern with varying high and low amplitude, combined with very low muscle tone. NREM sleep was characterised by low frequency waves (amplitudes usually less than 15 μV) and was scored when the power spectrum showed less than 0.2 μV²/Hz in the higher frequencies (10–30 Hz) and EMG activity was reduced. Data was scored as drowsing when the EEG signal displayed a

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synchronised pattern and the activity in the higher frequencies (10–30 Hz) of the power spectrum was between 0.2 and 1.0 µV²/Hz, based on Ruckebusch (1972) findings on cows and Williams et al. (2008) findings on horses. EMG signal during drowsing was reduced but could vary due to movements.

2.3. Statistical analysis

Readable data recorded from 8 cows was analysed for differences in transitions between vigilance states with a match-paired t-test. 1 cow did not show any REM sleep during the recording and was excluded from the transition calculation. Bonferroni correction was used for the repeated pair-wise comparisons. Results are presented as mean ± standard error. The statistical analyses were conducted with PASW 18.0 (SPSS Inc., Chicago, Illinois).

3. Results

Successful recordings of EEG, EOG and EMG were obtained from all 8 cows, even though they were allowed to move freely in the pens.

3.1. Polygraphic data

Different vigilance states showed individual variation in the polygraphic data on cow level, but shared the same typical features, so different vigilance states could be distinguished (Fig. 2).

NREM sleep data displayed low frequency signals with some slow wave activity and some K-complexes (Fig. 3, NREM sleep). Mean NREM sleep bouts lasted 5 ± 3 min. Data from drowsing showed mixed high and low frequency EEG signals. K-complexes often occurred during drowsing (marked in red in Fig. 3, Drowsing), and muscle activity varied and could change due to movement. The mean length of a drowsing period was 3 ± 2 min.

REM sleep bouts lasted 3 ± 1 min, and displayed a desynchronised pattern with varying high and low amplitude (Fig. 3, REM sleep). The typical muscle twitches during REM sleep caused artefacts seen as spike-waves in all signal traces. The muscle activity was markedly reduced and the regular spikes in the EMG trace in Fig. 3 (REM sleep) are artefacts from the heart beat, which could be seen in some of the REM sleep data.

Data from the alert wakefulness state (Fig. 3, Awake) resembled those from REM sleep with a desynchronised pattern with varying amplitude, but in contrast to REM sleep data, the alert wakefulness data often showed pronounced muscle activity and artefacts from the cow moving around were common when the cow was awake. The rhythmic chewing during rumination caused spike-wave artefacts which were seen in all signal traces, with an interruption every minute by straighter lines of about 4–5 s (Fig. 4, Rumination). This pattern also occurred when cows were eating (Fig. 4, Eating), but the spikes showed lower amplitude and the pattern was not as regular. The rumination sessions lasted 32 ± 12 min.

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**Table 1**

Ethogram for registered behaviour classes. The different body postures were combined with head positions, e.g. standing with head in awake position.

<table>
<thead>
<tr>
<th>Behaviour class</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body posture</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>Standing with at least 3 feet on the ground</td>
</tr>
<tr>
<td>Lying</td>
<td>Lying down, body to the floor</td>
</tr>
<tr>
<td>Head position</td>
<td></td>
</tr>
<tr>
<td>Awake</td>
<td>Head lifted from the ground, supported by the neck and in motion</td>
</tr>
<tr>
<td>REM*</td>
<td>Head leaning on the body or resting on the ground, not fully supported by the neck</td>
</tr>
<tr>
<td>NREM*</td>
<td>Head lifted from the ground, supported by the neck and motionless</td>
</tr>
</tbody>
</table>

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* NREM = Non-rapid eye movement sleep.
* REM = Rapid eye movement sleep.

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**Fig. 2.** Power spectrum for different vigilance states. Y-axis shows power (nV/Hz²) and x-axis frequency (Hz). For drowsing the power is between 0.2 and 0.5 nV/Hz in the shown frequencies, for NREM sleep the power never exceeds 0.2 nV/Hz in the shown frequencies and for REM sleep the power is below 0.2 nV/Hz. * NREM = non-rapid eye movement, REM = rapid eye movement.

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3.2. Vigilance state transitions

The cows changed vigilance state from drowsing to NREM sleep rather than to REM sleep, as seen in Table 2. From NREM sleep they would shift to drowsing rather than awake.

4. Discussion

Polygraphic EEG data recorded with surface-attached electrodes allowed different vigilance states in cows to be distinguished as described in earlier studies (Rechtschaffen and Kales, 1968; Ruckebusch, 1972). Cows' movements and muscle activity caused artefacts in the EEG data. However, this occurred mostly during awake. Other types of muscle activity, e.g. tension of the small muscles on the forehead, might also have contaminated the EEG signals but since the cows were relaxed during the sleep phases this was not an issue for the sleep scoring. The power spectrum showed the
activity levels for the different vigilance states but could not alone be used to fully determine the vigilance states, as the appearance of the signals is of equal importance.

Polygraphic data from cows during NREM sleep showed typical low frequency waves (amplitude < 10 µV) with some slow wave activity (SWA), as reported previously in adult cows (Ruckebusch, 1972) and calves (Hänninen et al., 2008; Takeuchi et al., 1998). Mean NREM sleep bout duration in cows was 5 ± 3 min, which is shorter than that reported earlier for cows by Ruckebusch (1972), but almost twice that of 3-month-old calves (Hänninen et al., 2008). Contrary to studies in sheep and horses (Ruckebusch, 1972; Williams et al., 2008), sleep spindles were not clearly distinguishable during NREM sleep. This might be due to more superficial recording and interference from muscle activity with our surface-attached electrodes. The filters used to filter out muscle activity from the muscles on the forehead might also have filtered out the sleep spindles since spindling is a burst of high frequency waves. Electrode placement also affects the possibility to see spindles in EEG.

Fig. 4. RUMINATION. Rumination appeared as a very rhythmic pattern with spike-like waves in all traces. These waves occurred during approximately 1 min intervals and were interrupted by the recurring sequence of swallowing and erupting a new bolus (encircled). EATING. Eating displaying the same rhythmic pattern as rumination, with profound muscle activity (EMG) but a less regular pattern than rumination. LOCOMOTION. Locomotion artefacts (EMG) resulting in a confusing pattern for all traces.

Table 2
Transitions between different vigilance states and rumination based on EEG recordings from 7 dairy cows. Table shows the mean percentage of times (SE) that each vigilance state or rumination was followed by a different vigilance state or rumination. Different letters within rows indicate statistically significant differences (p < 0.05) between the transitions.

<table>
<thead>
<tr>
<th>A shift from:</th>
<th>AWAKE</th>
<th>DROWSING</th>
<th>NREM*</th>
<th>REMb</th>
<th>RUMINATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>AWAKE</td>
<td>–</td>
<td>47.1 ± 13.6ab</td>
<td>5.7 ± 5.7 ac</td>
<td>0.0 c</td>
<td>47.1 ± 11.3 b</td>
</tr>
<tr>
<td>DROWSING</td>
<td>29.2 ± 12.7 ab</td>
<td>–</td>
<td>60.0 ± 11.5 a</td>
<td>2.4 ± 2.4 b</td>
<td>26.4 ± 4.1 b</td>
</tr>
<tr>
<td>NREM*</td>
<td>5.8 ± 4.7 a</td>
<td>37.8 ± 4.5 b</td>
<td>–</td>
<td>30.0 ± 5.7 b</td>
<td>26.4 ± 7.9 b</td>
</tr>
<tr>
<td>REMb</td>
<td>13.9 ± 10.9 ab</td>
<td>58.3 ± 14.1 a</td>
<td>22.2 ± 8.2 ab</td>
<td>–</td>
<td>5.6 ± 3.5 b</td>
</tr>
<tr>
<td>RUMINATION</td>
<td>33.7 ± 5.8 a</td>
<td>24.5 ± 9.8 ab</td>
<td>35.1 ± 12.6 ab</td>
<td>6.8 ± 4.9 b</td>
<td>–</td>
</tr>
</tbody>
</table>

a NREM = Non-rapid eye movement sleep.
b REM = Rapid eye movement sleep.
Earlier studies in calves (Hänninen et al., 2008) showed that transitions from NREM sleep to either REM or awake were more likely to occur than transitions from NREM sleep to rumination. Drowsing was not observed in calves (Hänninen et al., 2008), which explains the differences in transition between calves and cows. During most episodes of REM sleep, cow neck muscle tone was markedly reduced compared with that in alert wakefulness as can be seen in Fig. 3. This feature is common in most species during REM sleep, although it can be more or less pronounced (Zepelin et al., 2005). However, during a few episodes of alert wakefulness cows also showed relatively low muscle tone, which might cause errors when scoring. The mean REM sleep bout duration of 3 ± 1 min was in agreement with the 4 min reported for adult cows (Ruckebusch, 1972) and also with the 2 ± 0.2 min reported for 3-month-old calves (Hänninen et al., 2008).

We observed frequent K-complexes during drowsing in the cows. This feature is common in light NREM sleep in humans (Rechtschaffen and Kales, 1968) and based on the K-complex data drowsing in dairy cows could correspond to light sleep in humans. As K-complexes are produced in the frontal lobe they were visible not only in all EEG traces but also in the EOG trace. Eye movements may cause artefacts in the EEG traces (Gratton, 1998), but this was not the case with the K-complexes during drowsing.

The bout length for drowsing of 3 ± 2 min was rather short compared with the Ruckebusch (1972) figure of approximately 18 min per drowsing episode in cows. However, this is not surprising, since the scoring method used by Ruckebusch (1972) was less sensitive than the one used in this study and the Ruckebusch (1972) data was presented as mean values for three adult cows. The bout duration may also differ between breeds and lactation stages.

The artefacts in cow EEG caused by ruminating and eating were easily distinguishable, with rumination causing a very rhythmic pattern with 1 min intervals of spike-like waves, compared with the more irregular, yet rhythmic, artefact of eating. This pattern has also been observed in polygraphic data for young calves (Hänninen et al., 2008). The rumination periods lasted 33 ± 12 min, which correspond to previous findings for dairy cows (Nielsen et al., 2000). It is still unclear whether the animals can be asleep or whether they are always awake during rumination, with a number of studies disagreeing on this subject (Klemm, 1966; Bell and Itabisahi, 1973; Ruckebusch et al., 1974; Itabisahi, 1973). During chewing, EMG data contaminated the EEG traces, because of these artefacts it was not possible to analyse brain activity during chewing. However, there were no differences between the transitions in cows from rumination to awake, drowsing or NREM sleep, although cows were less likely to change from rumination to REM sleep than to awake.

Even though there are some dissimilarities between earlier studies on sleep in cows and the present study regarding sleep features and bout durations, the vigilance states can be separated based on the overall appearance of the polygraphic data.

5. Conclusions

It is possible to distinguish different vigilance states, by the overall appearance of the data, using polygraphic electrophysiological recording with surface-attached electrodes in freely moving dairy cows.

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