Sleep in Dairy Cows

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Abstract

Little is known about rest and sleep in dairy cows, but it has been shown in other species that the total amount of sleep, duration of sleep bouts and distribution of sleep over 24 hours differ depending on health status, age, pregnancy and lactation. Sleep recordings conducted on cows with surgically implanted electrodes have shown that they sleep four hours per 24-hour period. In this thesis, a non-invasive electrophysiological technique for recording sleep in dairy cows was developed and used to investigate variations in sleep pattern during the lactation cycle.

Non-invasive sleep recordings and behaviour observations were conducted during five hours in eight cows to validate the recording method. The data was scored for sleep and awake states according to standardised methods for human sleep scoring and earlier findings on cow sleep. The behaviours 'lying with head resting' and 'lying with head lifted and still' were compared with sleep recordings in 13 cows to evaluate the accuracy of sleep time estimates based on behavioural indicators. To investigate whether the research equipment caused a first-night effect in sleep time in dairy cows, nine cows were studied over three consecutive 24-hour periods. Sleep was also recorded in 19 dairy cows on seven occasions to investigate variations in sleep time during a lactation cycle.

It was possible to distinguish sleep and awake states in data recorded with the non-invasive electrophysiological technique in unrestricted dairy cows. Using behaviour estimates only, sleep was greatly overestimated because drowsing and sleeping can be performed in the same position. The electrophysiological recordings conducted during the first out of three consecutive 24-hour periods provide adequate data on total sleep time in dairy cows.

In conclusion, the results show that cows sleep less and ruminate more in early and peak lactation compared with late lactation and dry period. Cows lie down to rest and it is therefore important to ensure that they have enough time to do so.

Keywords: Behaviour, dairy cow, drowsing, EEG, lactation, NREM, REM, sleep

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Dedication

To all my four-legged friends.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ternman E., Hänninen L., Pastell M., Agenäs S., Nielsen P.P (2012). Sleep in dairy cows recorded with a non-invasive EEG technique. *Applied Animal Behaviour Science* 140(1-2), 25-32.

II Ternman E., Pastell M., Agenäs S., Strasser C., Winckler C., Nielsen P.P., Hänninen L. (2014). Agreement between different sleep states and behaviour indicators in dairy cows. *Applied Animal Behaviour Science* 160, 12-18.

III Ternman E., Hänninen L., Agenäs S., Nielsen P.P. First-night effect in dairy cow sleep studies (manuscript).

IV Ternman E., Nilsson E., Nielsen P.P., Hänninen L., Agenäs S. Sleep in dairy cows varies with stage of lactation (manuscript).

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The contribution of Emma Ternman to the papers included in this thesis was as follows:

- I Was involved in planning the practical study. Mainly responsible for conducting the experiment. Analysed and summarised the results in collaboration with supervisors. Was responsible for completing the manuscript with regular input from the co-authors.
- II Was involved in planning the practical study. Mainly responsible for conducting the experiment. Analysed and summarised the results in collaboration with supervisors. Was responsible for completing the manuscript with regular input from the co-authors.
- III Was involved in concept creation and planning of the practical study. Mainly responsible for conducting the experiment. Analysed and summarised the majority of the results. Was responsible for completing the manuscript with regular input from the co-authors.
- IV Was involved in concept creation and planning of the practical study. Mainly responsible for conducting the experiment. Conducted data analysis and manuscript writing in collaboration with supervisors.

Abbreviations

AMS - automatic milking system
AMR - automatic rotary system
DIM - days in milk
EEG - electroencephalogram
EMG - electromyography
EOG - electrooculography
NREM - non-rapid eye movement
REM - rapid eye movement
SRB - Swedish red breed
VMS - voluntary milking system

1 Introduction

Little is known about rest and sleep in dairy cows, but they lie down for 10-12 hours per day and prioritise lying over eating if they have been deprived of both (Munksgaard *et al.*, 2005). They need time for eating, drinking and milking, and also for performing social behaviour (Helmreich *et al.*, 2014; Gomez & Cook, 2010; Munksgaard *et al.*, 2005; Dado & Allen, 1994). Rumination is closely related to feed intake and is often carried out in a lying position (Albright, 1993). As both high yield and low rank place a load on the time budget of cows, there is a risk of rest and sleep time in some cows being compromised (Gomez & Cook, 2010; Nielsen *et al.*, 2000; Ketelaar-de Lauwere *et al.*, 1996).

Sleep is defined as a calm state with an elevated stimulus threshold and species-specific posture. The state is homeostatically regulated and should be quickly reversible, separating it from unconsciousness (Zepelin *et al.*, 2005). According to Zepelin *et al.* (2005), the distinction between awake and asleep in ruminants is not always well-defined, as they also show the intermediate state of drowsing. Drowsing in dairy cows has been described as an intermediate state with reduced sensory response compared with wakefulness, but not as reduced a response as during sleep (Ruckebusch, 1972). It was long believed that ruminants could not engage in sleep due to the rumen function (Bell, 1960; Balch, 1955). However in 1966, Klemm was able to record sleep in goats and all mammals studied since then have displayed sleep, although the classical definition of sleep does not fit all species (Siegel, 2005). It has been shown that lack of sleep alters the endocrine system, increases energy expenditure and impairs the immune function (Everson, 1995; Bergmann *et al.*, 1989; Rechtshaffen *et al.*, 1983). Sleep time may vary between and within species depending on health status (Opp, 2005), age (Takeuchi & Harada, 2002; Li & Satinoff, 1996), pregnancy (Ruckebusch, 1975a) and stage of lactation (Blyton *et al.*, 2002). It has also been suggested that sleep in animals may be affected by prior waking experiences, since this is common for humans (Langford & Cockram, 2010).

Different types of sleep and wakefulness can be objectively distinguished through different brain wave patterns and are often referred to as vigilance states (reviewed by Staunton, 2005). Vigilance states in cows are divided into alert awake, drowsing, non-rapid eye movement (NREM) and rapid eye movement (REM) sleep and have been assessed using invasive electrophysiological recordings of brain activity, eye movements and muscle tone (Ruckebusch, 1972). For non-human mammals, the term NREM sleep is used synonymously with slow-wave sleep (Zepelin *et al.*, 2005). A non-invasive technique for recording sleep in calves has recently been developed (Hänninen *et al.*, 2008), but existing techniques for recording sleep in adult cows have been invasive, with permanent implants.

Vigilance states can also be reflected in body postures. During REM sleep muscle tone is reduced, so the REM sleep position in cows has been suggested as lying down with head resting (Ruckebusch, 1972). No specific NREM sleep or drowsing posture, other than lying down resting, has been observed for adult cows. Sleep in calves has been defined as lying resting if this position is maintained for at least 30 seconds (Hänninen *et al.*, 2008). The behavioural postures for adult cows described in the Ruckebusch (1972) study are referred to as sleep postures, but no studies have been conducted to evaluate whether sleep time in cows can be estimated from these behavioural postures alone.

2 Background

2.1 Time budget for a lactating dairy cow

Many factors limit the time available for the necessary activities of dairy cows, and for the individual cow these factors vary during the time between two calvings. Helmreich *et al.* (2014) showed that the time range for milking in automatic milking systems (AMS), including time spent in the waiting area, was three hours per 24-hour period. In free stall houses, cows engage in feeding for approximately four hours per 24-hour period and spend two and a half hour per 24-hour period standing in an alley (Gomez & Cook, 2010; Dado & Allen, 1994). Rumination is positively correlated to feeding time and accounts for approximately seven hours per 24-hour period (Dado & Allen, 1994).

Lactating cows are highly motivated to lie down and prioritise lying down over feeding (Munksgaard *et al.*, 2005). Having enough time for lying is important, as reduced lying time can affect both production and welfare in dairy cows (Munksgaard *et al.*, 2005; Munksgaard & Løvendahl, 1993). Duration of lying bouts and total time lying are shorter in cows with high milk yield, and occupy 10-12 hours per 24-hour period (Helmreich *et al.*, 2014; Deming *et al.*, 2013; DeVries *et al.*, 2011). Milk yield and stage of lactation did not influence lying pattern in a study with 17 dairy cows, whereas increased milking frequency, from two to three times daily, increased the lying time before morning milking (Österman & Redbo, 2001). Deming *et al.* (2013) showed that increased frequency of feed push-ups and feed bunk space allowance are associated with longer lying duration. Those authors attributed this to less competition between the cows, as the feed is more readily available in the bunk. Time of delivery of fresh feed influenced lying pattern in a study with 48 post-peak lactation Holstein cows, although total lying duration was not affected (DeVries & von Keyserlingk, 2005).

Cows are social animals and any change in their social environment is reflected in behaviour changes. Regrouping of adult dairy cows has been shown to affect their social, feeding and lying behaviour on the day of regrouping, but not in subsequent days (von Keyserlingk *et al.*, 2008). However, standing behaviour was still increased on day nine after regrouping of primiparous cows (Hasegawa *et al.*, 1997).

Early studies on sleep in restrained cows showed that sleep occurred in a number of bouts during the night (Ruckebusch, 1975b; Ruckebusch, 1972). The diurnal rhythm in sleep distribution might be related to fewer disturbances in the barn environment during night-time, as it has been shown that cow behaviour is much affected by management routines.

2.2 Importance of sleep

The function of sleep is widely discussed and involves several aspects. NREM sleep may be important for recovery of both the brain and the body, as well as for conserving energy (Zepelin *et al.*, 2005). REM sleep time in growing animals is positively correlated to brain size, so the function of REM sleep might play a role in memory consolidation and brain development (Siegel, 2005).

Several factors affect sleep time within species; with increasing age sleep fragmentation increases and REM sleep time decreases in dogs (Takeuchi & Harada, 2002). Lactating women have a higher proportion of slow-wave sleep than non-lactating women (Blyton *et al.*, 2002). In dairy cows, sleep time decreases 24 hours pre-partum (Ruckebusch, 1975a). In the same study, the cow was allowed to suckle her calf for 24 hours on day 18 after calving and it was found that after separation the cow was distressed and sleep time was decreased (Ruckebusch, 1975a).

Many studies have reported the effect of sleep deprivation on physiological parameters in mammals. In one such study, energy expenditure increased in sleep-deprived rats and the rats developed skin lesions (Rechtshaffen *et al.*, 1983). In another, immune function was suppressed during sleep deprivation, and normally sterile tissues were invaded by pathogens in sleep-deprived rats (Everson, 1995). According to Ruckebusch (1974), dairy cows can engage in NREM sleep, but not REM sleep, while standing, and REM sleep deprivation may be achieved by forcing the animals to a standing position. During the deprivation period in that study, the NREM sleep time increased as the REM sleep was heavily reduced. With only two hours to eat or lie down, the cows were increasingly irritated by the presence of humans and time spent drowsing was halved. Furthermore, the cows lost weight, as eating time was not sufficient to cover energy requirement (Ruckebusch, 1974). Rebound for NREM and REM sleep deprivation occurred during the four days following the experimental period, with almost twice the total sleep duration observed before the deprivation. No rebound effect was seen for drowsing (Ruckebusch, 1974). Sleep time increases when the immune function is challenged (reviewed by Imeri & Opp, 2009). Cytokines, a group of immune signalling molecules, are also involved in sleep-wake regulation and increased levels of cytokines are known to increase NREM and reduce REM sleep (reviewed by Opp, 2005).

2.3 Recording sleep

Sleep and awake stages in electrophysiological data are scored in 30-second intervals according to the appearance of the signals recorded by encephalography (EEG), electromyography (EMG) and electrooculography (EOG), as described in the standardised manual for human sleep scoring by Rechtschaffen and Kales (1968). The EEG signal from an alert individual is characterised by a desynchronised pattern of mixed frequency. In the awake state, the muscle tone is pronounced and may change due to movement (Rechtschaffen & Kales; 1968; Ruckebusch, 1972). The same desynchronised EEG pattern is apparent in the EEG signal from REM sleep, while the muscle tone is significantly reduced. Rapid eye movements and muscle twitches are two additional features associated with REM sleep (Rechtschaffen & Kales 1968; Zepelin *et al*., 2005).

The typical EEG pattern for NREM sleep is characterised by delta waves, *i.e.* slow wave activity with low frequency. Muscle tone is often reduced during NREM sleep and has been shown to be as low as for REM sleep (Rechtschaffen & Kales, 1968). EEG data for periods of drowsing show a mix of high and low frequency waves (Ruckebusch, 1972). Muscle tone in drowsing is reduced compared with the awake state, but may increase for short periods due to re-positioning of the body (Ruckebusch, 1972). Sleep spindles, which consist of short sequences with high-frequency waves, have been described throughout NREM sleep in mammals (Zepelin *et al*., 2005), but also during drowsing in sheep and rumination in cows (Ruckebusch, 1972). K-complexes, consisting of a sharp negative wave followed by a sharp positive wave, are present in NREM (stage 2) sleep in humans (Rechtschaffen & Kales, 1968) and have been reported in NREM sleep in ponies (Hale & Huggins, 1980). K-complexes are also connected with transition between sleep stages and sleep and drowsing in humans (Rechtschaffen & Kales, 1968). NREM and REM sleep in humans display a cyclic pattern, beginning with NREM sleep for approximately 90 minutes before the first REM sleep episode appears

(Carskadon & Dement, 2000). This pattern is then repeated throughout the night, although the proportion of NREM and REM sleep shift. According to Ruckebusch (1972), cows also display this type of sleep architecture, with NREM and REM in a cyclical pattern during the night.

2.3.1 Behaviour methods

To date, direct or indirect observations have been the most common way of studying cow behaviour. Using direct observations, the behaviour of the cow is recorded by observing her in real time. Indirect recording methods include video recording and automatic collection of data using different types of loggers. Methods for studying behaviour may interfere with the cow's daily activities. Among the different options for recording behaviour, video recording is known to have the least possible effect on the cow. However, video recordings are time-consuming to analyse and not all behaviours can be recorded using this technique. New techniques allow the cow's activities to be recorded using loggers that automatically collect data on standing or lying behaviours, number of steps (Bewley *et al.*, 2010), rumination bouts and eating behaviour (Kononoff *et al.*, 2002), among other things. Rest and sleep in calves can be estimated using an accelerometer determining head position (Hokkanen *et al.*, 2011), but this technique has not been used for studying sleep in adult cows.

Behaviour studies on sleep have been conducted in numerous mammals, including large herbivores such as elephants (Tobler, 1992) and giraffes (Tobler & Schwierin, 1996) (Table 1). Standing sleep has been observed and confirmed using electrophysiological recordings in adult horses. During standing sleep the animal was motionless, the ears were still and the head closer to the ground than when awake (Williams *et al.*, 2008; Ruckebusch, 1972). Adult elephants and adult giraffes also showed standing sleep, while the young animals more often lay down and slept in a recumbent position, although not confirmed by electrophysiological recordings (Tobler & Schwierin, 1996; Tobler, 1992). In adult cows (Ruckebusch, 1972), the sleep posture has been described as lying down with head resting on the flank. Cows well-adapted to their surroundings rarely engage in sleep whilst standing, and REM sleep cannot be displayed if the animal is standing up (Ruckebusch, 1974). According to Ruckebusch (1972), closed eyelids are a sign of REM sleep, while partially closed eyes are a sign of NREM sleep. During drowsing, the upper eyelids are relaxed but the eyes are not fully closed (Ruckebusch, 1972). Even though behavioural sleep postures have been described for adult cows, no studies have confirmed that these postures can be used to assess sleep time.

Table 1. Sleep data recorded in herbivores using electrophysiological equipment or behaviour observation.

Species	**Type of recording**	**Behaviour recorded**	**Total sleep time**	**Bout duration NREM/REM**	**Reference**
Giraffe	Behaviour in captivity (24 h)	Sleep & resting behaviour	5 h	SS=5 min, RS=10 min/ PS=4 min	(Tobler & Schwierin, 1996)
Elephant	Behaviour in captivity (24 h)	Sleep & resting behaviour	4-6.5 h	RS=69 min/ REM= not specified	(Tobler, 1992)
Horses	EEG (12 h) Non-invasive	Sleep	n .a.	NREM=3.5 min/ REM= 1 min	(Williams *et al.*, 2008)
Horses	EEG (24 h) Invasive	NREM, REM sleep & drowsing	3 h	NREM=not specified/ REM=5 min	(Ruckebusch, 1972)
Horses	EEG (12 h) Invasive	NREM, REM sleep & feed regimes	3-5 h	Not specified	(Dallaire & Ruckebusch, 1974)
Sheep	EEG (24 h) Invasive	NREM, REM sleep & drowsing	4 h	NREM=not specified/ REM=5 min	(Ruckebusch, 1972)
Goat	EEG (15 h) Invasive	REM sleep & stomach contractions	n.a.	NREM=n.a./ REM=5 min	(Itabisashi, 1973)
Sheep	EEG (15 h) Invasive	REM sleep & stomach contractions	n.a.	NREM=n.a./ REM= 3 min	(Itabisashi, 1973)
Goat	EEG, recording h not specified Invasive	Somnolence & rumination	n.a.	Not specified	(Bell, 1960)
Cow	Behaviour (24 h)	Resting, neck relaxed	1.5 h	Not specified	(Norring *et al.*, 2012)
Calf	EEG (15 h) Non-invasive	Sleep	n.a.	NREM=3 min/ REM=2 min	(Hänninen *et al.*, 2008)
Calf	Activity meter (24 h)	Sleep	n.a.	Not specified	(Hokkanen *et al.*, 2011)

NREM = non-rapid eye movement sleep
REM = rapid eye movement sleep
SS = standing sleep
RS = recumbent sleep
PS = paradoxical sleep, sometimes used synonymously with REM sleep

2.3.2 Electrophysiological methods

Studies on sleep in adult cows have been performed on restrained animals with permanent implants recording electrophysiological data on brain activity, eye movements and muscle activity. Using this method, it is possible to record distinct data and to separate NREM and REM sleep from awake and drowsing (Ruckebusch, 1972). Whether the invasive method itself disturbs the behaviour of the cows is not known. There has been no non-invasive method for studying brain activity, eye movements and muscle tone in adult cows, but recently a non-invasive electrophysiological method for recording sleep in calves was evaluated with good results, allowing sleep to be recorded in freely moving calves (Hänninen *et al.*, 2008).

In a study performed on three cows, Ruckebusch (1972) showed that the cows slept lying down during night time in three sleep bouts, comprising in total 3.5 hours per 24-hour period. They also drowsed for 7.5 hours. According to Ruckebusch (1975b), cows sleep during night time when housed indoors, but during both day and night when on pasture. When brought in from pasture, they need 3-6 days to re-establish indoor sleep architecture.

In human subjects, sleep is recorded using non-invasive electrophysiological methods (EEG, EMG and EOG). Studies have shown that the method can cause adaptation artefacts in the recordings (Le Bon *et al.*, 2001; Wauquier *et al.*, 1991; Rechtschaffen & Verdone, 1964). The altered sleep pattern might be due to discomfort from the electrodes and a change of environment. To minimise the effect of change of environment on behavioural studies in animals, an adaptation period where animals acclimatise to their new surroundings is often allowed prior to the study. Parity (von Keyserlingk *et al.*, 2008; Hasegawa *et al.*, 1997) and temperament of the cows (Ruckebusch, 1975b) have been shown to influence acclimatisation time.

3 Aims

The overall aim of this thesis was to use a non-invasive electrophysiological technique to record sleep in freely moving dairy cows and investigate whether sleep pattern varies with stage of lactation.

Specific aims were to:

- Validate a non-invasive electrophysiological method for recording sleep and awake states in freely moving adult dairy cows (**Paper I**).
- Test whether behavioural sleep estimates correspond to electrophysiologically recorded sleep in adult cows (**Paper II**).
- Investigate whether the research equipment cause a variation in the sleep cycle of dairy cows (**Paper III**).
- Examine whether lying time, sleep time and sleep pattern in dairy cows vary during the lactation and the dry period (**Paper IV**).

4 Materials and Methods

All experimental facilities were certified for research purposes by the Swedish Board of Agriculture and by the Finnish Animal Experiment Board. The experimental procedures were all approved by the Uppsala Local Ethics Committee in Sweden and by the Faculty Board in Finland.

Non-invasive electrophysiological sleep recordings were conducted in all studies (**Papers I-IV**). The majority of the studies were conducted on cows in the university herd at the Swedish University of Agricultural Sciences. This herd was moved from Kungsängen Research Centre to the Swedish Livestock Research Centre, Uppsala Lövsta, in 2011 and recordings were conducted at both sites. In **Paper II**, recordings carried out in the Viiki Research Facility at the University of Helsinki were also included. The cows in the studies were recruited from different management systems; the two herds in Sweden were kept in tie-stall housing with milking twice a day or loose housing with automatic milking in a voluntary milking system (VMS DeLaval, Tumba, Sweden) at Kungsängen; and in loose housing with either VMS or twice daily batch milking in an automated rotary milking parlour (AMR DeLaval, Tumba, Sweden) at Lövsta. The Viiki Research Facility also ran a loose housing system with automatic milking (Lely Astronaut, Lely, Massluis, the Netherlands). Cows were fed roughage *ad libitum* and concentrate to meet their requirements for maintenance and milk production. When kept in loose housing systems, roughage was provided on a feeding table and the concentrate in automatic feeders.

All recordings were conducted in individual pens, as the equipment on the cow did not allow recordings in a loose house with AMS. The recording device is sensitive to tampering and the environment in the loose housing system would not be suitable. The harness used for securing the equipment cannot be used in AMS because of the high risk of harness getting tangled. Also, by recording sleep and awake states in individual pens many factors that may interfere with sleep time were eliminated. Details of recording occasions, number of cows included in the particular study and length of the studies are given in Table 4.

The cows selected for **Papers III** and **IV** were at minimum in their first lactation on the first recording occasion. Moreover, they were selected for being calm and easy to handle and willing to allow brushing of the head. The temperament of the cows and their acceptance of brushing were examined by one person approaching the cows in the loose housing system with a hand-held cow brush and brushing them for a couple of minutes on the head. If the cow approached walked away from the person, she was considered not suitable for the study. A total of 42 cows were selected with these criteria. Out of those two were excluded after exposure to the equipment because they became distressed when electrodes and wires were attached and did not calm down. For both cows, before excluding them, the electrodes and wires were removed and reattached after the cows were brushed and allowed time to calm down. This was done twice but the cows still showed signs of distress and they were considered unsuitable for the experiment. All equipment was removed and recording was terminated.

Prior to the recordings, the cow was moved from the standard system to an individual pen (3 m x 3 m) and equipped with a harness and a textile halter. When recording sleep and awake states for 24 hours or more (**Papers III** and **IV**), the cows were allowed to adapt to the recording environment, the harness and the halter for two days prior to the recording. In **Paper III,** the recordings were conducted in individual pens in an isolated room in the facility, avoiding disturbances from the surroundings. Two to three cows were recorded at the same time, in individual pens next to each other. To minimise the impact of climate on the behaviour of the cows, these recordings were conducted during winter time, when the indoor temperature can be kept rather constant at approximately 10 ºC. In **Paper IV**, the cows were placed in individual pens in the combined delivery and medical treatment section at Kungsängen and the medical treatment section at Lövsta. This is a quiet part of the facility that has diurnal management routines regarding milking and feeding. One to three cows were recorded at the same recording occasion and the recordings were always started around the time for morning milking. The environments were similar between the locations, but the management routines differed. Light hours at Kungsängen were 07.00-18.00 h, whereas light hours at Lövsta were 05.30-20.30 h. Silage and concentrate were fed separately and distributed at 06.30 and 17.00 h at Kungsängen and 05.30 and 19.00 h at Lövsta.

Figure 1. Equipment and electrode placement (illustration by Patrik Pesonius).

After being moved to the individual pen, the cow was shaved on the patches where the electrodes were to be attached, the patches were cleaned with alcohol and the electrodes were attached using a strong, fast-acting adhesive. Four EEG electrodes were placed vertically two by two in a square shape 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. A pair of electrodes was used for recording eye movements, one placed below and one above one eye. Activity of the neck muscle was recorded (Figure 1). When recordings were obtained from all three measuring sites, the term electrophysiological recording is used in this thesis.

Snap-on wires (Embla Systems, Broomfield, USA) were connected to the electrodes and to the portable recording device (Embla Titanium, Embla Systems Inc., Broomfield, USA), and impedance was checked before recording was started. Data was stored on the device until a recording session ended and was thereafter downloaded to a computer using the software RemLogic version 2.0.1 (Embla Systems, Broomfield, USA). Standing and lying behaviour was recorded in **Paper IV** using an IceTag automatic datalogger (IceTag, Icerobotics Inc., Edinburgh, UK). The logger was fastened on one hind leg using Velcro straps. Recordings were made every second and summarised in one-minute bouts by the IcaTagAnalyser software (IceTagAnalyser, Icerobotics Inc., Edinburgh, UK).

*Table 2. Ethogram used in **Papers I** and **II**. The different body postures were combined with head positions, e.g. lying with head lifted and still*

Behaviour class	**Description**
Body posture	
- Standing	Standing with at least 3 feet on the ground
- Lying	Lying down, body to the floor
Head position	
- Lifted and moving	Head lifted from the ground, supported by the neck and in motion
- Lifted and still	Head lifted from the ground, supported by the neck and motionless
- Not supported by neck	Head leaning on the body or resting on the ground, not fully supported by the neck

Direct behaviour observations were made in **Papers I** and **II** according to the ethogram in Table 2**.** In **Paper II**, the electrophysiologically recorded data were combined with the behaviour indicators shown in Table 3 to investigate the agreement between the methods.

Table 3. Behaviour estimates used to describe sleep from observations.

Electrophysiological state	**Behaviour indicator**
NREM sleep	Lying with head lifted and still
REM sleep	Lying with head resting against body or object
Total sleep	Lying with head lifted and still, or head resting
Awake	Lying with head lifted and moving

NREM = non-rapid eye movement, REM = rapid eye movement.

During all recordings, the cow was continuously monitored using video recordings or by one person sitting next to the pen, to ensure that the electrodes were in place, that the device was working appropriately and that the cow was not showing signs of distress. If a cow seemed distressed, the research staff entered the pen and brushed the cow with smooth strokes to calm her down. The cows showed individual preferences for where they liked to be brushed, so the brushing differed between cows. If this did not help within 10 minutes, recording was terminated and the cow was relieved of the electrodes and wires and was not disturbed by the research staff for five minutes. The electrodes and wires were then reattached and the recording continued if the cow did not show signs of distress.

When analysing the electrophysiologically recorded data, sleep and awake states were scored according to the Rechtschaffen and Kales (1968) standardised manual for human sleep states and the Ruckebusch (1972) findings on sleep in cows. In **Paper II**, sensitivity and specificity (Firk *et al.*, 2002) were calculated to test the predictive value of the behavioural estimates for each vigilance state. Sensitivity (True positive/(True positive + False negative)) describes how well the behavioural sleep estimate can predict occurrence of the actual vigilance state, while specificity (True negative/(True negative + False positive)) describes how well the behavioural sleep estimate can predict non-occurrence of the actual vigilance state. The differences between recording days in **Paper III** were analysed with a linear mixed model, with period (recording day 1-3) and the interaction between period and day-night included as fixed effects in the model. Cow was included as random effect. In **Paper IV**, difference in sleep and awake times, as well as standing and lying times, during the lactation period and the dry period were investigated on seven occasions; in the late dry period (two weeks before calving), in lactation week 2, 7, 13, 21, 36 and in the early dry period (four weeks after drying off). The statistical model included the fixed effects of farm and time of recording and the random effect of cow nested within farm using the statement 'random cow(farm)'. For the fixed effects, least-square means were calculated and differences between them were tested for significance using t-tests, applying Tukey-Kramer adjustments to avoid overestimation of differences. As milk yield and stage of lactation were closely associated in **Paper IV**, milk yield was excluded in the statistical analyses of the data. The statistical analyses were performed using the software R version 2.15.2 (R Core Team, 2013) in **Paper II**, and SAS (SAS 9.3, SAS Institute Inc., Cary, USA) in **Papers II** and **IV**. The values presented are least squares mean ± standard error of the mean (LSMeans ± SEM) unless otherwise stated.

Table 4. Details of recording location, cows included in the studies, recording length and period used in ***Papers I-V****. SRB=Swedish Red Breed*

Paper	Aim	Method	Location	Housing	Number of cows	Breed	Recording length (h) mean (SD)	Time period
Paper I	Validate non-invasive recording method	Non-invasive electrophysiological recordings and behaviour observations	Kungsängen, Sweden	Single pen	8	SRB	5(1)	Between 21.00 and 03.00 h
Paper II	Test behaviour indicators against electro-physiological data	Non-invasive electrophysiological recordings and behaviour observations	Kungsängen, Sweden and University of Helsinki, Finland	Single pen	5	Ayrshire	4(2)	Between 17.00 and 03.00 h
					8	SRB		
Paper III	Investigate day-to-day variation in sleep cycles	Non-invasive electrophysiological recordings	Lövsta, Sweden	Single pen in a quiet room	9	SRB	71(0.1)	3 x 24 hours
Paper IV	Examine total sleep time and sleep pattern during a production year	Non-invasive electrophysiological recordings and standing/lying behaviour	Kungsängen, and Lövsta, Sweden	Combined delivery and medical treatment section	19	SRB	24(1.5)	Late dry period (2 weeks before calving)
					19	SRB	23(1.7)	Lactation week 2
					19	SRB	24(0.0)	Lactation week 7
					19	SRB	24(2.8)	Lactation week 13
					19	SRB	24(0.0)	Lactation week 21
					19	SRB	24(0.2)	Lactation week 36
					18	SRB	24(0.0)	Early dry period (4 weeks after drying off)

5 Main findings

The non-invasive method for recording electrophysiological data during sleep and awake states was successfully evaluated in adult cows. Although individual differences occurred, the electrophysiological recordings contributed objective measurements of sleep time and distribution during the 24-hour period. The appearance of the sleep and awake states recorded with the non-invasive method in **Paper I** showed good agreement with data recorded in adult cows using the invasive technique (Ruckebusch, 1972) and the description of human sleep by Rechtschaffen and Kales (1968). Behavioural indicators of sleep were not sufficient to assess whether the cows were asleep, drowsing or awake resting. Interestingly, NREM, REM and total sleep time varied with stage of lactation and the cows slept most during the late dry period and late lactation. Research staff had to enter the pen approximately once per hour during daytime to brush the cows and replace the electrodes, as the cows rubbed them off against the interior or by interacting with the cow in the neighbouring pen. The staff only entered the pen when the cow was fully awake and, if she was in a lying position, she usually remained lying down. At night the cows were calmer and the staff only had to enter the pen once every two or three hours. When sleep recordings were conducted in the summer, the high temperature in combination with flies and the recording equipment clearly annoyed the cows. If the cow was in heat during a recording session, she was much more alert and gave the impression that lying pattern was more fragmented.

Figure 2 shows a 30-second window of electrophysiological recordings of sleep and awake states from one cow collected in **Paper I**. NREM sleep data displayed low-frequency signals with some slow-wave activity and possible K-complexes (Figure 2, NREM sleep). Data from drowsing periods showed mixed high and low frequency EEG signals and K-complexes often occurred during drowsing (encircled in Figure 2, Drowsing). Behavioural estimates for

NREM sleep in **Paper II** showed high sensitivity (81%) but low specificity (6%), indicating that even though cows do sleep when lying with head lifted and still, other vigilance states, *i.e.* drowsing, are also displayed in this position.

Data on REM sleep recorded in **Paper I** displayed a desynchronised pattern of both high and low amplitude (Figure 2, REM sleep). During REM sleep the neck muscle activity was markedly reduced. The behavioural estimates for REM sleep in **Paper II** showed high sensitivity (70%) and moderate specificity (41%) and by using the estimates REM sleep could be identified with good precision, although there was a risk of this indicator alone underestimating total duration of REM sleep.

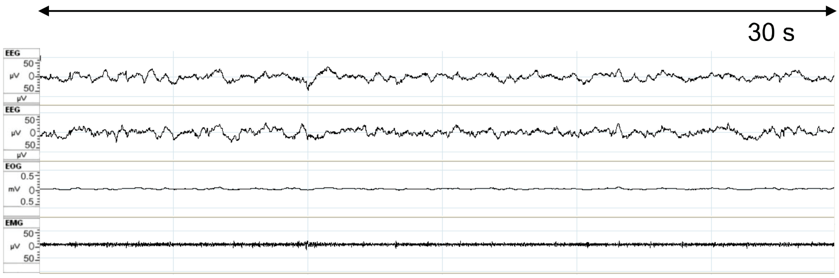
Data from awake periods in **Paper I** resembled those from REM sleep, with a desynchronised pattern with varying amplitude. However, in contrast to REM sleep data, the alert wakefulness data often showed pronounced muscle activity. During the awake state, it was common with muscle activity artefacts from the cow moving around (Figure 2, Awake). The chewing during rumination caused rhythmic artefacts which were seen in all signal traces, with an interruption every minute of about 4-5 seconds when the bolus was swallowed and a new bolus was erupted (Figure 3, Rumination). The pattern of eating resembled that of rumination, but showed less rhythmicity (Figure 3, Eating). As shown in Table 5, the bout duration for sleep, drowsing and rumination was similar in all studies (**Paper I-IV**). However, the awake bout duration was shorter in **Papers I** and **II**.

Table 5. Bout durations (min) ***Paper I-IV****, mean(SD).*

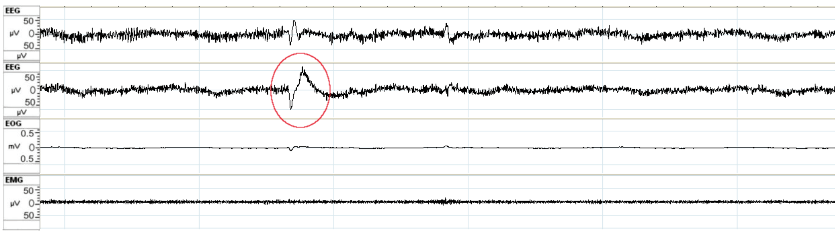
	NREM	REM	Drowsing	Awake	Rumination
Paper I	5(3)	3(1)	3(2)	8(11)	32(12)
Paper II	3(2)	3(1)	3(2)	9(13)	27(19)
Paper III	4(3)	4(2)	2(2)	21(33)	31(16)
Paper IV	5(2)	4(1)	3(1)	26(7)	32(8)

NREM=Non-rapid eye movement
REM=Rapid eye movement

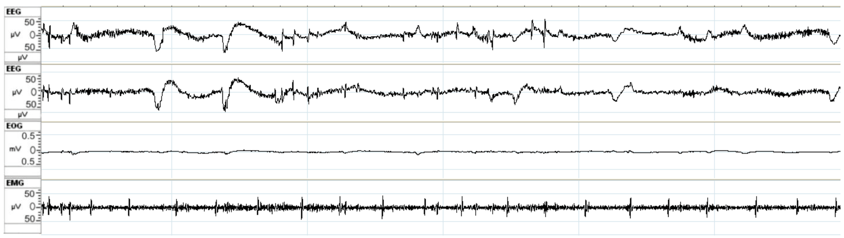
Total sleep and awake time showed great variation between cows, but did not differ between 24-hour periods (**Paper III)**. NREM sleep, REM sleep and drowsing times followed the same pattern, with the highest amount of sleep and drowsing during recording day 2, but the differences were not statistically significant. Number of bouts per recording day was higher for awake during recording day 2 (41 ± 8) compared with days 1 (34 ± 7, P<0.01) and 3 (37 ± 8, P<0.05). Average lying time was 11.9 hours with the shortest lying time in early lactation. Total sleep, NREM and REM sleep times per 24 hours were shorter ($P<0.05$) in early lactation (88 ± 12, 54 ± 10 and 34 ± 4 minutes, respectively) than in late lactation (137 ± 12, 86 ± 10 and 50 ± 4 minutes respectively; **Paper IV**). Drowsing time was longer ($P<0.01$) in late dry period (65 ± 5 minutes) compared with early dry period (42 ± 6 minutes) and lactation week 2, 7, and 13 (38 ± 5, 45 ± 5 and 41 ± 4 minutes, respectively). Rumination time was longer ($P<0.05$) in peak lactation (573 ± 23 minutes) than in late lactation (482 ± 23 minutes).



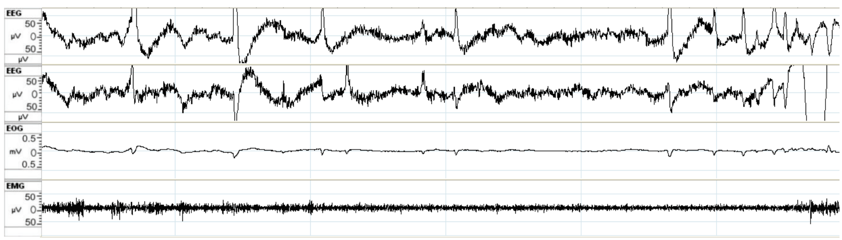
NREM SLEEP



DROWSING

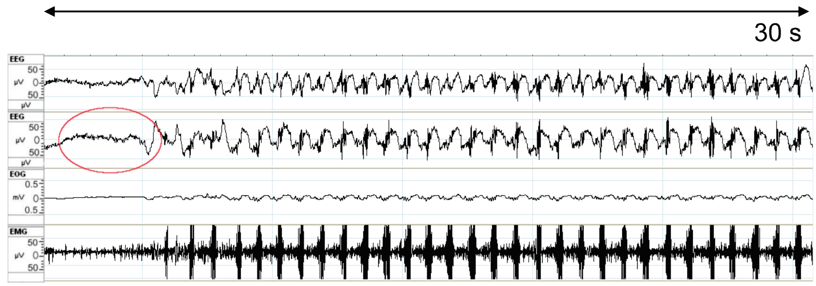


REM SLEEP

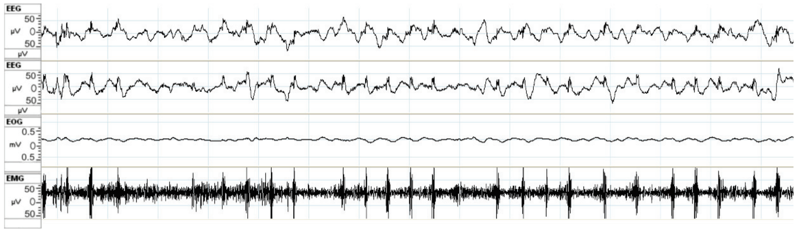


AWAKE

Figure 2. Data from one cow during different sleep and awake states collected in **Paper I**. **NREM SLEEP:** Displaying low frequency signals (EEG), often with low muscle tone (EMG). **DROWSING**: EEG with visually high frequencies, and recurring K-complexes (encircled). **REM SLEEP**: Desynchronised EEG pattern resembling that of alert wakefulness but with very low muscle tone (EMG). **AWAKE:** Desynchronised EEG pattern with marked muscle tone (EMG). *NREM = non-rapid eye movement, REM = rapid eye movement.



RUMINATION



EATING

Figure 3. Data collected in **Paper I**. **RUMINATION:** Very rhythmic pattern with spike-like waves in all traces for approximately 1-min intervals, followed by the recurring sequence of swallowing and erupting a new bolus (encircled). **EATING:** Similar pattern to rumination with profound muscle activity (EMG), but with less rhythmicity.

5.1 Case report

The night before the first recording day in the first-night effect study in **Paper III**, one cow became ill, with a high temperature, clotted milk and reduced milk yield. Milk yield on the previous day was 37.5 kg and milk yield during the three days of observation was 4 kg/day. She was treated with antibiotics (Procaine benzylpenicillin) and analgesic (meloxicam) and retained in the study, although the data collected were not included in the final analysis. Mastitis was later confirmed and diagnosed as *E. coli* mastitis. In addition to the non-invasive sleep recordings, standing and lying behaviour were also recorded using IceTag automatic data loggers. The cow was in lactation number four and in lactation week 21. Data on sleep and awake states from the previous lactation of this particular cow was collected in **Paper IV**. The electrophysiological data showed that total sleep duration during mastitis and fever was double that in the same phase (lactation week 21) in her previous lactation. NREM sleep time per 24-hour period was 104 minutes, 148 minutes and 101 minutes during the first, second and third recording days, respectively, whereas NREM sleep time per 24 hours during week 21 in the previous lactation was 92 minutes. REM sleep time was shortest (16 minutes) during the first recording day and increased to 18 minutes on recording day 2 and 25 minutes on recording day 3. REM sleep time in week 21 in the previous lactation was 54 minutes per 24 hours.

Drowsing time was increased during the first two recording days but decreased during the third recording day (130, 123, 80 minutes, respectively) although not to the same level as recorded in the previous lactation (66 minutes). Rumination time was profoundly reduced during the first recording day, only 20 minutes per 24 hours, but increased gradually to 3.4 hours during the second recording day and to 9.6 hours during the third recording day. Rumination time recorded in the previous lactation was 7.4 hours. During the first recording day, the cow in question lay down for 16 hours, which is two hours more lying time than in her previous lactation (14 hours). Lying time during the second and third recording days was 13 hours and 14 hours, respectively.

6 General discussion

The non-invasive electrophysiological method presented here was validated for recording sleep and awake states in unrestricted dairy cows. The behavioural estimates for sleep in adult cows could not be used to assess sleep time, so electrophysiological recordings were made to investigate the sleep pattern during different stages of lactation and the dry period in adult dairy cows.

The electrophysiological data recorded with a non-invasive electrophysiological method resembled data from earlier studies using surgically implanted electrodes (Ruckebusch, 1972) and data on human sleep (Rechtschaffen & Kales, 1968). The non-invasive sleep recording method for unrestricted cows allows sleep studies to be conducted with less limitations on cow behaviour than when surgically implanted electrodes are used and cows need to be tied.

6.1 Non-invasive electrophysiological sleep recordings

The electrophysiological data showed a similar pattern to the Ruckebusch (1972) data in all studies. Drowsing was displayed when the cow was lying with head lifted and still, which was the position also observed during NREM sleep. The masticatory muscle activity during rumination caused artefacts in all traces and vigilance state during rumination could not be established. Ruckebusch (1972) concluded that cows frequently drowse during rumination and that occasionally the drowsing progresses into NREM sleep. When NREM sleep occurs during rumination, the rate is slowed and a transition to REM sleep often follows when rumination ceases (Ruckebusch, 1972). If NREM sleep and drowsing may occur during rumination information might be concealed in data from rumination when recorded with the non-invasive method. This would explain the large discrepancy of NREM sleep duration during 24-hour periods in this thesis compared with that reported by

Ruckebusch (1972). REM sleep cannot be performed during rumination due to the body position and the reduced muscle tone, so even though NREM sleep time was short overall compared with earlier findings, the REM sleep duration was similar to that described by Ruckebusch (1972). REM sleep bouts were of similar length in all present studies, and total REM sleep duration was similar between Kungsängen and Lövsta. These findings indicate that the REM sleep duration is rather robust and that the non-invasive recording method can record REM sleep time with certainty. Awake bout duration was shorter in the first two studies than in the last two studies. However, the first studies were conducted during night time, and the awake bout durations were similar to the bout duration for awake during night in the last papers.

There are limitations to the non-invasive recording method, as it currently requires cows to be housed individually and involves close handling. Continuous monitoring was necessary to ensure that the equipment stayed in place and that the cows did not show signs of unease or stress due to the equipment. The close handling may have interfered with their behaviour and affected the data. The habituation process in dairy cows is highly dependent on the temperament of the cow (Ruckebusch, 1975b), and therefore cows included in the present studies were selected for being calm and easy handled. To avoid disturbing cows' sleep, research staff only entered the pen when the cows were awake. If the staff had to enter the pen when a cow was lying down, she usually remained in a lying position, indicating that she was little affected by the presence of the staff. The attitude and behaviour of the handler affect the behaviour of the cow and it has been shown that negative tactile handling increases restless behaviour in cows (Waiblinger *et al.*, 2002; Hemsworth *et al.*, 2000). For this reason, the research staff were careful to handle the cows particularly gently. According to Pajor *et al.* (2000), feed is more rewarding for cows than brushing. In the beginning of that study brushing appeared less gratifying for the cows than feed or no handling at all, but they became habituated to the treatment. The cows studied in this thesis were all accustomed to being brushed and this handling procedure allowed the recordings to be continued when cows responded well, but it might have affected the cow's time budget for that particular session. The cows frequently started to ruminate when they were brushed and during preparation when the electrical trimmer was used, and ruminating can be taken as a sign of comfort (Bristow & Holmes, 2007; Uvnäs-Moberg *et al.*, 2001).

At the end of a session the cows were increasingly disturbed by the electrodes and wires, and for some cows only a few electrodes were used during the last hour of the session. However, they did not seem more irritated at the end of the 72-hour sessions than at the end of 24-hour recordings, which could indicate that handling of the cows differed at the end of the recording sessions.

6.2 Use of behavioural indicators to estimate sleep time

The sleep postures described in dairy cows (Ruckebusch, 1972) and in dairy calves (Hänninen *et al.*, 2008) were not sufficient to estimate total sleep time in dairy cows. According to Ruckebusch (1972), cows sleep with closed eyes and drowse with half-closed eyes. Unfortunately, eye stance could not be recorded in this thesis, as the work was conducted at night with only a dim night light. Including eye stance in a previous behaviour estimate for sleep in calves did not strengthen the correlation between behaviour and electrophysiological data (Hänninen *et al.*, 2008).

The posture 'lying with head resting against flank' can be used to identify individual bouts of REM sleep, but not to accurately quantify REM sleep. It would overestimate total REM sleep time, as the cows also displayed NREM sleep and drowsing in this position. REM and NREM sleep times are correlated and thus under constant conditions it might be possible to calculate total sleep time from the REM sleep position. However, sleep deprivation changes the proportion of REM and NREM sleep and this change is dependent on the timing and duration of the deprivation (reviewed by Banks and Dinges, 2007). As it is not possible to know whether a cow is sleep-deprived without prior sleep recordings, the REM sleep position would not be a very precise metric to use in calculating total sleep time for cows.

6.3 Sleep and resting time

The variation in sleep time between cows was large, but in all cows sleep was displayed during day and night time, with a greater proportion at night. In earlier studies using invasive recordings, sleep was only displayed at night (Ruckebusch, 1972) whereas according to Ruckebusch (1975b), on pasture cows sleep during the day as well. Cows brought in from pasture to a tie-stall environment need 3-6 days to adjust to the indoor sleep pattern (Ruckebusch, 1975b). This relatively long adjustment time is probably due to the different management routines in pasture and indoor housing, which have a strong influence on cow behaviour. Access to feed and feeding management are associated with lying time in cows (Deming *et al.*, 2013) and heifers brought in from pasture to tie-stalls have difficulties in lying down compared with heifers brought in to individual pens (Jensen, 1999). The cows in the present study were moved between two indoor systems, loose housing with free-stall and individual pens, so their sleep pattern was probably not profoundly affected by the move. The high temperature in combination with flies and the recording equipment in the summer clearly annoyed the cows. Steensels *et al.* (2012) showed that high temperature decreases lying times and concluded that this is a consequence of heat stress. Decreased lying time reduces the possibility for the cows to rest and sleep.

In human sleep studies it is common practice to discard the first night of recordings due to the altered sleep pattern as a consequence of adaptation artefacts (Le Bon *et al.*, 2001; Agnew *et al.*, 1966). Adaptation artefacts occur if the subject is unaccustomed to sleeping with the somewhat uncomfortable electrodes and wires (Le Bon *et al.*, 2001; Wauquier *et al.*, 1991; Rechtschaffen & Verdone, 1964). In the work described in this thesis, the cows were allowed to adapt to the recording environment, the harness and the halter for two days prior to the recording. Sleep time showed a large variation between cows, but there was no significant effect of recording day for sleep time or for sleep distribution when sleep was recorded over three consecutive days. The variation between cows is probably more important to consider when studying sleep changes over time on several occasions rather than recording during consecutive 24-hour periods.

Cows in late dry period exhibited more drowsing than those in late lactation and early dry period. Lying times also increased with stage of lactation and this has been shown in other studies as well (Bewley *et al.*, 2010; Nielsen *et al.*, 2000), and could be an effect of body weight and late pregnancy. In the lactating cows studied in this thesis, the total sleep time was the longest, and rumination time the shortest, in late lactation. The opposite was shown for early lactation; NREM sleep, REM sleep and drowsing times were shorter and rumination time was longer in early lactation compared with late lactation. In women, lactation increases the amount of slow-wave sleep, although total sleep time is not affected due to a compensatory reduction in light sleep (stage 1 and 2 sleep) (Blyton *et al.*, 2002).

The longest rumination time in the present thesis was recorded in peak lactation, indicating increased feed intake. This means that they need to spend a lot of time feeding, reducing the time available for rest and sleep. In the present thesis, feed and water were readily available and the cows did not need to walk long distances to access different resources. Milking was performed in the pen and time taken for milking did not include waiting in a holding area without the possibility to lie down or to eat. Under these favourable conditions for their time budget, the cows spent on average 11.9 hours per 24 hours lying down, either ruminating, drowsing or sleeping. Lying time reported in this thesis is similar to that reported for high-yielding cows in a free-stall system

(Bewley *et al.*, 2010; Fregonesi & Leaver, 2001), but lower than the 14 hours per 24 hours described in the Ruckebusch study (1972). Lying behaviour is affected by not only milk yield and stage of lactation, but also by management routines (Deming *et al.*, 2013; DeVries & von Keyserlingk, 2005). It is important that cows in loose housing systems are provided with sufficient time to allow them to engage in rest and rumination in a lying position.

Several studies have shown that lack of sleep impairs the immune function (Everson, 1993; Rechtshaffen *et al.*, 1983). It has also been suggested that time in NREM sleep is prolonged during infection (Imeri & Opp, 2009). At the onset of recordings in Paper III one cow became ill and her data was excluded from the results. *E. coli* mastitis and a high body temperature coincided with increased NREM sleep time and drowsing in the cow affected compared with the same period in a previous lactation, while REM sleep time decreased to less than half the time recorded in the previous lactation. These infection-related sleep changes are in line with findings from other species in which infection was induced by injecting them with pathogens (reviewed by Imeri & Opp, 2009). The increase in NREM sleep is thought to be related to reduced energy expenditure, as the energy requirement during NREM sleep is smaller than when the animal is awake. The decreased amount of REM sleep might be linked to infection symptoms because shivering, which is crucial for generating a high body temperature, cannot appear during REM sleep (Imeri & Opp, 2009). Providing enough time for dairy cows to sleep may be important in order to avoid sub-clinical mastitis becoming a clinical infection.

The cows in this thesis were all hand-picked for having a calm temperament and this might have been reflected in their sleep pattern, as it has been reported that the resting behaviour of cows is affected by their temperament (Ruckebusch, 1975b; Ruckebusch, 1975a). If a cow was in heat during a recording session in this thesis, she was much more alert and gave the impression that her lying pattern was more fragmented. The same behaviour was observed close to parturition; a few of the cows were recorded 2, 5 and 6 days before calving, which increased their restless behaviour. Lying time has been shown to decrease compared with later in lactation in the first days after calving (Steensels *et al.*, 2012) and during heat (Esslemont & Bryant, 1976).

7 Conclusions

Using a novel non-invasive technique for recording sleep and awake states in freely moving dairy cows, it was possible to estimate REM sleep and rumination with good accuracy. However, the method needs to be developed further in order to distinguish awake, drowsing and NREM sleep during rumination, as the muscle artefacts from chewing override data on brain activity.

Sleep time in dairy cows varied during lactation, with the shortest sleep time displayed in early lactation. Cows in peak lactation showed the longest rumination time, indicating less available time for resting. The overall conclusions were that:

- Non-invasive electrophysiological methods for recording sleep and awake states in freely moving adult dairy cows can accurately estimate REM sleep time and rumination time.
- Behavioural estimates of sleep in adult cows can be used to assess resting time, but not sleep time.
- Non-invasive electrophysiological recordings carried out over consecutive days do not result in a statistically significant difference in sleep time between recording days, but there is a large variation in sleep time between cows.
- Sleep time in lactating dairy cows increases with stage of lactation. The shortest sleep time occurs in early lactation and dry cows in late pregnancy display the greatest amount of drowsing and lying rumination.

8 Populärvetenskaplig sammanfattning

Bakgrund

Det finns inte så mycket kunskap om behovet av sömn hos mjölkkor men man vet dock att de ligger ner 10 till 12 timmar per dygn. Möjligheten att ligga är viktig för mjölkkor; de väljer hellre att ligga ner än att äta om de inte haft möjlighet att göra någondera under åtta timmar. Under ett dygn använder mjölkkor i lösdriftsbesättning i genomsnitt fyra timmar för att äta, en halvtimme för att dricka och upp till tre timmar för att bli mjölkade. Kor är sociala djur och ägnar två timmar per dygn åt att umgås med andra kor. Dessutom behöver de två och en halv timme för att förflytta sig mellan olika delar av stallet. Studier på sömn hos kor från 1970-talet visade att de sover liggande, fyra timmar per dygn. Enligt de tidiga studierna dåsar också kor sju timmar av dygnet. Dåsa är en form av vila där kon inte riktigt är vaken men heller inte sover och enligt sömnstudierna från 1970-talet dåsar kor 7 timmar per dygn och detta sammanfaller många gånger med idissling. Idissling i sig är kopplat till foderintag och i genomsnitt idisslar kor sju timmar per dygn, ofta när de ligger ner. De flesta däggdjur sover vid flera tillfällen under dygnet, och studier på sömn hos kor visade att de sov nattetid uppdelat på ett antal tillfällen.

Mjölkavkastningen hos kor har ökat avsevärt sedan 1970-talet vilket innebär att dagens kor behöver ägna mer tid åt att äta vilket kan utgöra en konflikt med tid för sömn och vila. Från kalvning och sex till åtta veckor framåt ökar mängden mjölk kon producerar varje dag tills den når den högsta dygnsproduktionen. Under den här tiden behöver kon äta mycket för att täcka energibehovet för mjölkproduktionen. Man brukar säga att en ko under den här tiden varje dag gör av med motsvarande energimängd som en maratonlöpare gör den dagen maratonloppet genomförs. Den första tiden efter kalvning är också då som kon är som känsligast för infektioner. Studier har visat att immunförsvaret kraftigt försämrades och att råttor ökade sitt energibehov efter att ha hållits vakna större delen av dygnet. Det finns därför skäl att tro att sömn och återhämtning är extra viktigt för kor vid tiden efter kalvning. Mellan sex och åtta veckor innan det är dags för nästa kalvning har kons mjölkproduktion ofta avtagit betydligt och hon slutar att producera mjölk, antingen av sig själv eller för att man slutar mjölka henne. Hon är då högdräktig men mjölkas inte och behöver inte äta så mycket som när hon producerar mjölk, det blir därför mer tid över till att ligga ned och vila.

Sömntid och de olika sömnstadierna REM- (rapid eye movement) och NREM- (non-rapid eye movement) sömn kan fastställas genom att mäta hjärnaktivitet, ögonrörelser och muskelaktivitet. I de tidiga studierna på sömn hos kor mättes sömn genom att registrera elektiska signaler i hjärnan. Metoden innebar att hål borrades i skallbenet och elektroder fördes in genom hålen och vilade direkt mot hjärnan. Med den mätmetoden behövde korna vara uppbundna med begränsad möjlighet att röra sig. Nyligen utvecklades en teknik för att mäta sömn på kalvar där elektroderna limmas på huden. Den här mätmetoden är icke-invasiv, det vill säga att den inte innebär något kirurgiskt ingrepp, och djuret kan röra sig fritt.

Det övergripande syftet med det projekt som presenteras här var att anpassa den icke-invasiva tekniken för mätning av sömn hos vuxna kor och undersöka om sömntiden hos dagens mjölkkor ändras under tiden mellan två kalvningar. Totalt utfördes fyra studier i SLU:s besättningar på Kungsängens forskningscentrum (studie 1, 2 och 4) och på Lövsta forskningscentrum (studie 1, 3 och 4), samt i Helsingfors Universitets besättning på Viks försöksgård (studie 2). Korna var alltid i ensamboxar under sömnmätningarna på grund av att mätutrustningen var känslig och för att vi ville veta hur mycket de sov när de inte hade någon konkurrens om liggplats.

Sömnmätning hos mjölkkor

I den första studien utvärderades om metoden som utvecklats för att mäta sömn hos kalvar även går att använda för att mäta sömn hos vuxna kor. Sömn hos åtta mjölkkor mättes under fem timmar samtidigt som kornas beteende studerades så att metoden kunde utvärderas. Data från kornas hjärnaktivitet kunde delas upp i vakenhet, dåsning, REM- och NREM-sömn och data på muskelaktivitet gjorde att idissling kunde identifieras. Signaler från tuggmusklerna var så kraftiga när korna åt och idisslade att de störde alla andra signaler. Analysmetoden behöver därför utvecklas för att kunna filtrera bort muskelaktiviteten under idissling så att det går att fastställa om kon är vaken, sover eller dåsar när hon idisslar.

Tidigare studier har angett ”ligga ner med huvudet vilandes på flanken” som sömnspecifik kroppsposition för mjölkkor så i den andra studien undersöktes om den här positionen kan användas för att fastställa total sömntid hos kor. Totalt 13 kor deltog i den studien och kornas beteende studerades samtidigt som sömn- och vakenstadier registrerades under fyra timmar med den nya mätmetoden. Korna låg alltid ned och sov och när de sov i REM-sömn hade de huvudet vilandes på flanken eller så låg de helt utsträckta så att huvudet vilade på boxgolvet (Bild 1). När korna låg ned med huvudet upplyft dåsade de eller sov i NREM-sömn och det gick inte att skilja de två åt genom att enbart titta på dem. Det går alltså inte att se på kon om hon sover eller bara vilar.

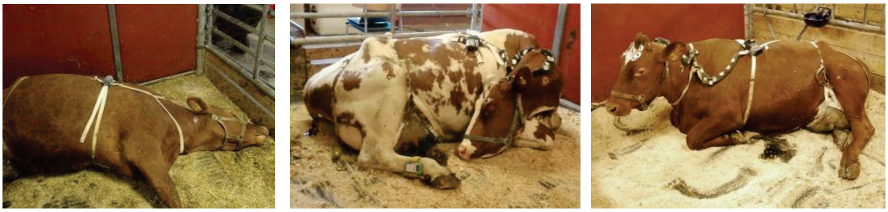


Bild 1. Kroppspositioner för sömn hos mjölkkor i box. Kon sov i REM- eller NREM-sömn i de första två positionerna och hon kunde både dåsa och sova i NREM-sömn i den sista positionen

Sömnstudier på människor har visat att den första natten med mätutrustningen inte ger någon bra bild av sömntider och -mönster. Anledningen kan vara att det är nytt och obekvämt att sova med elektroder och sladdar, och att man då inte sover på samma sätt som när man vant sig vid utrustningen. Det finns en risk att kornas sömn påverkas av utrustningen så den tredje studien utfördes under tre på varandra följande dygn för att bedöma om mätmetoden i sig stör kornas sömn. Sömn- och vakenstadier registrerades med den nya mätmetoden hos nio kor och skillnader i sömntid mellan dygnen undersöktes. Resultaten visade att sömntiden skiljer sig mycket åt mellan kor men att sömnmätningar utförda under ett dygn är tillräckligt för att få tillförlitlig information om total sömntid hos en individ.

Total sömntid hos mjölkkor

Flera faktorer kan påverka sömntid och -mönster hos en individ och man har sett att kvinnor som producerar mjölk har mer NREM-sömn än kvinnor som inte producerar mjölk. Hälsostatus och ålder är ytterligare faktorer som har visat sig påverka sömnbehovet. Eftersom sömn och vila också är viktigt för återhämtning ville vi studera om sömnen hos mjölkkor ändrades inom tidsintervallet för två kalvningar. Sömn mättes med den nya mätmetoden under ett dygn vid sju olika tillfällen på 19 kor. Korna låg ned i genomsnitt 11,9 timmar per dygn och sov 1 timme och 52 minuter per dygn, uppdelat på ett antal tillfällen under både dagen och natten. De sov som kortast två veckor efter kalvning, 1 timme och 28 minuter, och hade längst sömntid strax innan sinläggning, 2 timmar och 16 minuter.

De sömntider för kor som presenterats i detta projekt kan betraktas som minimitider eftersom det eventuellt är möjligt att kor kan idissla och sova samtidigt. Korna låg ned 11,9 timmar och det är rimligt att hävda att de behöver ha möjlighet att ligga ned minst så lång tid per dygn för att ha möjlighet att tillgodose sitt sömnbehov.

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