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Characterising Sleep Spindles in Sheep

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Characterising Sleep Spindles in Sheep

Abstract

Sleep spindles are distinctive transient patterns of brain activity that typically occur during non-rapid eye movement (NREM) sleep in humans and other mammals. Thought to be important for the consolidation of learning, they may also be useful for indicating the progression of aging and neurodegenerative diseases. The aim of this study was to characterise sleep spindles in sheep (*Ovis aries*). We recorded electroencephalographs (EEG) wirelessly from 6 sheep over a continuous period containing two nights and a day. We detected and characterised spindles using an automated algorithm. We found that sheep sleep spindles fell within the classical range seen in humans (10- 16 Hz), but we did not see a further separation into fast and slow bands. Spindles were detected predominantly during NREM sleep. Spindle characteristics (frequency, duration, density, topography) varied between individuals, but were similar within individuals between nights. Spindles that occurred during NREM sleep in daytime were indistinguishable from those found during NREM sleep at night. Surprisingly, we also detected numerous spindle-like events during unequivocal periods of wake during the day. These events were mainly local (detected at single sites) and their characteristics differed from spindles detected during sleep. These 'wake spindles' are likely to be events that are commonly categorised as 'spontaneous alpha activity' during wake. We speculate that wake and sleep spindles are generated via different mechanisms, and that wake spindles play a role in cognitive processes that occur during the daytime.

Statement of Significance

Sleep spindles provide an indication of brain health and function. In this study we characterise sleep spindles in sheep (*Ovis aries*) for the first time. We found that sleep spindles in sheep are similar to those found in humans in many respects (such as density, duration and frequency) and occurred mainly during NREM sleep. Interestingly however, we also saw spindles during wake in the day. Spindles detected during wake were characteristically distinct from those occurring during sleep. We suggest that wake and sleep spindles are generated via different mechanisms and may have different functional roles. Wake spindles may be a component of cognitive processes that occur during the daytime, such as memory retrieval and attention.

Introduction

Sleep spindles are transient and distinctive patterns of brain activity that typically occur during non-rapid eye movement (NREM) sleep in humans. They have been documented in several other animal species, including mice (Kim et al., 2015), rats (Eschenko et al., 2006), dogs (Iotchev et al., 2017), cats (Contreras and Steriade, 1996), monkeys (Takeuchi et al., 2016) and ferrets (McCormick and Bal, 1997). Sleep spindles are considered to play a key role in memory consolidation and have been studied in relation to both learning ability and cognitive impairment (Clemens et al., 2005; Eschenko et al., 2006; Iotchev et al., 2017). Interplay between multiple circuits (the thalamus, cortex, and hippocampus) is known to result in spindle generation (Lüthi, 2013). These same circuits are active during learning in wake (Vukadinovic, 2011). Therefore, it is thought that the functional status of these brain networks can be inferred by the characteristics of spindles observed in EEG recordings (Ciric et al., 2017; Lüthi, 2013).

The quantitative definition of a sleep spindle is not universally agreed, although there is a consensus of opinion that they occur in the 10 – 16 Hz frequency range (Andrillon et al., 2011; Gibbs and Gibbs, 1941). Spindles are characterised by a waxing and waning shape and are generally considered to last between 0.3 and 3 seconds (Gibbs and Gibbs, 1941). In humans, the classical 10 – 16 Hz spindle band is often subdivided into fast (> 13 Hz) and slow (< 13 Hz) spindles. This distinction is made because fast and slow spindles are considered to be generated via different mechanisms (Ayoub et al., 2013; Mölle et al., 2011), resulting in different temporal relationship to slow waves (SWs; Mölle et al., 2011). In sleep, the most prominent EEG feature is the presence of SW reflecting an oscillation between the activated (upstate) and inactivated (downstate) states of cortical neurons

(Contreras and Steriade, 1995). In humans, fast spindles occurring on the SW upstate are thought to have a closer association with memory and learning than slow spindles that occur on the SW downstate (Mölle et al., 2011). Fast spindles are found predominantly in the centroparietal cortex, while slow spindles are more dominant at frontal regions (Andrillon et al., 2011). Among animal models, however, the distinction between fast and slow spindles is less clear. For example, in the macaque (*Macaca fuscata*) the fastest spindles are found in the frontal and central regions, whilst slow spindles are found predominantly at the back of the cortex (Takeuchi et al., 2016). This is the opposite to that found in humans. The apparent function of fast and slow spindles also varies. In dogs only slow spindles (< 13 Hz) predict learning ability (Iotchev et al., 2017), whilst in rats only fast spindles (above 12 Hz) correlate with learning (Eschenko et al., 2006).

Here we used sheep (*Ovis aries*) as a large animal model to study sleep spindles. Sheep have a number of advantages over other species for studying the neurobiology of sleep. They have human-like brain anatomy with elaborately convoluted cortices. They are diurnal, and their sleep structure is more-similar to humans than that of rodents (Toth and Bhargava, 2013). Their husbandry and welfare is easily managed, particularly when compared non-human primates (Morton and Howland, 2013). Techniques for EEG monitoring in sheep are already well established (N Perentos et al., 2016) and telemetric data collection allows continuous longitudinal recording across night and day. We used automated detection methods to identify spindle characteristics in sheep from recordings made continuously over a 34-hour period. We analysed our data according to sleep state and provide a detailed characterisation of sleep spindles in sheep. We show that many of the measures used to characterise human spindles can be used in sheep, and that these measures provide consistent markers of an individual's brain state between different nights.

Because we recorded continuously, we were also able to make direct comparisons of spindles that occur during day and night, and between wake and sleep occurring in both of these periods. We recorded local spindles during wake and provide evidence to show that these are characteristically divergent from the spindles that typically arise during NREM sleep.

Methods

Sheep. Six female merino sheep were used for these recordings. All procedures were conducted at the Preclinical Imaging and Research Laboratories of the South Australian Health and Medical Research Institute (SAHMRI) followed the requirements of the SAHMRI Animal Ethics Committee including the Australian Code for the Care and Use of Animals for Scientific Purposes (8th Edition 2013). All sheep were genetically normal but were part of a flock that included transgenic animals. Accordingly, all handling of these sheep conformed to physical containment conditions as approved by the Institutional Biosafety Committee and the Office of the Gene Technology Regulator (OGTR, Australia). At the time of surgery, sheep were aged ~6 years and their mean weight was 85 ± 3kg. After surgery, they were housed together in a covered outdoor area with natural lighting, in individual pens separated by smooth transparent Perspex partitions.

Surgery. The surgery for implanting electrodes was carried out as described by Perentos et al (2016). Briefly, subdural electrodes (3 mm diam. x 1 mm deep Ag/AgCl discs, NDimension (Science and Engineering Ltd), Cambridge, UK) were implanted at sites across the cortex (Fig. 1A). One of these, positioned at the midline over the transverse sulcus, served as a reference electrode. Eight recording electrodes were positioned bilaterally at locations

corresponding approximately to the post cruciate gyrus (anterior 1, A1), the ansatus sulcus (anterior 2, A2), front third of the ectolateral sulcus (central, C) and the lateral sulcus near the anterior part of the entolateral sulcus (posterior, P). Stainless steel coils were implanted in the dorsal splenius muscles to record neck electromyography (EMG). Electrodes also were positioned at the inner and outer canthi of each eye to record the electrooculogram (EOG). A subdural electrode for online referencing was positioned 10 mm posterior to the bregma at the midline. Wires from all electrodes were terminated at a nano-miniature multi-pin male strip connector (NPD-18-WD-18.0-C-GS, Omnetics Connector Corporation, Minneapolis, USA). When all electrodes were implanted, the connector and wires were enclosed in a 3D-printed polyamide (nylon) chamber (G.E. Baker (UK) Ltd, Bury St Edmunds, UK) fitted to the head of the sheep. During recordings a top stage was fitted to the chamber. This stage held a transmitter amplifier (W2100-HS16, Multichannel Systems Gmbh, Germany), mated to the Omnetics connector, and a battery capable of supporting recording for ~24 h. Signals were transmitted wirelessly. Recordings were conducted 1-4 months post-surgery.

Recording. Recordings were made using a wireless telemetry system (Advanced W2100-System, Multichannel Systems Gmbh, Germany) at a sampling frequency of 1 kHz. EEG data were recorded from 8 cortical electrodes were designated left and right anterior 1 and 2 (A1-L, A1-R and A2-L, A2-R, respectively), left and right central (C-L, C-R), and left and right posterior (P-L, P-R) channels (Fig. 1A). EOG and EMG data were also recorded. Battery changes to the on-sheep telemetry equipment were needed for continuous recording. We chose to do this during the middle of the day at the same time as facility animal husbandry activities were performed. As a result, this period in the middle of each day was omitted from the analysis. Thus, for the day, 9 h recordings were used that comprised of the first

four-hours after sunrise and the last five-hours before sunset. The night recordings were uninterrupted.

Pre-processing. Data were collected in serial 1 h epochs. These were then down-sampled to 250 Hz. Once down-sampled, they were compiled offline into three files (two nights and one day). The EEG data were re-referenced offline using a common average reference for all EEG channels that was subtracted from each channel. Recordings were then imported into MATLAB for spindle detection and characterisation.

Code Accessibility. The code/software described in the paper is freely available online at [https://uk.mathworks.com/matlabcentral/fileexchange/73390-spindle-characterisation-characterising-sleep-sp-in-sheep]. The code is available as Extended Data.

Spindle detection. Automated spindle detection was performed using custom MATLAB scripts. The detection algorithm was run in MATLAB 2018b, on a 64-bit Windows 10 desktop computer. This was originally based upon the SWA-toolbox spindle-detection functionality (Mensen et al., 2016). The adaptations we made to this method include numerous alterations to how spindles were detected, characterised, and processed, as well as data management (see Extended Data 1). In brief, recordings were imported into MATLAB from a text file. In the first processing step, following the SWA-toolbox methodology, a wavelet transform obtained frequency powers between 5 - 16 Hz (Friess et al., 2015). Next, possible spindles were highlighted if the mean power within this range exceeded a threshold (calculated separately for each channel and each sheep: 180% of the mean 5 - 16 Hz power in the night 2 recording). Although night 1 and night 2 recordings were very similar, the night 2 recording was used as the 'baseline' to avoid a possible first-night effect (Toussaint et al., 1995). We made changes to the SWA-toolbox method by altering the way in which

the start and endpoints of spindles were determined to avoid instances where single spindles were incorrectly detected as separate events or where multiple spindles were detected as single spindles. In our method, start and end of each spindle was defined by locating the point at which the mean spindle-range power dropped below 80% of the prominence of its peak, or below 120% of the channel mean power. Next, a discrete fast Fourier transform (FFT) using a Hanning window and 1024 frequency bins for each spindle was used to determine power spectral density and to find the peak frequency and peak frequency power. Detected spindles lasting less than 0.3 seconds or more than 3 seconds were discarded. Spindles with a sufficiently high power within the detection range but with a peak frequency outside of this range were also discarded. Noise-spikes were removed if they contained sudden spikes of power greater than 10 times the mean variation in voltage in the spindle. Finally, in order to be included, a filtered spindle needed to contain at least 3 positive peaks. We allowed our algorithm to detect spindles between 5 to 16 Hz because spindles in other animal models have been found at lower frequencies than those in humans (Contreras and Steriade, 1996; Iotchev et al., 2017). However, we found no evidence of a clear separation in spindle frequency bands (data not shown), therefore in this paper we have focussed on spindles falling into the classical range of 10 to 16 Hz.

Sleep stages: Sleep scoring was performed with semi-automatic analysis using SleepSign software (Kissei Comtec, Matsumoto, Japan) for the night 2 and day periods (e.g. Fig. 2A). Seven vigilance stages were separated based on previously published criteria (N Perentos et al., 2016). Wake (W) was defined by low voltage high frequency EEG accompanied by increased EMG activity and intensive but irregular ocular movements. W with concurrent rumination (WU) was additionally defined by EOG and EMG signals containing regular mastication (N Perentos et al., 2016). NREM sleep was characterized by high voltage SWs,

decreased muscle tone compared to W, and occasional slow rolling eye movements. In NREM sleep with concurrent rumination (U) EMG and EOG channels contain signal consistent with mastication. Based on the delta power, NREM sleep was further separated into 'light' and 'deep' NREM sleep (S1 and S2, respectively). We also differentiated light and deep NREM sleep accompanied by rumination (U1 and U2, respectively). The threshold between S1/S2 and U1/U2 was defined based on the 50% of the maximum delta power. During rapid eye movement (REM) sleep, low voltage high frequency EEG activity was accompanied by rapid ocular movements appearing in bursts. Muscle tone in this stage is reduced compared to NREM sleep but sometimes interrupted by transient muscle activity. Quantitative EEG analysis shows no difference between sleep states with or without rumination (data not shown). The scored data were used to investigate differences in spindle characteristics between vigilance stages, excluding any epochs that had been flagged as containing artefacts.

Sleep cycles: Sleep cycles were automatically identified using the results from the semi-automatic sleep stage scoring. Sleep cycles in sheep are shorter than those in humans (N Perentos et al., 2016). We defined sleep cycles as starting after a minimum of 30 seconds of wake, followed by a period of NREM sleep lasting at least 2 minutes that either terminated with wake or at least 1 minute of REM sleep. Spindle density within sleep cycles was compared by examining the first three sleep cycles for each sheep that lasted at least 18 minutes. This length of sleep cycle was chosen empirically as it was long enough to quantify spindle density changes, but not so long as to be a rare occurrence. Each sleep cycle was split into 3-minute periods. Spindle density during each 3-minute period was quantified. Periods of 3-minutes allowed enough time for reliable and stable counts of spindles. Spindle

densities were then normalised as proportions of the total number of spindles detected for each sheep.

Spindle density throughout night: Spindle density was calculated for each hour period of night 2. This was then normalised (for every channel, in each sheep) according to the amount of time spent in NREM sleep in each hour period.

Spindle-Slow wave relationship: To detect SW activity, EEG was filtered between 0.5 and 2 Hz (Berliersage and Achermann, 2010). To find the SW peak-to-peak amplitude, a 3 second-window either side of every spindle centre was created. The positive and negative peaks on the SW located closest to the spindle centres were found. The difference between these two peaks gave the SW peak-to-peak amplitude for each spindle.

Simultaneous spindles. Spindles frequently occurred in multiple channels. For every spindle, any spindle in another channel that overlapped with it in time was classified as occurring 'simultaneously'. The number of spindles occurring simultaneously for each spindle was recorded, as were the identities of the channels in which each spindle occurred. Spindles can therefore be differentiated into those that occurred 'locally', i.e. in just one channel at a time, or 'simultaneous', i.e. in more than one channel. Simultaneous spindles were then further separated into groups: those occurring in two channels simultaneously (to investigate links between any two particular channels) or those occurring in four or more channels simultaneously (to look at widespread global spindle events). We have developed a method for visualising these data in two different ways. First, for every sheep, the number of simultaneous spindles shared between channels was calculated as a proportion of the total number of simultaneous spindles. Second, the number of simultaneous spindles was

quantified as a proportion of the total number of simultaneous spindles in each channel separately.

Night/day and sleep/wake spindle differences. Spindles were classified by time of occurrence into night or day spindles, and by vigilance stage. This produced the following four vigilance groups: night sleep, day sleep, night wake, and day wake. Characteristics of spindles were compared in each group. These were spindle density, mean frequency, mean duration and mean spindle-SW phase angle.

State space analysis for spectral properties of day wake spindles. State space analysis is a technique used to investigate boundaries and transitions between states of consciousness (Diniz Behn et al., 2010). We used it to determine if wake epochs that contained spindles were any 'sleepier' than general wake epochs. To do this, we calculated ratios of power detected in different frequency bands for every epoch. We used two power band ranges to calculate the state space ratio (SSR). For SSR1 we used 6.5 - 9 Hz / 0.5 - 9 Hz, and for SSR2 we used 0.5 - 20 Hz / 0.5 - 100 Hz. These ratios were based upon previous studies conducted on mice (Diniz Behn et al., 2010). Our ranges differ from those used by that group because we increased the upper bound of SSR2 to 100 Hz, to utilise the high frequencies of our recordings. We found no reason to change these ratios further as they provided clear separation between the NREM and wake sleep states. To form an 'all epoch' state map, all epochs (10 s) scored as wake or NREM sleep were collated for an entire night (3731 potential epochs per sheep). FFTs were performed, as before, on all raw EEG channels within each epoch. The mean power was calculated for all channels, and then the state space ratios for each epoch were determined. To form the spindle-occurrence state map, 10 second epochs centred on every spindle occurrence were collated for all local spindles

occurring in both wake and NREM sleep. The ratios were then calculated in two different ways. The first was the same as for the 'all epoch' state space map; the mean of the FFT output was calculated from all channels. In the second, instead of taking the mean of all channels only the FFT output from the channel that contained the detected spindle was used. To create density maps, the two-dimensional ratio space was gridded and the number of epochs with ratios falling in the state-space of each grid-unit was summed. The grid-unit size was 0.025. For each sheep, the values within each grid-unit were divided by the total number of epochs, giving a proportional density per grid-unit (totalling 1). The maps for all 6 sheep were then summed together, such that the sum of all the units in this new grid space equalled 6. The grid space size was 41x41, giving a total of 1681 grid-units. For purposes of visualisation, thresholds were used to indicate contours around the highest density areas. If the density spread was even throughout the grid each grid would contain a value of 6/1681. The lower and upper thresholds were set as 2.5*(6/1681) and 5*(6/1681), respectively. These values were chosen because they provided easy an interpretable indication of the extent of the wake and NREM sleep states.

Statistical Analysis.

All statistical analyses were performed in RStudio (Version 1.1.463). Unless otherwise stated, data are shown as mean ± s.e.m. One-way repeated measure ANOVAs were used to test differences in spindle characteristics between sheep and channels (repeated factor). Levene's Test was used to check homogeneity of variance. Data were logged where necessary to achieve normal distributions. To investigate spindle density during sleep cycles or throughout the night, density values were normalised in each channel by calculating the densities as proportions of the total density during the three cycles, or the entire night,

294 respectively. Two-way ANOVAs were used to test for differences in proportions of spindle
295 density between sleep cycles or 3-minute windows within each sleep cycle. Five of the 6
296 sheep had 3 sleep cycles lasting over 18 minutes; 1 had only 2 sleep cycles longer than 18
297 minutes. Therefore, data from this sheep were not included in the sleep cycle density
298 analysis. A one-way ANOVA was used to test differences in proportional spindle density
299 between hours in the night. Wilcoxon signed rank tests were used to make pairwise
300 comparisons between vigilance groups to test spindle density differences for local and
301 simultaneous spindles because the variance between groups in these data was not
302 homogeneous. Generalised linear models using Gamma distributions were used to test the
303 correlation in spindle density between vigilance groups. Normal distributions allowed linear
304 models to be used to test correlations in mean spindle frequency and duration between
305 vigilance groups. Sleep scoring information was not needed for testing the difference in SW
306 peak-to-peak amplitude between local and simultaneous spindles, therefore this analysis
307 could be performed for all recording periods (as a repeated factor). P or alpha < 0.05 was
308 accepted as significant throughout.

309

310 **Results**

311 **Spindle detections.** Our algorithm detected sleep spindles in the sheep EEG within the 10 to
312 16 Hz classical range (Fig. 1B and C). We found spindles as both global events occurring in all
313 channels (Fig. 1D and F) and local events occurring in only a single channel (Fig. 1E and G).
314 Mean spindle density for all sheep (N = 6) during NREM sleep ranged from 5.9 ± 1.3 spindles
315 per minute (channel A1-R) to 3.5 ± 0.3 spindles per minute (channel P-L; Extended Data
316 Figure 1-2). There was a non-significant trend for spindle density per minute of NREM to

vary between channels ($F_{(7, 35)}$ = 2.0, p = 0.076, Extended Data Figure 1-2). Spindles were present in the entire 10 to 16 Hz frequency range. Spindle frequency differed between channels ($F_{(7, 35)}$ = 2.8, p = 0.019), although this was driven solely by lower frequency spindles in the C-R channel (Extended Data Figure 1-2). Neither spindle duration nor power differed significantly between channels (Extended Data Figure 1-2).

Individual variance between sheep in spindle characteristics was high. We found that spindles differed significantly between sheep in density ($F_{(5, 35)}$ = 9.7, p < 0.0001), frequency ($F_{(5, 35)}$ = 13.6, p < 0.0001), duration ($F_{(5, 35)}$ = 2.9, p = 0.028), and power ($F_{(5, 35)}$ = 5.1, p = 0.0013). This was not due to a single outlier sheep (see Extended Data Figure 1-1).

Macrostructure. At night, spindle density occurred almost exclusively during NREM sleep episodes (Fig. 2A, B & C). The presence of rumination during a sleep stage did not alter any spindle characteristics. Spindle density did not change during or between the first three sleep cycles (Fig. 2D), and remained stable throughout the entire night (Fig. 2E)

Simultaneous spindles. Inspection of the topography of simultaneous spindles using our visualisation method shows that sheep vary in channel connectivity for both paired simultaneous spindles (Fig. 3B), and for four or more simultaneous spindles (Fig. 3C). During sleep, there were more ~ 50% more simultaneous spindles (Fig. 3F, and Extended Data Figure 4-1) than local spindles (Fig. 3F, and Extended Data Figure 4-1). Taking the means of the simultaneous spindle connectivity maps shows dominance of the A1 channels (Fig. 3D & E). Dominance of the A1 channels is also visible in Fig. 3G & H, where each row represents the proportions of simultaneous spindles relative to the total in each channel separately. Here, the A1 dominance can be seen such that, even in the posterior channels, relative to their own maximum, they have a high propensity to share simultaneous spindles with the

A1 channels. This A1 dominance appears to be as strong as the propensity for spindles to occur simultaneously in their neighbouring channels.

Simultaneous vs local spindle-SW power. Simultaneous spindles (> 1 spindle occurring simultaneously) were associated with more powerful SWs than local spindles ($F_{(1, 269)} = 543$, $p < 0.0001$). The mean simultaneous spindle SW peak to peak power was 0.085 ± 0.0031mV while the mean for local spindles was 0.073 ± 0.0027 mV.

Night/day and sleep/wake differences. Spindle densities were similar for both nights (Fig. 4A and C). More spindles occurred during NREM sleep than in wake. Within NREM sleep, simultaneous spindles occurred more often than local spindles (Extended Data Figure 4-1). Within wake, however, there were more local spindles than simultaneous spindles. Interestingly, there were far more day wake spindles than there were night wake spindles (arrows in Fig.4 B' and C'). These day wake spindles tended to occur locally and predominantly in specific channels (see for example Fig. 4J), although the identity of these high-density channels was not consistent between different sheep. The channel bias in high spindle density was not apparent during NREM sleep in any of the sheep (Fig. 4G and H).

Spindles detected in wake during the day were distinct from those detected during NREM sleep in many respects (Extended Data Figure 4-2). Day NREM sleep spindles and night-time NREM sleep spindles were highly similar in terms of spindle density (Extended Data Figure 4-2A, t-value = 13.05, $p < 0.001$), frequency (Extended Data Figure 4-2C, t-value = 11.64, $p < 0.001$), and duration (Extended Data Figure 4-2E, t-value = 3.72, $p < 0.001$. Day wake spindles and day NREM sleep spindles were similar only in spindle duration (Extended Data Figure 4-2F). There was no correlation between any other characteristics of day wake spindles and day NREM sleep spindles (Extended Data Figure 4-2B, D and H).

363 It was apparent that local spindle detections during wake could occur without any
364 significant SW activity (Fig. 5A). By contrast, local spindles recorded during the day but
365 detected during epochs scored as NREM sleep were embedded in SW activity (Fig. 5B). State
366 space mapping of all scored wake and NREM sleep epochs during the day showed a very
367 clear separation between wake and NREM sleep states with little overlap (Fig. 5C). When
368 considering only epochs that contained spindles, the clear separation between the states
369 remained (data not shown). To assess whether spindle detections during wake occurred
370 during episodes of local sleep, the same sleep state analyses were performed using only the
371 spindle-containing channel. Again, an obvious separation between wake and NREM sleep
372 remained (Fig. 5D), further supporting our finding that spindle events can be found during
373 clear wakefulness.

374

375 **Discussion**

376 We have carried out a comprehensive characterisation of sleep spindles in sheep. Although
377 sleep spindles have been reported in many animal species at a wide range of different
378 frequencies (e.g. mouse, 8-16 Hz (Kim et al., 2015) rat, 12-14 Hz (Sitnikova et al., 2012), dog,
379 12-15 Hz (Jeserevics et al., 2007), cat, 7-14 Hz (Pare et al., 2017), sloth, 6-7 Hz (Voirin et al.,
380 2014), opossum, 8-11 Hz (Van Twyver and Allison, 1970), and monkey, 12-18 Hz (Takeuchi et
381 al., 2016)), a detailed characterisation of spindles in most of these species is lacking,
382 primarily because of the challenges of recording EEG in animals. Our long-duration high
383 quality recording enabled a direct comparison of sheep sleep spindles with those seen in
384 humans. Sleep spindles in sheep are similar to those found in humans in many respects,

385 including their frequency range (10-16 Hz), duration (between 0.5 and 3 s) and density of (1-
386 10 per min) within NREM sleep (Purcell et al., 2017).

387 A better understanding of the role and function of sleep spindles is needed, not only
388 in normal subjects but also in individuals with neurological conditions, particularly those in
389 which cognitive function is abnormal. It would be extremely useful to be able to determine
390 how characteristics of spindles change with age, and whether or not they can be used as
391 biomarkers of disease. Unfortunately, the large variation in spindle density, power and
392 frequency between individuals means that changes in these characteristics are difficult to
393 measure in humans unless large cohorts are used (Purcell et al., 2017). We found such
394 individual differences to also exist in sheep. This reduces the usefulness of spindles in cross-
395 sectional studies as a diagnostic biomarker, unless the impairments are severe. It has been
396 suggested ,however, that by taking advantage of the high intra-individual night-to-night
397 consistency (Cox et al., 2017) it would be possible to use spindles to track progression of
398 neurodegeneration longitudinally (Clawson et al., 2016; Seibt et al., 2016). Spindle density
399 has been reported to change during aging (Purcell et al., 2017) as well as during
400 neurodegenerative diseases such as Parkinson's disease (Emser et al., 1988), Alzheimer's
401 disease (Kam et al., 2019) and Huntington's disease (Wiegand et al., 1991). Indeed, there is
402 *post hoc* evidence to suggest that sleep spindles can be used as early predictors of
403 neurodegenerative disorders (Latreille et al., 2015). We have shown that, in sheep, despite
404 the considerable individual variation, spindle characteristics remain consistent from night-
405 to-night. We have developed novel methodology for investigating the topology of
406 simultaneous spindle occurrences. In humans, it is thought that topology of simultaneous
407 spindles indicates underlying information about the connectively of the brain, and are
408 therefore highly sensitive to neurodegeneration (De Souza et al., 2016). Using our method,

409 we saw simultaneous spindles more often in the anterior recoding channels, as is also found
410 in humans (Botella-Soler et al., 2012). Over longer timescales, a change or breakdown in
411 simultaneous spindle topology may indicate structural brain changes as a result of
412 development, aging, or disease (Clawson et al., 2016). Our visualisation of simultaneous
413 spindle data can highlight such changes. Furthermore, spindles occurring in specific regions
414 of the brain may occur because those specific brain regions of the brain are important in
415 memory and learning tasks (Yordanova et al., 2017). Therefore, short-term increases in
416 spindle density relative to other regions may indicate an intact ability to learn and reprocess
417 memories. This is detectable using our simultaneous spindle visualisation methodology.

418 A key component of spindle generation is considered to be their association with
419 SWs. SWs are thought to synchronise simultaneous spindle events (Andrillon et al., 2011)
420 which underly mechanisms of memory consolidation during sleep (Kim et al., 2017;
421 Yordanova et al., 2017). In accord with findings in both humans (Andrillon et al., 2011) and
422 in mice (Kim et al., 2015), our results showed that simultaneous spindle events were
423 associated with stronger SWs than local spindles events. Interestingly, during wake in the
424 day we found a surprising number of these local spindle events. Spindles are generally
425 considered to be events that occur only during sleep. We exclude the possibility that these
426 wake spindles were occurring during transient periods of sleepiness or during local sleep
427 because epochs in which they occurred were undeniably wake-like. These wake spindles
428 also appeared to occur predominantly in single channels, although there was no consistency
429 between sheep in which channel they would dominate. Furthermore, the characteristics of
430 wake spindles differed from those detected during sleep. While it is possible that wake
431 spindles are a sheep-specific phenomenon, we think this is unlikely given the similarity of
432 sheep sleep spindles to those detected in humans and other animals. Rather, given the lack

433 of previous descriptions of spindles during wake, it is more likely that oscillations in this
434 frequency range during wake are usually classified as alpha activity. Some other studies
435 show that characteristics of alpha activity during wake are different from those of sleep
436 spindles (Barman et al., 1995; Başar et al., 1997). Alpha activity is historically considered to
437 be associated with the idling state of the brain in wake, but there are a growing number of
438 studies linking alpha waves to many elements of sensory and cognitive processing (Başar,
439 2012; Palva and Palva, 2007; Sadaghiani and Kleinschmidt, 2016). These processes include
440 memory retrieval, attention, and consciousness (Klimesch, 2012; Kwok et al., 2019), and are
441 related to behaviours that are well studied in animal models (Bizon et al., 2012; Boly et al.,
442 2013; McBride and Morton, 2018). Given that both alpha oscillations and sleep spindles are
443 associated with memory and cognitive processing, the possibility that the wake spindles we
444 detect play a role in daytime cognitive processes is intriguing. In humans, rare reports of
445 sleep spindles during wake are considered to be a sign of abnormal brain function or aging
446 (Iyama et al., 1992). It would be particularly interesting to investigate the presence of wake
447 spindles in neurodegenerative diseases involving the caudate nucleus and cortex, such as
448 Huntington's disease where an increase in sleep spindles has been reported (Wiegand et al.,
449 1991).

450 To date, long-term EEG monitoring in human subjects in order to track
451 neurodegeneration has not been validated. Sheep would make an excellent species for
452 tackling such challenges, particularly since long-term (up to 4 years) stable recordings can be
453 made in sheep and they are also an established model for studying human neurological
454 disease and dysfunction (Morton, 2018; N. Perentos et al., 2016). This work provides
455 baseline details of sleep spindle characteristics in sheep, as well as methodology that can be
456 used to investigate changes in these characteristics over time.

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582

583 **Figure Legends**

584 **Figure 1. Overview of spindle detection in sheep.** Eight recording channels and a reference
585 electrode were surgically implanted onto the surface of the sheep brain (**A**). Sleep spindles were
586 observed in both raw waveforms (**B**, upper) and when filtered between 10 - 16 Hz (**B**, lower). A
587 spectrogram of the sleep spindle in **B** is shown in **C**. Raw traces for each of the eight recording
588 channels show an example of a global sleep spindle event (highlighted in red) occurring
589 simultaneously in multiple channels (**D**), and a local sleep spindle occurring in a single channel (**E**).
590 The colour scale shows the proportion of the detection threshold. This is scaled to the power in each
591 channel so that a value of '1' represents the power threshold that needs to be exceeded in order for
592 a spindle to be detected. This figure is extended in Extended Data Figure 1-1, and Extended Data
593 Figure 1-2. A = anterior, C = central, P = posterior, R = reference.

594

595 **Figure 2. Spindle density correlates primarily with non-rapid eye movement sleep**. A hypnogram
596 for a single sheep is shown between sunset (t = 0) and sunrise (t = 10) in **A**. Data are shown as mean
597 ± s.e.m. Power of spindle detections from the same EEG recording is shown in **B**. A black dot marks
598 each spindle detection. Detections from all channels are included. The proportional density of
599 spindles detected (N = 6 sheep) in each vigilance stage is shown in **C**. Proportional densities (N = 5
600 sheep) of spindles for the first three sleep cycles lasting 18 minutes are shown in **D**. Proportional
601 densities (N = 6 sheep) of spindles throughout a single night (normalised for amount of NREM sleep
602 in each hour period) are shown in **E**. W = wake, WU = wake with rumination, R = REM sleep
603 (highlighted in red), NR = NREM sleep. NREM sleep is split into four subgroups (light green = S1, light
604 blue = U1, dark green = S2, dark blue = U2).

605

606

Figure 3. Local and simultaneous spindle topography. Spindles that occurred locally (only in one channel) are shown as the proportion of the total number of local spindles that occurred, for each sheep (**A**). The size of each black dot represents the number of local spindles occurring in a particular channel. In **B**, the data represent the occasions on which spindles occurred simultaneously in 2 channels. The size of the black dot is again representative of the number of spindles occurring at each electrode for each sheep. The pairing of spindles is shown by the colour and size of the lines linking two dots. Thicker lines and darker colours mean the likelihood of spindles firing simultaneously in those two channels is higher. The mean of spindle pairings for all sheep (N = 6) is shown in **C.** Data in **D** show the distribution of spindles when 4 or more spindles occur simultaneously. Again, the proportion of spindles occurring in a particular channel is shown by the size of the back dot, and likelihood of simultaneous occurrence of spindles is shown by pseudocoloured lines linking the dots. Meaned data for spindles occurring simultaneously in 4 or more channels is shown in **E** (all sheep; N=6)**.** Histograms in **F** show how many channels were involved in each spindle event, for each sheep. In **G** (for pairs of spindles) and **H** (for spindles occurring in 4 or more channels) the relationship between the channels in which simultaneous spindles occurs is shown as heat maps. For **G**, where data from 2 simultaneous spindles are shown, the rows represent the total number of spindles occurring in a particular channel, the columns show the channel in which the second spindle occurs. For the data shown in H are similarly represented, for spindles that occur in 4 or more channels.

Figure 4. Spindles during the night and day for a single sheep. Cumulative spindle plots show the rate of spindle occurrences during night 1 (**A**), day 1 (**B**), and night 2 (**C**). The change in local and simultaneous spindle density as day sleep returns to day wake can be seen the enlarged plot **B**’ (indicated with an arrow). A similar shift from sleep to wake, but in the night, is shown in **C**’. Simultaneous spindles (occurring in more than one channel at a time) are shown in black. Local

spindles are shown in red. Hypnograms in **D-F** show the changes in vigilance stage for the same sheep, during night 1 (**D**), day 1 (**E**), and night 2(**F**). Spindle density in each channel for this sheep is shown for night 2 NREM sleep (**G**), day 1 NREM sleep (**H**), night 2 wake (**I**) and day 1 wake (**J**). The breaks in the data and axes at 5 hours in the day recordings for panels B and E indicate the times at which battery changes occurred. This figure is extended in Extended Data Figure 4-1, and Extended Data Figure 4-2.

Figure 5. Clear separation of daytime EEG spectral states in epochs containing spindle detections. Local spindles were detected during the day in both wake (**A**) and NREM sleep (**B**). Averaged sleep space state maps of spectral power during the day (**C**; all sheep and all channels) show a clear separation of wake (black) and NREM sleep (blue) states in epochs in which spindles were detected. The solid areas and dashed lines show the ratios where the two sleep states are dominant (with a threshold of 5 (solid) or 2.5 times (dashed) above baseline). The separation of the solid filled areas remains clear when only epochs from individual channels in which a spindle was detected are included (**D;** all sheep, spindle channels only). SSR1 is the ratio of power of 6.5-9 Hz/0.5-9Hz; SSR2 is the ratio of power of 0.5-20 Hz/0.5-100Hz.

Extended Data Legends

Extended Data 1 - Code Files

The spindle detection code is included here. The file 'basicSpindleWorkflow', gives an example of how the code can be run. The code is also available online at the following URL: [https://uk.mathworks.com/matlabcentral/fileexchange/73390-spindle-characterisation-characterising-sleep-sp-in-sheep].

Extended Data 2 - Figure 1-1

Figure 1-1. Examples of inter-sheep differences in spindle characteristics. Whisker plots show spindle characteristics during NREM for each sheep (N=6). Spindle density (**A**) frequency (**B**) spindle length (**C**) and spindle power (**D**). All data are taken from night 2. Spindle characteristics vary widely between individuals, and this is not due to a single outlier.

Extended Data 3 – Figure 1-2

Figure 1-2. General spindle characteristics in sheep.

Extended Data 4 – Figure 4-1

Figure 4-1. Paired Wilcoxon rank sum tests between simultaneous(sim.) or local spindles, and between vigilance group for differences in spindle density (per minute).

Extended Data 5 - Figure 4-2

Figure 4-2. Correlations in spindle characteristics between night/day and sleep/wake periods. Spindle density (per minute) correlations are shown between night sleep and day sleep (**A**), and between night sleep and day wake (**B**). Spindle density is plotted separately for all eight channels in each sheep. Each sheep is identified by a unique symbol: ○, ●, □, ■, △, or ▲. Solid red lines show the linear regression. Dashed red lines show the 95% confidence bounds. Mean spindle frequency correlations are shown between night sleep and day sleep (**C**) and between night sleep and day wake (**D**). Mean spindle duration correlations are shown between night sleep and day sleep (**E**) and between night sleep and day wake (**F**).