Automated detection and characterisation of rumination in sheep using in vivo electrophysiology

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Running title: Detection and characterisation of rumination

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Abstract

Rumination is a precisely timed process that occupies a large part of a sheep’s day. The complex motor coordination required to chew and swallow means that quantification of rumination may provide a surrogate marker for effective motor function. Here, data from 24 h in vivo electrophysiological recordings, collected as part of an earlier study, were reanalysed for chewing- and swallowing-related activity. The electroencephalographic (EEG) and electromyographic (EMG) data were collected from sheep with surgically-implanted electrodes. An algorithm was designed to detect coordinated, rhythmic muscle activity. This could distinguish episodes of eating from those of rumination. Normal sheep spent ~29% of their time ruminating. Rumination comprised ~40 s bouts of regular (~1.7 s⁻¹) chewing interspersed by ~6.5 s intervals during which time no chewing took place. Eating was significantly less regular than rumination, with quicker chewing (~2.7 s⁻¹). Biomarkers for measuring progression of disease would be invaluable for studying neurodegenerative disease such as Huntington’s disease (HD). To test the feasibility of using rumination as such a biomarker, we also made recordings from two neurologically impaired sheep. These showed deviations from the pattern of rumination and eating seen in normal sheep. This validates not only our use of rumination as a measure of normal motor function, but also as a surrogate biomarker for measuring motor dysfunction in impaired sheep.

Keywords: dysphagia; mastication; electromyogram; electrooculogram; electroencephalogram.

Abbreviations: HD, Huntington’s disease; EEG, electroencephalogram; EMG, electromyogram; EOG, electrooculogram; E_{inc}, incorporated EMG and EOG data.
1. Introduction

Chewing and swallowing require precise coordination of the tongue, mouth and throat muscles to enable food to be processed and delivered to the stomach without any entering the trachea and lungs. Chewing and swallowing are regulated by both voluntary and autonomic systems under central control (Miller, 1986; Bieger, 1993). Because of this, they are susceptible to dysfunction in conditions that affect motor coordination. They are therefore of interest as markers for the progression of such conditions in both animal and human subjects (Horner et al., 1994; Bine et al., 1995; Daniels, 2006; Heemskerk & Roos, 2010; Weber and Pearce, 2013). Dysarthria (difficulty in speaking) and dysphagia (difficulty in swallowing) are of major importance in many neurodegenerative diseases, such as HD, Parkinson’s disease and Alzheimer’s disease, wherein loss of the ability to speak, and particularly swallow, typically heralds the end of life.

Sheep (*Ovis aries*) typically spend 9-11 h a day grazing and, depending on diet, 8 h or more ruminating as an essential initial stage for digesting the high fibre diet (Gordon, 1965; Broom and Fraser, 2007). Given existing models of neurodegeneration in sheep (Morton and Howland, 2013; Pouladi et al., 2013), we wondered if rumination might be used as a marker for dysphagia.

Rumination takes place in the rumen and reticulum, where food separates into solid and liquid material. The solid component (the cud) becomes degraded into progressively smaller particles, more accessible to microbes in the reticulorumen. Rumination in the polygastric sheep differs from monogastric digestion in that food, once ingested, must be repeatedly regurgitated, chewed, and mixed with saliva. This occurs in a regular cycle of rumination, where regular bouts of chewing are separated by inter-bout intervals of a few seconds in which the cud is swallowed and a new bolus is regurgitated. Most rumination takes place while the animal is at rest. Indeed,
rest periods are essential for effective rumination, and stress may cause sheep to stop ruminating (Arney, 2009).

The complex motor coordination required by chewing and swallowing during rumination is similar to that during eating in other mammalian species (Bieger and Neuhuber, 2006). Various techniques have been used in past studies to monitor rumination in sheep, often involving a transducer attached below the jaw to monitor chewing activity (e.g. Kaske et al., 2002). The biomechanics of mastication during rumination have also been studied using superficially implanted (subcutaneous) electrodes in the masseter muscles (Dejongh et al., 1989; Hirakawa et al., 2001). Here we have used EMG data from electrodes implanted in periorbital and dorsal neck muscles to detect coordinated activity related to chewing. The periorbital electrodes, although intended for recording activity in the ocular muscles (electrooculogram, EOG), were also well situated for recording activity in the jaw muscles (Fig 1; see also Muthukumaraswamy, 2013).

We wanted to develop a measure that could be used as an objective marker for the progression of motor dysfunction in sheep models of human neurodegenerative disease (Morton and Howland, 2013). In this study, we developed an automated procedure for detecting rumination and eating within electrophysiological data collected as part of an earlier study from sheep with chronically-implanted electrodes (Perentos et al., 2015). Here we present this algorithm and use it to characterise, differentiate, and quantify eating and rumination in neurologically normal sheep. We have validated our methodology by comparing these measures in normal sheep with those from two cases of neurologically abnormal sheep that displayed chewing-related abnormalities.

2. Materials and Methods
2.1. Animals

All procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act (1986), and the University of Cambridge ethical review board. Procedures are described in detail in Perentos et al. (2015).

The animals used in this study were locally-sourced Welsh Mountain ewes (n = 4), heterozygous $CLN5^{+/}$ Borderdale sheep (3 castrated male, 2 ewes), and a single male homozygous (affected) $CLN5^{-/-}$ Borderdale sheep. The data from the Borderdale sheep used here were part of a data set that was published previously (Perentos et al., 2015). Only the application of the algorithms used to reanalyse these data is new. The Borderdale sheep were obtained from Lincoln University, New Zealand, where they had been bred and reared in accordance with procedures approved by the Lincoln University Animal Ethics community in compliance with the New Zealand Animal Welfare Act (1999), and in accordance with US National Institutes of Health guidelines. The Borderdale sheep were transported to the UK by air, and EEG implantation and recordings commenced after a three month acclimation period, as described previously (Perentos et al., 2015). Sheep were housed in an outdoor paddock until implantation, and subsequently in a barn with windows allowing natural lighting which was supplemented by an artificial diurnal lighting cycle (6 am – 6 pm). The sheep had ad libitum access to hay feed and water with additional pellet feed from 8 – 9 am. The average age of the sheep at implantation was 14.7 ± 2.2 months.

Two of the sheep were neurologically abnormal. One was an unaffected male Borderdale sheep that experienced neurological damage to the facial nerve during implantation surgery. Human carriers of the CLN5 mutation are neurologically normal, and the $CLN5^{-/-}$ sheep also show no sign of disease (Frugier et al., 2008; Perentos et al., 2015). The other was a $CLN5^{+/}$
Borderdale sheep exhibiting ovine Batten disease symptoms, that was implanted as part of the study mentioned above (Perentos et al., 2015). Clinical signs of disease in affected CLN5+/− sheep are blindness, and poor motor coordination (Frugier et al., 2008). (For further details, see Perentos et al. (2015) for references). These two sheep were included here for comparison with the normal animals.

2.2. EEG implant surgery

Food was withheld for 12 h before surgery. Anaesthesia was induced using Alfaxalone (Alfaxan®, Jurox; 3 mg kg⁻¹, i.v.), and maintained using an isoflurane/oxygen/nitrous mixture administered using a Manley ventilator (stroke vol. 300 ml and ventilation rate 15 – 20 min⁻¹). Isoflurane was maintained at 2 – 3%, end-tidal CO₂ at 25-30mgHg. Intravenous fluids were supplied at a rate of 5 ml kg⁻¹ h⁻¹ (lactated Ringers, Hartmann’s Solution 11 by Aquapharm). Vital functions were recorded at 5min intervals, and blood gases sampled every 30 min throughout the procedure.

Surgery was conducted under aseptic conditions. A midline incision was made at the midpoint between the ears and extended to the occipital end of the skull. Bleeding was controlled using electrocautery. The cranial skin was retracted by blunt dissection, and any remaining connective tissue was scraped off the skull. The skull was cleaned with 3% hydrogen peroxide, and immediately rinsed with sterile saline. Craniotomies were drilled 10 mm either side of the midline on both hemispheres, at positions to allow EEG recording from the frontal, central and occipital cortices (Fig. 1A). These were positioned 30 and 20mm anterior to (frontal), 10mm anterior to (central), and 10mm behind (occipital) the bregma (where bregma is defined as the intersection of the midline skull suture separating the frontal bones, and the transverse suture.
between the frontal and parietal bones, corresponding to the primate bregma). The electrodes were either uninsulated intracortical stainless needle electrodes (length 12 mm) or, for three of the Welsh Mountain ewes, subdural silver/silver chloride discs (3 mm diam, NDimension (Science & Engineering) Ltd., Cambridge, UK). Electrodes of either type were similarly effective for collecting data of the kind used in this study. Needles were inserted through the craniotomies and secured with plastic screws. For placement of disc electrodes, a small incision was made in the dura at the base of each craniotomy, and an electrode was passed through the cut to rest on or near the surface of the brain. Electrodes were implanted in each dorsal neck muscle for EMG recording. For the three Welsh Mountain ewes that were implanted with subdural disc electrodes, an additional bipolar EMG electrode was implanted in the right dorsal neck muscle. Electrodes primarily intended for recording electrooculographic activity (EOG) were implanted in the outer and medial canthi of either the left or right eye. These electrodes were also well positioned for recording activity in the deep and superficial parts of the masseter muscle (Fig. 1B). The EOG electrodes were tunnelled subcutaneously, and EMG wires were sutured to the neck muscles. All EEG electrodes were secured in place with sterile bone cement containing gentamicin (Deputy CMW2, Johnson and Johnson, UK). Electrodes were terminated at a 16-pin Omnetics micro circular connector (Omnetics Corporation, Minneapolis, USA), exteriorised at the dorsal neck area. The incision was sutured and sealed with a wound plaster spray (Wound Plast, Kruuse, Langeskov, Denmark). After surgery, the animals were allowed to recover for 7 – 10 days in their home environment, at which time sutures were removed and recordings commenced.

2.3. Data acquisition
During data acquisition, sheep were held individually in pens, adjacent to, and in sight of other familiar sheep. Sheep wore a pediatric ambulatory amplifier device (Siesta 802, Compumedics Ltd., Australia) in an instrumentation jacket (Lomir Biomedical Inc., Montreal, Canada). Data were sampled at 256 Hz, and hardware band-pass filtered at 0.15 – 128 Hz. Twenty-four hour recordings were stored on-board the device for subsequent downloading (see Perentos et al., 2015). Sheep were monitored and video recorded throughout electrophysiological recordings. On completion of recordings, data were saved to hard disk in European Data Format (EDF) using Scan software (Compumedics Ltd., USA), and imported into Spike2 (Cambridge Electronic Design Ltd., Cambridge, UK) for analysis.

2.4. Data transformation

Data sampled from EEG, EOG and EMG electrodes were DC-balanced using the sampling interval (1/256 s) as the time constant. A baseline for each trace was established by visually selecting fifty 10s epochs, distributed throughout the full period of recording. These were selected on the basis of their indicating that the sheep was calm and stationary at the time of recording (i.e. there was no overt movement-related or chewing-related activity in the EOG or EMG), and that the data were artefact-free. Epochs were incorporated into the baseline sample only when both EOG and EMG fitted these criteria. Baseline EEG epochs were selected as conforming to the same criteria as those for EOG and EMG, with the additional condition that selected epochs were free from overt slow-wave activity (high amplitude oscillations below 4 Hz). Baselines were used subsequently in an artefact rejection procedure described below. The EOG and EMG channels were both duplicated, and one copy of each was root mean square (rms) transformed (EOG_{rms} and EMG_{rms}) using the sampling interval (1/256 s) as time constant. The
EOG and EMG were incorporated in a further data channel \( E_{inc} \) as the square root of the product of the two rms-transformed channels:

\[
E_{inc} = \sqrt{(EOG_{rms} \times EMG_{rms})}
\]

Data are mean ± s.e.m., unless described otherwise.

### 2.5. Artefact rejection

An automated procedure was used to reject artefactual data. Artefact rejection was conducted on serial 10 s epochs throughout the data. Two criteria were employed: (i) drop-outs in sampling, i.e. sustained zero amplitude after DC balancing, for more than 0.5 s, and (ii) transient amplitudes exceeding 100 s.d. of the amplitude of the baseline trace described above. Epochs that contained artefacts detected by either criterion were marked for exclusion from further processing. However, if an epoch with artefact was both preceded and followed by the same defined process (i.e. eating or ruminating) it was counted as being part of that process, since its exclusion would break an episode of eating or ruminating.

### 2.6. Detection of Rumination in EEG data

In cases when chewing-related activity was evident in the EEG, the EEG trace was analysed in the same way as the EOG, incorporating it with the EMG (i.e. \( \sqrt{(EEG_{rms}\times EMG_{rms})} \)), as outlined above, to establish whether this signal could also be used to detect rumination.

### 3. Results

Recordings of EEG, EOG and EMG data were collected from 10 sheep during 24 h recording sessions (Fig. 2A). Data were recorded in two sessions from seven sheep, and in three sessions
from one sheep. These recordings were made between 14 and 155 days post-surgery. Repeat recordings were separated by between 7 and 91 days, and were balanced for recording quality. The remaining data were collected in a single session for each of the two remaining sheep that were neurologically compromised. One of these was the affected CLN5<sup>−/−</sup> sheep, from which data were collected 461 days after implantation surgery, by which time disease symptoms had developed. The other was the sheep that sustained nerve damage during surgery, from which data were collected 13 days post-implantation.

### 3.1. Detection of coordinated neck and jaw muscle activation

Episodes of rumination and eating could be identified in all of the recorded data files (Figs. 2, 3). Data were analysed in serial 10 s epochs through the full recording session. The EOG and EMG traces were rms transformed, and the two transformed traces were incorporated as the square root of their product (see Materials and Methods). The resulting $E_{inc}$ trace reflects the degree of coordination between jaw and neck muscle activity.

Autocorrelation histograms were computed for $E_{inc}$ data (Fig. 2B, C). The contents of these histograms reflect the correlation of the amplitude of the signal at a given point in time with the amplitude of the same signal at times either side of that point within a ±1 s window. Thus a central peak in the histogram simply reflects correlation of the instantaneous amplitude with itself, whereas a secondary modal peak in the histogram, lateral to the central peak, reflects rhythmic activity with a latency equal to the time of the secondary peak (see Fig. 2B, C). Autocorrelation was considered significant if the rise to such a peak, above a trough immediately lateral to the central peak, exceeded one standard deviation of all values across the histogram.
3.2. Detection and differentiation of eating and rumination

Autocorrelation of coordinated neck and jaw activity ($E_{inc}$) was significant in $32.2 \pm 3.4\%$ of epochs per 24 h recording. For each data file, an interval histogram (Fig. 4A, B) was created. These interval histograms indicated two distinct ranges of chewing frequency. These corresponded to ruminating, and eating (or foraging), as confirmed by assessment of a sample of the video recordings made during electrophysiological recordings. Hence, these ranges of chewing frequency were used to distinguish periods of rumination and eating in the data. During rumination (Fig. 4A, C), activation of the jaw and neck muscles, evident in the EOG and EMG traces respectively, was coordinated and regular. During eating (Fig. 4B, D), activation in these muscles was coordinated, but irregular, and faster than during rumination (see Table 1).

3.3. Characteristics of rumination

An episode of rumination was defined as a period of 10 min or more when bouts of regular chewing were interspersed at regular intervals by inter-bout intervals lasting a few seconds. Rumination episodes were identified in all recordings, as periods when the autocorrelation of the $E_{inc}$ trace was significant, and the modal interval corresponded to that selected for rumination, as described above (Fig 4A). A second, shorter, modal interval was used to identify eating (Fig 4B).

Rumination accounted for approximately 29% of daily activity. On average, normal sheep ruminated ~14 times per day (24 h; Table 1), with an average episode duration of approximately half an hour. Therefore, the average rumination episode comprised ~37 rumination bouts. The longest rumination episode was 1 h 45 min; the average maximum duration of a rumination episode per sheep was ~65 min.
The duration of rumination episodes varied significantly over the 24 h recording period (Fig. 5; GLM ANOVA, $F_{23,96} = 1.86$, $P = 0.02$). Curve estimation found the episode durations to best fit a cubic distribution pattern ($F_{3,247} = 8.0$, $P < 0.001$). The longest rumination episodes occurred at night time, when artificial lighting was switched off, peaking in the early hours of the morning, between 01:00 and 05:00 am.

Within rumination episodes, bout durations were determined by identifying the periods of minimal absolute deflection in the $E_{inc}$ trace (Fig. 6A). This was achieved by first plotting the maximum full-scale deflection in 2 s windows throughout each episode, DC-balancing the resulting trace with a time constant of 60 s, truncating the amplitude at zero, and smoothing the trace with a time constant of 5 s. Troughs in the resulting trace (Fig. 6B) marked the mid-points of the inter-bout intervals. The closest chewing events detected in the $E_{inc}$ trace before and after such a trough indicated the end of the preceding rumination bout, and the start of the next respectively. Using these criteria, rumination was shown to be comprised of bouts with an average duration of ~40 s separated by intervals of ~6 s (Table 1).

Bout durations increased over the course of rumination episodes (Fig. 6C). By analysing bout durations in successive 10 minute blocks through rumination episodes, the average bout duration was found to increase, from $39.3 \pm 0.6$ s in the first 10 min of rumination, to $54.2 \pm 5.4$ s after 90 min of rumination (Fig 6C; GLM ANOVA, $F_{9,497} = 2.08$, $P = 0.029$). However, inter-bout intervals remained remarkably unaltered over rumination episodes.

### 3.4. Characteristics of eating

Periods of eating could be clearly identified in all but one of the neurologically normal sheep. For that sheep, the percentage of time when eating was detected (2.5%) was minimal relative to
time spent ruminating (19.9%) compared to the other sheep (38.9% and 28.6% respectively; see Table 1). For the remaining eight animals, time spent eating was in direct proportion to the time spent ruminating \( (p(14) = 0.45, P = 0.041, \text{ single-tailed}) \); the average percentage of time spent ruminating, relative to the time spent eating, was \( 79.4 \pm 29.4\% \), consistent with previous findings (Broom & Fraser, 2007).

3.5. Detection of rumination and eating in EEG data

Chewing-related activity was evident in 22 EEG traces (Fig. 7A), for which we also had EMG recordings. In these cases, the power spectrum of the EEG data was dominated by low frequency oscillations associated with chewing (Fig. 7B). Using the same procedure as described above for generating the \( E_{\text{inc}} \) trace by combining the EOG and EMG, an incorporated trace could also be produced by combining such an EEG trace with the EMG \( (i.e. \sqrt{(E_{\text{rms}} \times \text{EMG}_{\text{rms}})}) \). Episodes of rumination and eating could be detected by replacing the EOG in the rumination detection algorithm by this ‘contaminated’ EEG data.

3.6. Characteristics of rumination and eating in neurologically impaired sheep

Data from two neurologically abnormal sheep were examined. One of these exhibited difficulty with chewing as a result of facial nerve injury during surgery. The other was a CLN5\(^{-}\) sheep exhibiting symptoms of Batten disease, including epileptogenic EEG activity, as we have reported previously (Perentos et al., 2015).

The first of these sheep ruminated only once during a single day of recording, spending 4% of its time ruminating. The duration of the single rumination episode detected in this animal (53 min) was within the normal range for maximum rumination episode duration in the
neurologically normal animals (Table 1). Although the amounts of time this animal spent ruminating (3.9%) and eating (5.9%) were greatly reduced relative to those in the normal animals (28.6% and 38.9% respectively), these were nonetheless in similar proportion to each other; the time spent ruminating was equivalent to 66.3% of the time spent eating. Bout durations (24 s), inter-bout intervals (6 s), and chewing rate during ruminating (2.55 s⁻¹) and eating (2.99 s⁻¹), did not differ significantly from those in the normal sheep. Thus, what distinguished this sheep from the normal sheep was the absolute amount of time spent eating and ruminating. In contrast, the proportion of time the CLN5⁺/− sheep spent ruminating (29.7%) was consistent with the normal sheep (see table 1), as were the number (13) and average duration of its rumination episodes (36 ± 5 min). Rumination bout durations (44.0 ± 2.0 s), inter-bout intervals (5.2 ± 0.6 s), and chewing frequencies during ruminating (1.70 ± 0.03 s⁻¹) were also consistent with those for normal sheep (see Table 1). What distinguished the CLN5⁺/− sheep from the normal sheep was the variability in inter-bout intervals during ruminating (Fig. 8). In the CLN5⁺/− sheep, this variance (24.9 ± 3.0) was much greater than that in the normal sheep (7.7 ± 0.7, GLM χ²₁ = 23.0, P<0.001).

4. Discussion

We have developed an automated procedure for detecting, characterising, and quantifying rumination and eating from electrophysiological data in sheep. We found these activities occurring to a similar extent to those reported elsewhere (Gordon, 1965; Broom and Fraser, 2007). In the neurologically normal sheep, the characteristics of rumination, including episode duration, bout duration, and inter-bout interval, were consistent with those reported previously using manual methods for identification and measurement of rumination from data collected
electrophysiologically (Perentos et al., 2015) or visually (Moquin et al., 2010). Our approach is distinct from that of others in that we did not collect or analyse our data manually. Our method is fast and automated, and delivered accurate results whilst eliminating operator variability from the analysis. This provides a simple method for studying motor control in both normal sheep and in sheep models of human neurodegeneration, which, if applied to data acquired over a sufficient period of time would provide a marker for the progression of motor dysfunction. Chewing and swallowing are complex motor functions in both human and non-human vertebrates (Miller, 1986; Bieger, 1993). The precise timing required by these processes in rumination makes it useful for detecting small changes in motor coordination. This principle could be translated to human subjects. Longitudinal studies of chewing while a subject consumes food requiring prolonged rhythmic chewing would provide valuable information for tracking the progression of disease in diseases where swallowing and chewing deficits are problematic, such as HD (Morton and Howland, 2013).

In these recordings, most rumination was detected during the night. The average duration of rumination episodes was longest in the early hours of the morning, between 1am and 5am, and declined into the start of farm staff working hours. Periods of rest are essential for effective rumination, and stress may cause sheep to stop ruminating. This property of rumination can be used as an indicator of good animal welfare (Arney, 2009). Restricting rumination to low risk periods may be an adaptive strategy in a foraging species under risk of predation (Gregorini et al., 2006). Here, although there is no pressure from predators, rumination was more concentrated at night when there was no distraction from the routine activities of the facility where the sheep were housed. Previously (Perentos et al., 2015), we reported that, whilst diurnal, sheep spend relatively little time sleeping, and that the EEG during periods of rumination includes slow wave
activity similar to that during non-REM sleep. This sleep-like state during rumination may compensate for the otherwise limited opportunity for sleeping.

Bout durations increased over the extent of rumination episodes, so that bouts towards the end of an episode were significantly longer than those at the start of rumination. We have previously reported variation in bout durations with regard to neurological status in Batten disease affected sheep compared to unaffected sheep (Perentos et al., 2015). Inter-bout intervals were not influenced by episode length. This represents a potentially useful parameter distinguishing the oral and gastric components of rumination.

Muscle activity, particularly associated with chewing, is commonly detected in human EEG recordings (e.g. McAdam and Whitaker, 1971), and routinely leads to the rejection of data in both human and animal studies to preserve data integrity (Schomer and Lopes da Silva, 2012). Here, when chewing-related activity was present in the EEG, we show that this EEG artefact from muscle activity may still provide useful data for detecting activity related to chewing during rumination, despite being compromised for EEG analysis. By applying our algorithm to the combined EOG and EMG, it is likely that any signal containing sufficient information related to mouth/neck movements could be used to detect eating and rumination. Thus it is likely that non-invasive electrodes attached to the skin to detect jaw and neck muscle activity, as have been used to study swallowing in human subjects (Watts and Kelly, 2015), would be as effective for collecting data as were the implanted electrodes we used. The use of non-invasive electrodes would be worth investigating in future studies.

Two neurologically impaired sheep exhibited abnormal activity. These sheep were affected in contrasting ways. One of the subject animals experienced difficulty with chewing following complications during surgery. When we examined the data from this sheep, we found that eating
and rumination were qualitatively similar to the normal sheep. That is, bout lengths were not significantly different, inter-bout intervals were similar, and episode length was comparable to the longer rumination episodes in the normal animals. However, this animal spent much less time eating and ruminating, although the time spent ruminating relative to time spent eating was similar to that of the neurologically normal sheep. Thus the difficulties experienced by this sheep seem to have primarily affected eating. Reduced rumination was likely to be due to the fact that there was less in the stomach to digest. The other impaired animal was an affected CLN5<sup>−/−</sup> sheep which exhibited Batten disease symptoms. Batten disease is a progressive neurological disease in which end stage patients need to be fed parenterally. There was no difference in either the time this sheep spent ruminating, or the average duration of rumination bouts compared to the normal sheep. However, irregularity in rumination, detected as increased variability in inter-bout intervals, distinguished this sheep from the normal sheep. It is unclear whether this phenomenon reflects peripheral nerve degeneration, deficits in central command, or gross anatomical (i.e. muscle tissue) changes at the rumen and/or the reticulum. Nevertheless, these comparisons between neurologically normal and abnormal sheep demonstrate that our algorithm is capable of detecting abnormalities in rumination and eating behavior on different levels.

Our algorithm for detection and characterisation of rumination is validated by the consistency of our findings with previous work (Gordon, 1965; Broom & Fraser, 2007; Moquin et al., 2010; Perentos et al., 2015). We demonstrate that these data can be used to characterise both normal and abnormal feeding behavior in sheep. The sensitivity of our technique is sufficient to extract subtle properties from the data, such as increasing bout durations in long rumination episodes. This automated procedure is a valuable tool for quickly, accurately, and
objectively detecting deviations from normal patterns of activity in sheep models of human neurodegenerative disease.

5. Conclusions

Using a novel algorithm for detecting coordinated chewing-related activity in electrophysiological data, rumination is identified as rhythmic chewing, occurring in regular bouts of approximately 40 s, separated by regular intervals of a few seconds. Episodes of rumination may be prolonged, enduring for >1 h. This algorithm distinguishes rumination from eating, in which chewing is faster and less regular. We demonstrate that rumination in sheep experiencing difficulty with chewing because of disturbed motor coordination is distinct from that in normal sheep. We conclude that rumination could serve as a valuable marker for motor dysfunction in sheep models of human neurological disease such as HD.
Figure Legends

**Figure 1. Positions of electrodes.**
Electromyographic activity was sampled from EOG electrodes implanted periorbitally beneath the skin (⊙), and from EMG electrodes implanted in the dorsal neck muscles (○), and EEG data were sampled from subdural silver/silver chloride disc electrodes. The approximate positions overlaying the locations of EEG electrodes on the left and right cortex (●; F1, F2, Frontal; Cn, Central; Oc, Occipital), and the reference and ground electrodes (⊙ and □ respectively) are shown in A. In B, the locations of muscles used for chewing, the superficial and deep parts of the masseter muscle (Ms, Md), and the temporalis muscle (T), are shown with respect to the positions of the EOG electrodes. The pterygoid muscles, attached to the insides of the lower jaw, and also involved in chewing, are not shown. Also shown, delimited by dashed lines, are the positions of the malar (m), depressor (d), and zygomatic (z) muscles.

**Figure 2. Rumination recorded from EOG and EMG electrodes.**
Data from a 24h recording is shown in A. Times of episodes of eating and rumination are indicated by the grey and black boxes respectively below the electrophysiological traces. Autocorrelation histograms of the incorporated EOG and EMG traces are shown in B and C for activity during eating and rumination respectively. The data displayed in B and C are selected from the episodes denoted respectively as B’ and C’ in A. The solid horizontal lines in B and C indicate the mean, and the dashed lines ± 1 s.d. of the amplitudes in the histograms. In each autocorrelation histogram, a secondary modal peak and lateral trough are indicated by p and t.
respectively. The EOG and EMG data used to generate the autocorrelation histograms shown in B and C, are displayed in D and E respectively.

Figure 3. Characteristics of rumination.
Shown in A is a recording of EEG, EOG and EMG, during a single typical rumination episode, lasting for ~45 min (this is the episode denoted as C’ in Fig 2A). Three bouts, denoted B’ in A, are expanded in B. The rumination bouts are indicated by the grey bars below the electrophysiological traces. Asterisks indicate neck muscle activation at the start of each rumination bout. This can be seen clearly, also marked by an asterisk, in C, which illustrates the expansion of the segment denoted as C’ in B. A single chewing interval in C is indicated by the black bar.

Figure 4. Distribution of chewing intervals in eating and rumination.
Modal chewing intervals were computed in 10 s epochs through each recording session, and an interval histogram was generated (shaded grey in A and B; data here were obtained from one sheep during a single recording session). Areas delineated by the solid black line in A and B indicate numbers of epochs included in episodes subsequently defined as ruminating and eating respectively. Two example 10 s epochs contributing to these respective ranges are shown in C and D. Also shown, in E, for comparison, is a 10 s epoch sampled when the sheep was awake, but not chewing.

Figure 5. Circadian distribution of rumination.
In A, the average durations of rumination bouts from all sheep (19 recordings from 10 sheep) are plotted over a 24 h recording cycle commencing at 10:00 am. The time assigned to each rumination episode was the starting point for the episode. The rumination episodes detected in all of the 24 h recordings are plotted in B. Lights in the barn where the sheep were maintained were switched on at 6 am, and off at 6 pm.

**Figure 6. Detection and distribution of rumination bout lengths and inter-bout intervals through rumination episodes.**

In A, the incorporated EMG and EOG trace shows chewing during rumination. The trace in B reflects the absolute chewing activity detected in A. Minima in this trace correlate with cessation of chewing. The shaded bands mark inter-bout intervals (IB). The bout duration (BD) is the time spent chewing. In C, the mean duration of rumination bout durations (■) and inter-bout intervals (●) are plotted for 10 min periods through detected rumination episodes (mean values shown ±s.e.m. where this is of sufficient magnitude to be discernible).

**Figure 7. Detection of rumination in EEG data.**

Rumination could be detected in the incorporated EEG and EMG (Einc). In A, the EMG (top trace) is presented when incorporated with an EEG trace from a central electrode, and another from a frontal electrode. These EEG recordings contained differing levels of chewing-related activity, as is evident in the power spectrum analysis presented for each trace in B. The autocorrelations of the two incorporated traces are presented in C.

**Figure 8. Rumination in a normal and a neurologically impaired sheep.**
Shown in A is the combined EMG and EOG trace ($E_{inc}$) recorded during a rumination episode lasting ~45 min in a neurologically normal sheep. In B, the corresponding trace is shown for a rumination episode of similar duration (~40 min) in the affected CLN5<sup>−/−</sup> sheep. The grey bar beneath each trace indicates the period identified automatically from the start to the end of the rumination episode.
Acknowledgements

We thank Dr Polly Taylor, Roger Mason and Prof Abigail Fowden for assistance with instrumentation, surgery technique and anaesthesia support, and Robin Cumming and Marino Krstulovi for technical support, animal handling and care, and data processing. We thank Prof. David N. Palmer and Ms Nadia Mitchell for breeding and supply of the Batten disease animal.

Author contributions

Data were collected by AUN, NP and AQM. Surgical procedures were carried out by AUN, NP and AJM. AUN processed and analysed the data in close consultation with NP and AJM. AUN, NP and AJM were responsible for interpretation of the analyses. All authors contributed to the final presentation of findings.

Funding

This work was funded by CHDI Inc. (AJM). Costs in New Zealand relating to the rearing and genotyping of the CLN5 animal from which one of the data sets used here were derived were provided by grants from the Neurological Foundation of NZ and the Batten Disease Support and Research Association (Nadia Mitchell and David Palmer, Dept. of Molecular Biosciences, Faculty of Agricultural and Life Sciences, Lincoln University, Christchurch, New Zealand) and CHDI Inc. (AJM).
References


Table 1. Mean values (±s.e.m.) for parameters extracted from identified periods of rumination and eating

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rumination</th>
<th>Eating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of episodes per 24 h recording period</td>
<td>14.5 ± 1.0</td>
<td>25.5 ± 1.6</td>
</tr>
<tr>
<td>Time spent per 24 h recording period (% of total time)</td>
<td>28.6 ± 2.5</td>
<td>38.9 ± 3.5</td>
</tr>
<tr>
<td>Episode duration (s)</td>
<td>1735 ± 71</td>
<td>1331 ± 70</td>
</tr>
<tr>
<td>Maximum episode duration (s)</td>
<td>3901 ± 365</td>
<td>4302 ± 651</td>
</tr>
<tr>
<td>Bout duration (s)</td>
<td>40.7 ± 0.5</td>
<td>NA</td>
</tr>
<tr>
<td>Inter-bout interval (s)</td>
<td>6.4 ± 0.2</td>
<td>NA</td>
</tr>
<tr>
<td>Chewing rate (s⁻¹)</td>
<td>1.7 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
</tbody>
</table>

NA = not applicable
Figure 1
Figure 2

B. Eating
Autocorrelation of $E_{inc}$ (%)

C. Ruminating
Autocorrelation of $E_{inc}$ (%)

D. Eating episodes

E. Ruminating episodes

A. EEG (mV)
EEG (mV)
EOG (mV)
EMG (mV)

Eating and rumination episodes
Figure 3
Figure 4

A. Ruminating

B. Eating

C. Ruminating

D. Eating

E. Not chewing
Figure 6

A

Chewing (E\textsubscript{inc})

0.1mV

B

Absolute chewing activity

10s
IB
BD
IB

C

Duration (s)

60
40
20
0

0 10 20 30 40 50 60 70 80 90 100

Time from start of rumination (min)

(247) (147) (93) (55) (30) (15) (6) (3) (3) (3)

(number of episodes)
Figure 7

A

EMG (mV)

E_{inc} Frontal (mV)

E_{inc} Central (mV)

Time (s)

B

Power (% of total)

Frequency (Hz)

C

Normalised autocorrelation of E_{inc}

Frontal

Central
Figure 8

A. Normal sheep

B. Affected CLN5⁻/⁻ sheep