

## Research Paper

# Sleep in two free-roaming blue wildebeest (*Connochaetes taurinus*), with observations on the agreement of polysomnographic and actigraphic techniques

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## ABSTRACT

Most studies examining sleep in mammals are done under controlled conditions in laboratory/zoological facilities with few studies being conducted in their natural environment. It is not always possible to record sleep polysomnographically (PSG) from animals in their natural environments, as PSG is invasive, requiring the surgical implantation of electrodes on the surface of the brain. In contrast, actigraphy (ACT) has been shown to be a minimally-invasive method to objectively measure overall sleep times in some mammals, although not revealing specific sleep states. The aim of this study is two-fold, first, to measure sleep polysomnographically in free-roaming blue wildebeest (*Connochaetes taurinus*) under the most natural conditions possible, and second, to establish the degree of concordance between ACT and PSG recordings undertaken simultaneously in the same individuals. Here we examined sleep in the blue wildebeest, in a naturalistic setting, using both polysomnography (PSG) and actigraphy (ACT). PSG showed that total sleep time (TST) in the blue wildebeest for a 24-h period was 4.53 h ( $\pm 0.12$  h), 4.26 h ( $\pm 0.11$  h) spent in slow wave (non-REM) sleep and 0.28 h ( $\pm 0.01$  h) spent in rapid eye movement (REM) sleep, with 19.47 h ( $\pm 0.12$  h) spent in Wake. ACT showed that the blue wildebeest spent 19.23 h ( $\pm 0.18$  h) Active and 4.77 h ( $\pm 0.18$  h) Inactive. For both animals studied, a fair agreement between the two techniques for sleep scoring was observed, with approximately 45% of corresponding epochs analyzed being scored as both sleep (using PSG) and inactive (using ACT).

## 1. Introduction

Behaviourally, sleep is defined by sustained quiescence in species-specific postures accompanied by reduced responsiveness to external stimuli (Tobler, 1995; Lima et al., 2005; Zepelin et al., 2005). Electrophysiologically, mammalian sleep is usually comprised of two states: rapid-eye-movement (REM) sleep and slow wave or non-rapid eye movement sleep (non-REM) (Tobler, 1995; Lima et al., 2005; Lesku et al., 2006; Voirin et al., 2014; Berry et al., 2015). The two major states of sleep, non-REM and REM, alternate in a sleep cycle that is repeated one or more times during a sleep episode (Zepelin et al., 2005; Petersen, 2007; Capellini et al., 2008). Sleep architecture can be defined as the distribution of the sleep cycles within a sleep episode, and also refers to the duration of these episodes and the phasing of sleep across the daily cycle (Lima et al., 2005), with sleep architecture varying across mammalian species (Zepelin et al., 2005; Capellini et al., 2008; Voirin et al., 2014). There are studies that claim episodes of behavioural

quiescence (rest) in invertebrates, and non-mammalian vertebrates present functional equivalents, and perhaps even homologues, of mammalian sleep (Rosenwasser, 2009). This in turn has blurred the traditional distinction between “sleep-wake” and “rest-activity” cycles (Rosenwasser, 2009). Comparative studies of mammalian sleep may provide insights into the function and evolution of sleep by identifying factors correlated with variations in sleep architecture (e.g. Siegel, 2005; Lesku et al., 2006, 2009).

Most studies examining sleep in mammals, specifically “wild” or non-traditional laboratory animals, have been performed under controlled conditions in laboratory or zoological facilities with few studies being conducted on mammals in their natural environment (e.g. Rattenborg et al., 2008, 2016; Lesku et al., 2011; Lyamin et al., 2012; Voirin et al., 2014). The assumption made by the studies conducted in controlled conditions is that the interspecific differences in sleep observed in controlled environments somehow explains similar differences in the wild (Lesku et al., 2009).

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It is not always possible to measure and record sleep polysomnographically (PSG) from animals in their natural environments, as PSG is invasive, requiring the surgical implantation of electrodes on the surface of the brain. In contrast, actigraphy (ACT) has been shown to be a non-invasive method to objectively measure sleep and assess sleep disorders in humans (Sadeh and Acebo, 2002; Ancoli-Isreal et al., 2003; Kanady et al., 2011; Shambroom et al., 2012). Furthermore, validation studies in humans, have shown that the concordance in sleep scoring between ACT and PSG ranges from 83 to more than 90% (Sadeh and Acebo, 2002; Ancoli-Isreal et al., 2003; Kanady et al., 2011; Shambroom et al., 2012). While ACT is now commonly used in the study of sleep in humans, limited studies have made use of this methodology to study sleep in other mammals (e.g. Davimes et al., 2016, 2018; Gravett et al., 2017). The aim of this study is thus two-fold, first, to measure sleep polysomnographically in wild free-roaming blue wildebeest (*Connochaetes taurinus*) under the most natural conditions possible, a species in which sleep has not been studied previously, thereby adding to the comparative dataset available for analysis. Second, this study will aim to establish the degree of concordance between ACT and PSG recordings taken simultaneously, to determine whether using ACT alone can provide a less-invasive method with which to record sleep times in wild, free-roaming species where the use of PSG is not possible.

## 2. Materials and methods

### 2.1. Experimental animals and site location

Two wild, free-roaming, adult male blue wildebeest were located and captured opportunistically in the Dinokeng Game Reserve, South Africa, with the assistance of Wildlife Assignments International (WAI). Each male had an estimated body mass of approximately 250 kg and both were estimated to be between 8 and 15 years in age by an experienced wildlife veterinarian. Permits from the Gauteng Provincial Government were obtained for the capture and transport of the animals from the wild. All animals were treated and used according to the guidelines of the University of the Witwatersrand Animal Ethics Committee (AES number: 2014/53D), which parallel those of the National Institutes of Health (NIH) for the care and use of animals in scientific experimentation.

The two adult male blue wildebeest were housed individually in 200 m<sup>2</sup> enclosures (100 m<sup>2</sup> of which was indoor and 100 m<sup>2</sup> of which was outdoor) on the premises of WAI and allowed to acclimatize for three weeks. The animals were kept in the indoor part of the enclosure at night and allowed out in the mornings and spent the day in the outdoor part of the enclosure. The WAI enclosures were cleaned every morning and the animals were fed lucerne and teff hay once a day and water was also made available ad libitum. Each animal was implanted with a Neurologger® (Vysotski et al., 2006) that allowed the recording of EEG and EMG without cables or restraint and two Actiwatch Spectrums (Philips Respironics) for activity recordings. After the surgical procedure (see below for details of the procedure), the animals were kept in the enclosures described above for 5 days to allow post-surgical recovery and observation. Following this recovery period, the animals were moved to a larger 2-hectare enclosure (20,000 m<sup>2</sup>, or 4.942 acres), where they were housed together, but without any other animals and with no access to this area by potential predators. The enclosure consisted of a fenced piece of land within the Dinokeng Game Reserve that encompassed part of the natural habitat of the blue wildebeest. The animals had access to lucerne, teff hay and water; however, the animals preferred to graze on the available natural vegetation.

### 2.2. Recording period and environmental conditions

The period during which the recording took place was from February 26 to March 9, 2016. The average temperature for the recording period was 24 °C, with a mean maximum temperature of 37 °C, a mean

minimum temperature of 12 °C, and an average daily rainfall of 0.21 mm. This weather data was recorded at the Pienaars River weather station (5 km from the experimental site), and was provided by the Agriculture Research Council (ARC) of South Africa. Sunrise times for the recording period ranged from 05:59 (February 26) to 06:06 (March 9), and sunset times from 18:41 (February 26) to 18:28 (March 9). Sunrise and sunset times were obtained from freely accessible online databases (<https://www.timeanddate.com>).

### 2.3. Devices: Neurologger®

The Neurologger® (model: Neurologger 1, Evolocus LLC) used in the current study weighed 78 g, had approximate dimensions of 66 × 36 × 10 mm (weight and dimensions include wax covering and batteries), and allowed simultaneous recording of six different channels (one EMG, two EEG and three for the accelerometer x, y and z planes). The Neurologger® had an onboard memory of 8 GB in the form of a removable microSD card and was powered by two Lithium ion batteries (Lithium Primary Battery, SW-AA11, 3.6 V, Tekcell, Vitzrocell Co. Ltd.). The sampling rate for each of the channels was set at 500 Hz (electrodes – LiFY Colorflex Li-HF 0.06 mm<sup>2</sup>; Conductor configuration: Cu 30 × ø0.05 mm; Insulation: PVC; impedance approximately 1.1 Ω). The Neurologger® unit was coated with two layers of biologically inert wax (SasolWax 1276, Sasol, Johannesburg, South Africa) and sterilized in a container containing formalin pellets for 48 h prior to implantation.

### 2.4. Devices: Actiwatches

Activity was logged in each animal using two subcutaneously implanted actiwatches, one on the side of the neck and one on the upper hindleg. The actiwatch is a wristwatch size, ambulatory device that is commonly used for measuring sleep in humans (e.g. Ancoli-Isreal et al., 2003; Shambroom et al., 2012). Within each actiwatch is a piezoelectric accelerometer device connected to a microchip that sums and records the number of acceleration events per minute. The Actiwatch Spectrum (Philips Respironics) was used in the current study. These devices have a mass of 25 g and approximate dimensions of 35 × 35 × 12 mm (weight and dimensions include wax covering). Each actiwatch was calibrated and programmed (data acquisition rate set at 1 min intervals) with the Philips Respironics Actiware 5 software, prior to implantation. The wristbands from the actiwatches were removed, and the watches were insulated with standard electrical insulation tape and covered with two coats of biologically inert wax (SasolWax 1276, Sasol, Johannesburg, South Africa) and sterilized in a container containing formalin pellets for 48 h prior to implantation.

### 2.5. Surgical procedure

After the initial acclimatization to the WAI enclosure, surgical implantation of the Neurologger® and actiwatch devices were performed. With the help of an experienced wildlife veterinarian, the animals were anesthetized with weight-appropriate doses of thiofentanyl (4 mg of A30–80®, Wildlife Pharmaceuticals, South Africa) and azaperone (40 mg Stresnil®, Janssen Pharmaceutica, South Africa). Once safely immobilized, the animals were placed in sternal recumbency and then prepared for surgery. The head of the blue wildebeest was secured in an upright position through ropes tied to the horns and anchored to a roof beam. This prevented movement of the head during surgery and allowed for the accurate placement of the EEG and EMG electrodes. The regions of the head and neck that would be manipulated during the surgery were shaved, and the skin cleaned with soap and water and disinfected with chlorhexidine (CHX, Kyron Laboratories, South Africa). During the surgical procedure the animal's heart rate and oxygen saturation were monitored. The animal received oxygen throughout the surgical procedure via a nasal catheter.

Under aseptic conditions, a mid-sagittal incision of the skin overlying

the skull was made after which, the skin and underlying temporalis muscle were reflected to expose the region of the skull overlying the presumed visual cortex (based on comparative estimations of the location of visual cortex in domestic Artiodactyls). Using a cordless Dremel drill, four 2-mm-diameter holes were made (for placement of the EEG electrodes), with a fifth hole drilled posteriorly on the midline of the occipital crest for placement of the ground electrode. The four holes were drilled approximately 1 cm apart, and 2 cm lateral to the sagittal suture (two holes on each side of the midline) over the presumed visual cortex. The EEG electrodes were inserted in each of the holes in such a manner that the tips appeared to rest firmly on the surface of, but did not appear to pierce, the dura mater, as no cerebrospinal fluid was observed to leak from the opening. Once inserted, the electrodes were secured in place with dental cement. A 15 cm subdermal pocket was created on the left side of the neck for the placement of the Neurologger® unit, and the electrodes were fed through a subcutaneous tunnel, of approximately 35 cm in length, from the neck to the skull. In addition to the EEG electrodes, two EMG electrodes were sutured into the nuchal musculature approximately 2 cm apart, near the location of the Neurologger® unit.

Subsequent to the Neurologger® implantation, each animal was implanted with two actiwatches. The two implantation sites (the right side of the neck and hind-leg) were shaved, washed and disinfected with chlorhexidine (CHX, Kyron Laboratories, South Africa). A small incision (approximately 5 cm in length) was made at each of the implantation sites and a subcutaneous pocket extending approximately 10 cm ventral from the incision sites was created. One actiwatch was placed in each of these pockets.

All incision sites were sutured by means of intradermal and superficial stitches and sterilized with the topical antiseptic Necrospray® (Centaur Labs, Johannesburg, South Africa). Following the surgical procedure, each animal received weight appropriate doses of antibiotics and analgesics (8 ml of Draxxin®, Zoetis and 9 ml of Ketofen®, Zoetis) and was returned to the WAI acclimatization enclosure. Once inside the enclosure the anesthesia was reversed by administering weight appropriate doses of naltrexone (50 mg of Trexonil®, Wildlife Pharmaceutical, South Africa), and the animals were closely monitored until they were able to stand and move around freely. The animals were examined each morning for 5 days by the attendant veterinarian, and were considered to have recovered well from the surgery.

## 2.6. Sleep recording and data analysis

Following the five-day recovery period, the animals were moved to the 2 hectare (141.2 × 141.2 m), natural enclosure. The animals were allowed to acclimatize to the new environment for two days, subsequently 72 h of continuous sleep recording commenced in both individuals simultaneously.

## 2.7. Polysomnography (PSG) analysis

The recorded data on the Neurologger® microSD was converted to float32 format and imported into Version 7.02 of the Spike 2 software (Cambridge Electronic Designs, UK) for visual scoring and analysis. Prior to scoring, DC remove was applied to all channels. The PSG data was visually scored in 1 min epochs (to align with the output obtained from the actiwatches, see below) for 72 h and designated as the states of Wake, non-rapid eye movement sleep (non-REM), or rapid eye movement sleep (REM), based on the characteristics of the EEG and EMG as described in detail below. An epoch was only assigned to one of these three states if the state occupied greater than 50% of the epoch. The following variables were calculated and averaged across the recording period for each blue wildebeest: the percentage time spent in each state (Wake, non-REM sleep, and REM sleep) per 24 h, the percentage of REM sleep in total sleep time (TST), the percentage time spent in each state during the light period (sunrise to sunset) and during the dark period

(sunset to sunrise). These variables were adapted from the analysis of Rattenborg et al. (2008) and Voirin et al. (2014) for ease of comparison. Additionally, the number of episodes and the mean duration of the episodes of each state for the entire 24 h, and the respective light and dark periods were calculated. An episode was defined as a succession of consecutive epochs of one state. From the 1-min scored data the modal state for 5-min was calculated and used to determine the onset and duration of the major sleep bouts for all animals. A sleep bout was defined as a period lasting at least 10 min (two consecutive 5-min sleep bouts without waking) and included either/both non-REM and REM sleep epochs. The power spectrum for each of the defined states was calculated with the aid of the Spike 2 computer program (Hanning window, FFT number 512, sampling frequency 500 Hz, segment length 1.024 s). For the spectral power analysis, 10 clearly defined electrophysiological episodes each of Wake, non-REM sleep and REM sleep, as determined through visual evaluation of the recordings, from each animal ( $n = 20$  for each state) were used.

## 2.8. Actigraphy (ACT) analysis

Phillips Respironics Actiware 5 was used to retrieve the recorded data from each of the implanted actiwatches. The data was exported to Microsoft Excel where it was scored and analysed. Data obtained from the neck and leg actigraphs were scored concurrently in 1-minute epochs as either Active or Inactive. For an epoch to be scored as Active either the neck or leg actigraphs had to have an activity score greater than zero. Inactive epochs were scored when both the neck and the leg actigraphs had an activity score equal to zero. The percentage time spent in each state (Active and Inactive) for the 24 h, light (sunrise to sunset) and dark periods (sunset to sunrise) was calculated for all animals over the same recording days PSG analysis was undertaken (see above). Total time spent Active/Inactive was determined for the 24 h, light and dark periods for both animals. From the 1-min scored data the modal state for 5-min was calculated and used to determine the onset and duration of the major Active/Inactive bouts for both animals.

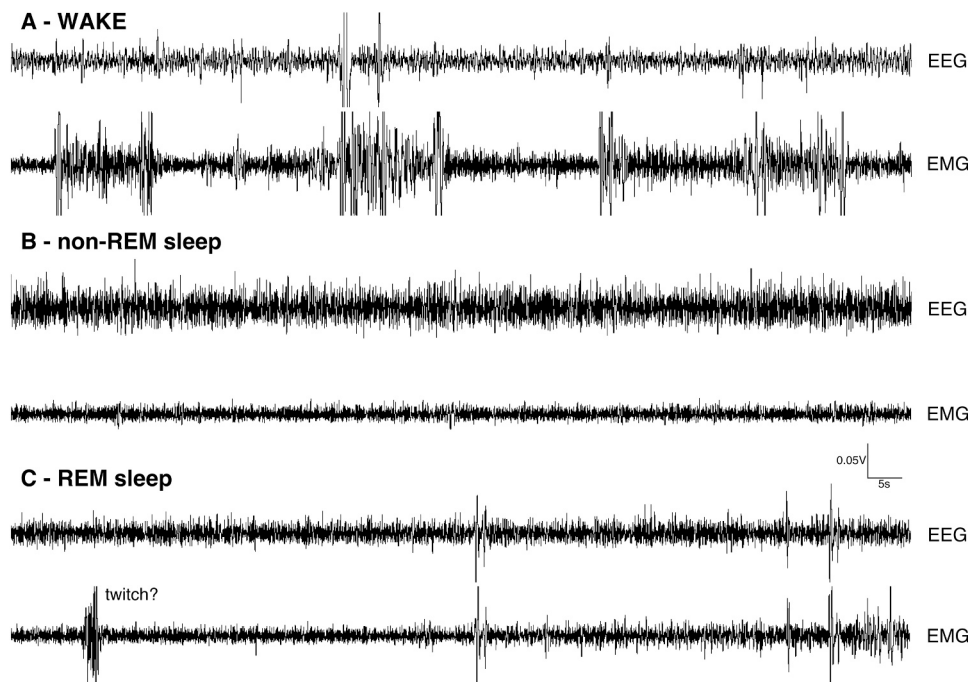
## 2.9. Concordance between polysomnography and actigraphy

In order to determine the degree of agreement between the two recording techniques (PSG and ACT, where Wake was considered equivalent to Active, and non-REM sleep plus REM sleep was considered equivalent to Inactive) we used Cohen's Kappa ( $\kappa$ ), the variable of interest is categorical, which is a statistical method designed to take into account chance agreement (Watson and Petrie, 2010). When Cohen's kappa equals 1, there is complete agreement between the two techniques, while a kappa value equal to zero suggests that the agreement is no better than that which would be obtained by chance alone (Watson and Petrie, 2010). According to Watson and Petrie (2010) (adapted from Landis and Koch, 1977) there is no formal scale for Cohen's kappa, but suggest the following levels of agreement: Poor if  $\kappa < 0.00$ , Slight if  $0.00 \leq \kappa \leq 0.20$ , Fair if  $0.21 \leq \kappa \leq 0.40$ , Moderate if  $0.41 \leq \kappa \leq 0.60$ , Substantial if  $0.61 \leq \kappa \leq 0.80$  and Almost perfect if  $\kappa > 0.80$ . All statistical analyses, where possible, were conducted using IBM SPSS Statistics software version 23 and PAST 3 (Hammer et al., 2001).

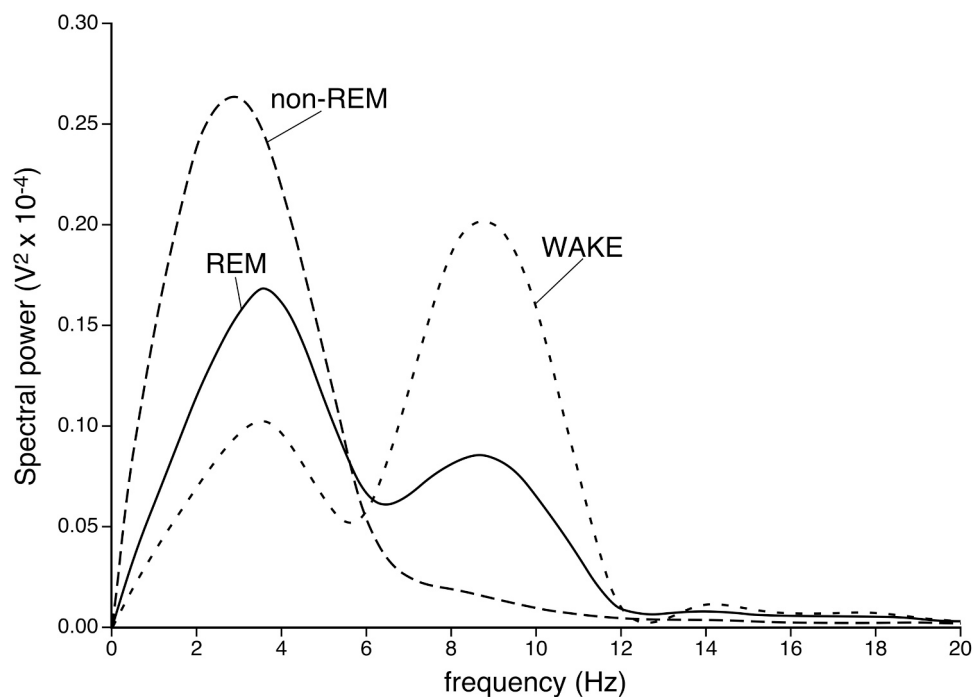
## 3. Results

### 3.1. State definitions

The recording of EEG and EMG allowed the definition of three distinct states in the blue wildebeest, wake, non-REM and REM sleep. Wake was characterized by low amplitude, high frequency EEG, with the EMG exhibiting a high and variable voltage and frequency (Fig. 1A). During Wake, the power spectra range was 0.1–12.5 Hz with a peak power at 8.7 Hz (Fig. 2). Non-REM sleep was identified by the low frequency and high-amplitude of the EEG, while the EMG voltage



**Fig. 1.** Polygraphic traces illustrating examples of the electroencephalogram (EEG) and electromyogram (EMG) in **Wake**, **non-REM** sleep and **REM** sleep states in the blue wildebeest (*Connochaetes taurinus*). (A) Wake is characterized by a low-voltage, high-frequency EEG and high amplitude and variable EMG. (B) non-REM sleep is characterized by a low-voltage, high-frequency EEG signal accompanied by a high frequency, low amplitude EMG signal. (C) REM sleep is characterized by a high frequency, low amplitude EEG signal, resembling that of waking, accompanied by a low-amplitude EMG signal that contained large, high amplitude infrequent spikes indicative of muscle twitches.



**Fig. 2.** Graph indicating the spectral power range characteristic of **Wake**, **non-REM** and **REM** sleep in the blue wildebeest.

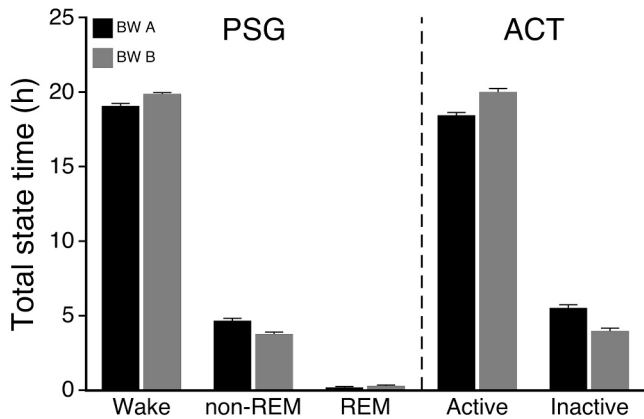
decreased, but was not atonic (Fig. 1B). Episodes of non-REM sleep showed a power spectra range of 0.1–6.7 Hz with a peak at 2.9 Hz (Fig. 2). REM sleep was associated with low amplitude mixed frequency EEG that resembled the Wake EEG, while the EMG activity exhibited consistent low amplitude nearing atonia (Fig. 1C). The power spectra during REM sleep showed a range of 0.1–12 Hz with a peak at 3.51 Hz, and also exhibited hippocampal theta activity with a range of 6.5–10.6 Hz and a peak at 8.6 Hz (Fig. 2).

### 3.2. Time spent in Wake, non-REM sleep and REM sleep states

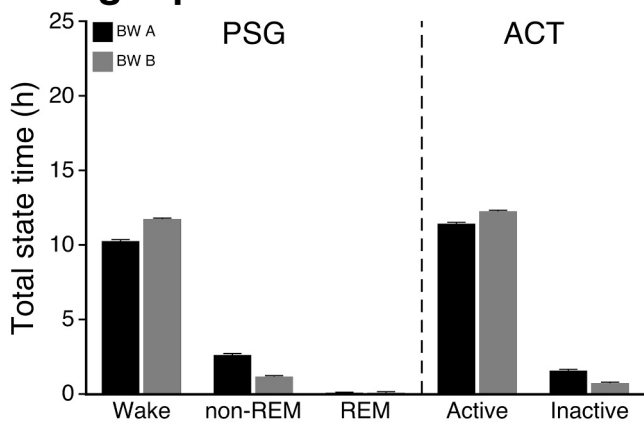
Analysis of the polysomnographic recordings revealed that the average total daily sleep time (TST) for the blue wildebeest studied was 4.53 h ± 0.12 h (mean ± SD). On average for each day, 19.47 h (±0.12 h) was spent in a state of Wake, 4.26 h (±0.11 h) spent in a state of non-REM sleep, and 0.28 h, approximately 17 min (±0.01 h), spent in a state of REM sleep (Fig. 3A). TST during the light period was 1.41 h (±0.06 h), with 11.59 h (±0.06 h) spent in Wake, 1.32 h (±0.05 h) in non-REM sleep and 0.09 h (±0.01 h) in REM sleep (Fig. 3B). During the dark period, the TST observed was 3.12 h (±0.10 h), with 7.88 h



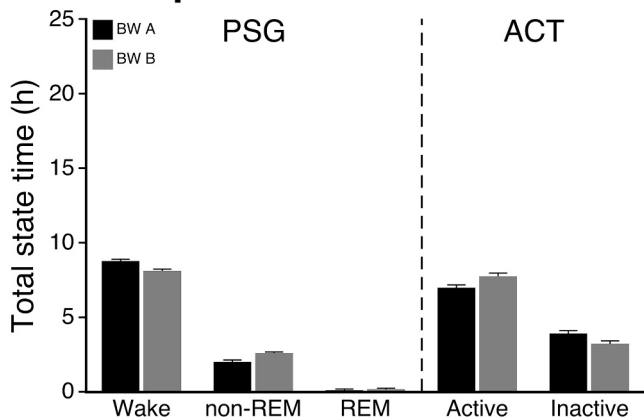
### A - 24 hours



### B - light period



### C - dark period



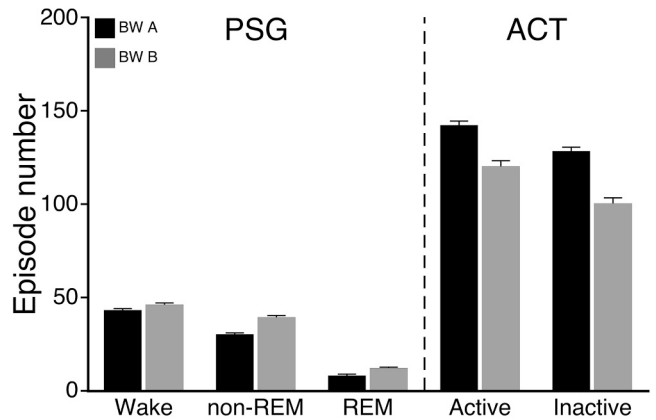
**Fig. 3.** Histograms depicting the total amount of time spent in each of the polysomnographically (PSG, Wake, non-REM and REM) and actigraphically (ACT, Active, Inactive) defined states for (A) the 24 h period, (B) the light period, and (C) the dark period, for both recording methods. BW A – blue wildebeest animal A, BW B – blue wildebeest animal B.

(±0.09 h) spent in Wake, 2.94 h (±0.08 h) spent in non-REM sleep and 0.18 h (±0.02 h) spent in REM sleep (Fig. 3C). REM sleep determined as a percentage of TST was 6.07% over the 24 h period, 3.24% during the light period, and 5.88% during the dark period.

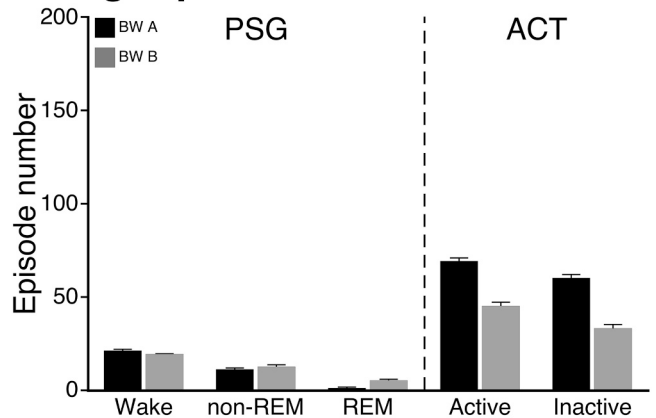
### 3.3. Number and average duration of episodes of wake and sleep states

The average number of daily episodes for Wake was 44.50 (±0.56), non-REM sleep was 34.17 (±0.82) and REM sleep was 9.67 (±0.41) (Fig. 4A). The average number of episodes during the light period was 20 (±0.3) for Wake, 12 (±0.48) for non-REM sleep and 3.33 (±0.25) for REM sleep (Fig. 4B). During the dark period, the average number of

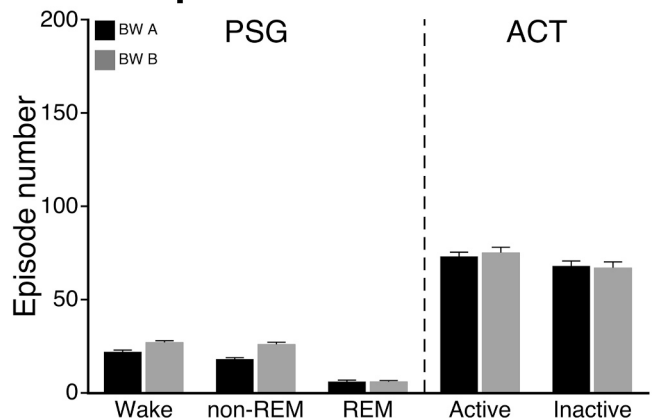
### A - 24 hours



### B - light period



### C - dark period

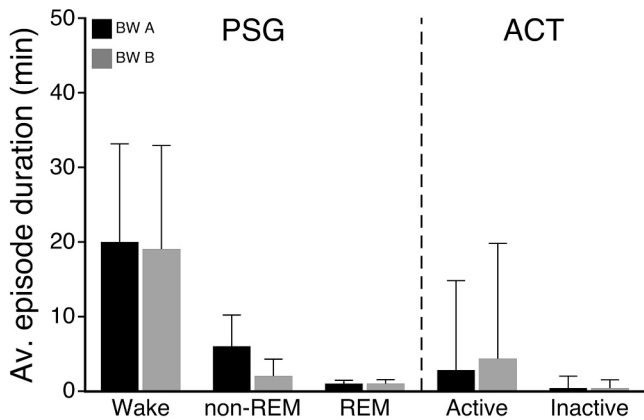


**Fig. 4.** Histograms depicting the average number of episodes for each of the polysomnographically (PSG, Wake, non-REM and REM) and actigraphically (ACT, Active, Inactive) defined states for (A) the 24 h period, (B) the light period, and (C) the dark period, for both analytical methods. BW A – blue wildebeest animal A, BW B – blue wildebeest animal B.

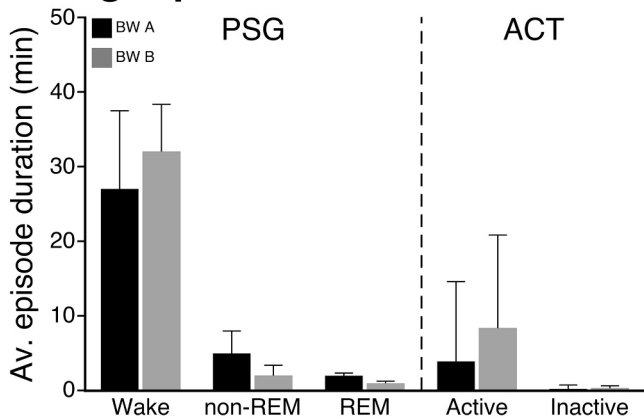
episodes was 24.50 ( $\pm 0.57$ ) for Wake, 22.17 ( $\pm 0.74$ ) for non-REM sleep and 6.33 ( $\pm 0.5$ ) for REM sleep (Fig. 4C). The average duration of the episodes for the 24 h period was 19.58 min ( $\pm 12.00$  min) for the Wake state, 3.79 min ( $\pm 3.03$  min) for the non-REM sleep state, and 1.05 min ( $\pm 0.42$  min) for the REM sleep state (Fig. 5A). During the light period, the average duration of the episodes for Wake was 29.22 min ( $\pm 5.67$  min), non-REM sleep was 3.7 min ( $\pm 2.05$  min), and REM sleep

was 1.08 min ( $\pm 0.26$  min) (Fig. 5B). For the dark period, an average duration of 11.88 min ( $\pm 10.00$  min) was found for the Wake state, 3.85 min ( $\pm 2.32$  min) for the non-REM sleep state, and 1.04 min ( $\pm 0.50$  min) for REM sleep (Fig. 5C). Using 5-min modal times, the average daily onset of the main sleep bout (longest consecutive period of non-REM and REM sleep without any Wake) for both wildebeest occurred between 03:30 and 04:30 and lasted an average of  $43.33 \pm 9.83$  min

### A - 24 hours



### B - light period



### C - dark period

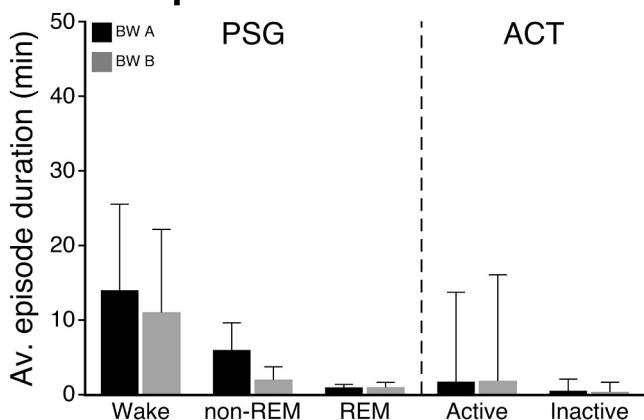


Fig. 5. Histograms depicting the average episode duration for each of the polysomnographically (PSG, Wake, non-REM and REM) and actigraphically (ACT, Active, Inactive) defined states for (A) the 24 h period, (B) the light period, and (C) the dark period, for both analytical methods. BW A – blue wildebeest animal A, BW B – blue wildebeest animal B.

### 3.4. State transition probabilities

Transitions between the physiologically identified states were counted and summed for both animals for the entire recording period (Fig. 6). From the Wake state, the animals only transitioned to non-REM sleep (100% of transitions), with no occurrences (0%) of transition from Wake to REM sleep. From non-REM sleep we observed that the state transitioned mostly to Wake (71.75%), with 28.25% of transitions from non-REM sleep being to REM sleep. From REM sleep, 59.18% of state transitions were to non-REM sleep, while 40.82% of state transitions were to Wake.

### 3.5. Actigraphy (ACT) recordings

Using actigraphy methods we determined two states, Active and Inactive. The average daily time spent Inactive, as scored with actigraphy (see methods), was 4.77 h ( $\pm 0.18$  h), while 19.23 h ( $\pm 0.18$  h) was spent Active (Fig. 3A). During the light period 1.16 h ( $\pm 0.05$  h) was spent Inactive, while 11.84 h ( $\pm 0.05$  h) was spent Active (Fig. 3B). During the dark period, 3.6 h ( $\pm 0.18$  h) was spent Inactive while 7.4 h ( $\pm 0.18$  h) was spent in the Active state (Fig. 3C). The daily average number of episodes for Active was 131 ( $\pm 2.24$ ) and 114 ( $\pm 2.38$ ) for Inactive (Fig. 4A). For the light period, 57 ( $\pm 1.5$ ) episodes were scored as Active, while 47 ( $\pm 1.59$ ) episodes were scored as Inactive (Fig. 4B). During the dark period, there was on average 74 ( $\pm 2.37$ ) episodes spent Active and 68 ( $\pm 2.48$ ) episodes spent Inactive (Fig. 4C). The average duration of these episodes on a daily basis was 3.53 min ( $\pm 13.05$  min) for Active and 0.4 min ( $\pm 1.30$  min) for Inactive (Fig. 5A). During the light period, the average episode duration was 5.72 min ( $\pm 10.40$  min) for Active and 0.29 min ( $\pm 0.36$  min) for Inactive (Fig. 5B). During the dark period, the average duration for Active episodes was 1.84 min ( $\pm 12.86$  min) and Inactive episodes was 0.47 min ( $\pm 1.27$  min) (Fig. 5C). Using the actigraphy scoring criteria, the average daily onset of the main sleep bout (longest uninterrupted period of Inactive) for both wildebeest occurred between 04:30 and 05:00 and averaged 49.16 min ( $\pm 10.68$  min).

### 3.6. Concordance between polysomnography (PSG) and actigraphy (ACT)

In general, actigraphy methods tend to overestimate the total sleep

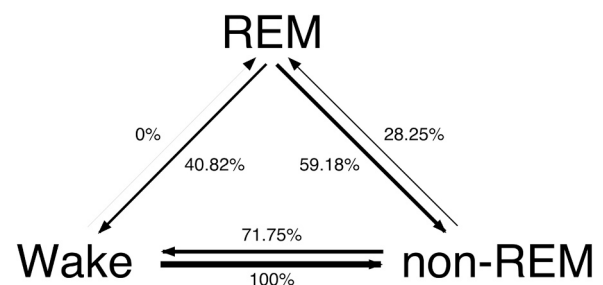


Fig. 6. Flow diagram illustrating the occurrence of state transitions (represented as a percentage) between Wake, non-REM sleep and REM sleep states with the pooled data from both animals studied over the 72 h recording period analyzed.

time compared to PSG methods due to the inability of ACT to distinguish quiet wake from sleep states (Paquet et al., 2007). This was observed for the two blue wildebeest in the current study, where the total Inactivity measured by ACT for 24 h was 4.77 h ( $\pm 0.18$  h), while TST (non-REM plus REM sleep) using PSG was 4.53 h ( $\pm 0.12$  h), ACT overestimating TST by a daily average of 14.2 min ( $\pm 12.98$  min); however, when each day for each animal was analyzed individually, actigraphy did not always appear to overestimate the amount of time spent asleep (Fig. 7). The ACT scoring for Animal A, on average for the 3 days analyzed, overestimated daily TST by 37.6 min ( $\pm 19.08$  min), for the light period by 3 min ( $\pm 11.77$  min), and the dark period by 34.6 min ( $\pm 21.17$ ). In the case of Animal B, ACT scoring underestimated daily TST by 9.66 min ( $\pm 16.69$  min), and by 33 min ( $\pm 9.83$  min) in the light period. The ACT scoring overestimated the TST in the dark period by 23 min ( $\pm 18.22$  min) in Animal B (Fig. 7). Despite these differences, it appears, when examining across the entire recording period that ACT provided a reasonably accurate measurement of TST in both animals studied.

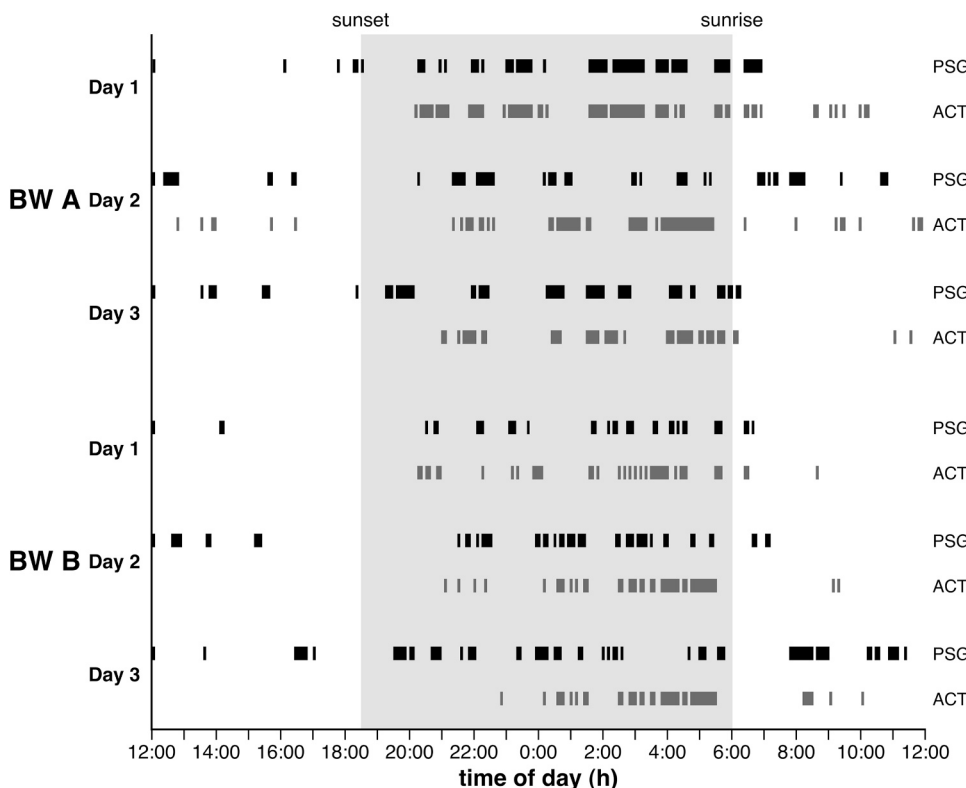
When we examined the data in finer detail, to determine the extent of overlap between PSG scoring and ACT scoring for the individual epochs, a different picture emerged (Table 1). When examining the 1 min epoch data for both animals over the 3 days recordings (6 days), a total of 1632 1 min epochs were scored as sleep with the PSG data, while a total of 1720 1 min epochs were scored as inactive with the ACT data; however, the number of corresponding 1 min epochs that were scored as both sleep (PSG) and inactive (ACT) numbered only 738 (Table 1). Thus, only 45.2% of the 1 min epochs scored as sleep with PSG were simultaneously scored as inactive with ACT. Similarly, for the 5 min epoch data, a total of 326 epochs were scored as sleep (PSG) and 337 scored as inactive (ACT), with 147 corresponding epochs (45.1%) being scored as sleep and inactive (Table 1).

A Cohen's  $\kappa$  was run to determine the extent of agreement between PSG and ACT scoring methods regarding sleep/inactivity, over the 3 days of recording for both blue wildebeest, for the measure of TST (inactive equivalent compared to non-REM plus REM sleep) and time spent awake (Wake compared to Active). There was fair agreement

between the two techniques for sleep measurements,  $\kappa = 0.306$  (95% CI, 0.281–0.330),  $p < 0.0001$ . The agreements were different for the individual animals, with animal A showing a fair agreement between the techniques for TST estimation,  $\kappa = 0.388$  (95% CI, 0.355–0.421),  $p < 0.0001$ , whereas animal B showed a slight agreement between the techniques for TST estimation,  $\kappa = 0.198$  (95% CI, 0.163–0.234),  $p < 0.0001$  (Table 2).

#### 4. Discussion

The present study examined sleep in two blue wildebeest, in a naturalistic setting, by simultaneously employing two different measures of sleep, polysomnography (PSG) and actigraphy (ACT). Our recordings indicate that the blue wildebeest displays the typical non-REM and REM states of sleep observed in most mammals studied, without any additional non-typical sleep states being observed. Moreover, they slept mostly during the dark period, in the early hours of the morning, and exhibited polyphasic sleep, which could be considered typical for an artiodactyl based on studies of domestic species (Tobler, 1995). PSG showed that daily TST in the blue wildebeest for a 24-h period averaged approximately 4.5 h, with approximately 4.3 h spent in non-REM sleep and 0.3 h (18 min) spent in REM sleep, with the remainder of the 24-h period, approximately 19.5 h spent in Wake. This amount of sleep appears to be what one would expect based on the body mass of the blue wildebeest in comparison to measures of total sleep time in other Artiodactyls (Siegel, 2005; also see below). ACT showed that the blue wildebeest spent on average approximately 19.2 h Active and 4.8 h Inactive each day. Thus, the total daily sleep amount was similar between the two methods, but ACT analysis indicated a slightly higher amount of time spent in sleep/Inactive, by around 15 min each day. This indicates that during the analysis of the ACT data a portion of quiet waking is likely the cause of the slightly inflated total daily sleep time. However, when examining the correspondence of the scoring methods in fine detail, it appears that the two methods only have a fair level of agreement, with only approximately 45% of epochs scored as sleep with



**Fig. 7.** Plots of 5 min epochs scored as sleep (non-REM/REM, black) using polysomnography (PSG) or inactive (dark grey) using actigraphy (ACT) for both blue wildebeest (BW A and BW B) for the three days of recording (Day 1, Day 2, Day 3). These plots show the match and mismatch of the two scoring techniques. The light grey region represents the period between sunset and sunrise. Note that the vast majority of sleep occurred during the hours leading up to sunrise, but occasional sleep/inactive episodes could occur at different times throughout the 24-h period.

**Table 1**

Cohen's  $\kappa$  showing the agreement between polysomnography (PSG) and actigraphy (ACT) recordings, for the individual days of recording for each blue wildebeest, for the measure of total sleep time (combined non-REM and REM compared to Inactive) and wake (Wake compared to Active). The statistically significant results are indicated by an asterisk.

Animal	Day	Cohen's $\kappa$	Level of agreement	p value	Std. error	95% CI	
						Lower limit	Upper limit
A	1	0.596	Moderate	0.000*	0.025	0.546	0.645
	2	0.241	Fair	0.000*	0.034	0.175	0.307
	3	0.301	Fair	0.000*	0.035	0.232	0.370
B	1	0.273	Fair	0.000*	0.044	0.188	0.359
	2	0.313	Fair	0.000*	0.036	0.242	0.383
	3	0.034	Poor	0.188	0.038	0	0.109

**Table 2**

Cohen's  $\kappa$  showing the agreement between polysomnography (PSG) and actigraphy (ACT) recordings, for the individual days of recording for each blue wildebeest, for the measure of total sleep time (combined non-REM and REM compared to Inactive) and wake (Wake compared to Active). The statistically significant results are indicated by an asterisk.

Animal	Day	Cohen's $\kappa$	Level of agreement	p value	Std. error	95% CI	
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A	1	0.596	Moderate	0.000*	0.025	0.546	0.645
	2	0.241	Fair	0.000*	0.034	0.175	0.307
	3	0.301	Fair	0.000*	0.035	0.232	0.370
B	1	0.273	Fair	0.000*	0.044	0.188	0.359
	2	0.313	Fair	0.000*	0.036	0.242	0.383
	3	0.034	Poor	0.188	0.038	0	0.109

PSG being simultaneously scored as inactive with ACT.

#### 4.1. Comparisons to sleep in other Artiodactyla

The vast majority of systematic studies of mammalian sleep have been conducted in only a few domesticated species including rats, mice, cats, dogs, monkeys and humans within well controlled settings (Lesku et al., 2006; Siegel, 2008). Very few studies of mammalian sleep have been undertaken in their natural habitat (Rattenborg et al., 2008; Voirin et al., 2014; Yetish et al., 2015; Davimes et al., 2018). Along with the cetaceans, the blue wildebeest is a species assigned to the superorder Cetartiodactyla; however, comparing sleep between blue wildebeest (or indeed other terrestrial Artiodactyls) and cetaceans is not tenable due to the specialized nature of cetacean sleep phenomenology (Lyamin et al., 2008). Thus we, for the most part, restrict our comparisons to other members of the terrestrial Artiodactyla.

Within the Artiodactyla, the majority of polysomnographically conducted sleep studies have been undertaken on the domesticated species belonging to this order. The TST per day reported for the cow (*Bos taurus*) was 3.97 h (Ruckebusch, 1972), sheep (*Ovis aries*) 3.85 h (Ruckebusch, 1972), goat (*Capra aegagrus*) 5.4 h (Bell and Itabisashi, 1973), pig (*Sus scrofa*) 7.82 h (Ruckebusch, 1972; Robert and Dallaire, 1986), and in the non-domesticated Arabian oryx (*Oryx leucoryx*) 6.60 h in winter and 3.77 h in summer (Davimes et al., 2018) and in the current study blue wildebeest 4.5 h. Behaviourally, the TST of the giraffe (*Giraffa camelopardalis*) was reported to be 4.6 h (Tobler and Schwierin, 1996). Thus, the result of an average daily total sleep time of 4.5 h for the two blue wildebeest investigated in the current study appears to be within the range of daily total sleep time reported for phylogenetically related terrestrial species, even though the current study was undertaken in as close to natural conditions as possible, which has been shown to lower total sleep time in mammals (Rattenborg et al., 2008; Yetish et al., 2015).

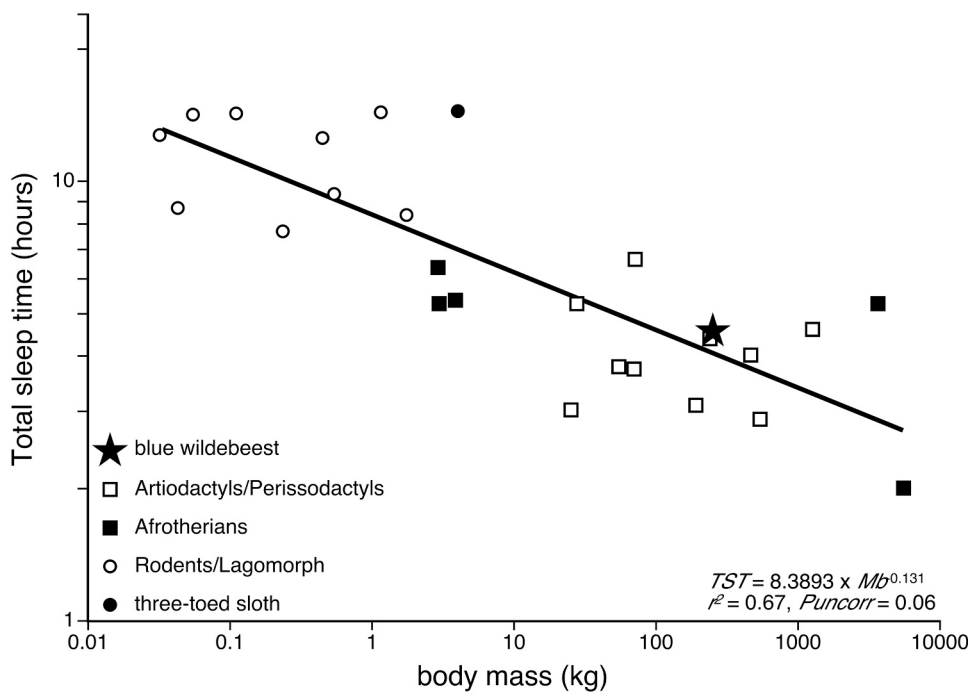
If we broaden our comparison to encompass all mammalian herbivores in which sleep has been recorded, as suggested by Siegel (2005) (the data provided includes other species of Artiodactyla, Perissodactyla, Rodentia, domestic rabbit, three-toed sloth and Afrotheria), we find that the total sleep time for the blue wildebeest is very similar to what would be predicted based on their body mass in comparison to

other herbivores (Fig. 8). It should be noted here that the regression calculated in Fig. 8, approaches, but is not, statistically significance ( $P_{\text{incorr}} = 0.06$ ) and was not undertaken with methods that account for phylogenetic relationships. However, if one were to examine TST in blue wildebeest from a the concept that total sleep time is correlated to body mass, irrespective of phylogenetic affinities, then the findings of the current study do not contradict this concept.

Concerning the percentage of TST occupied by REM sleep, the blue wildebeest spent 6.18% of TST in REM sleep, which is comparable with the 4.7% observed behaviourally in the giraffe (Tobler and Schwierin, 1996) and 4.18% observed physiologically during winter sleep in the Arabian oryx (Davimes et al. (2018), but see Davimes et al. (2018) concerning suppression of REM sleep in the summer in Arabian oryx). In contrast, the domestic Artiodactyls (cow – 18.91%, sheep – 14.72%, pig – 29.13%, goat – 13.15%) have substantially higher percentages of TST occupied by REM sleep. Thus, there may potentially be a domestic vs wild dichotomy in the artiodactyls, where while total sleep times appear to be consistent across both domestic and wild artiodactyl species, the proportion of sleep occupied by REM is substantially higher in the domestic species compared to the wild species. Indeed, the three wild artiodactyl species (blue wildebeest, giraffe and Arabian oryx) appear to have approximately 15 min of REM sleep per day, while the domesticated cow, sheep and goat have approximately 45 min of REM sleep per day, with the domestic pig having about 2 h 15 min of REM sleep per day.

The average daily amount of REM sleep for a given mammalian species appears to be strongly related to maturity at birth, with precocial mammals having less REM as adults, while altricial mammals have more REM sleep as adults (Siegel, 2005); however, here we have noted that domestication may be a factor in increasing the amount of daily time spent in REM sleep, at least in the Artiodactyls. This may be a result of the artificial selection processes leading to the domestic varieties of mammals, where neotenus, or juvenile, features are artificially selected for as they increase the tameness and manageability of the animals for human purposes – “the domesticated phenotype” (e.g. Diamond (2002) and Agnvall et al. (2018)). Perhaps the increased amount of REM sleep, both in actual terms and as a percentile of total sleep time, in domestic artiodactyls is part of the domesticated phenotype. An analogy may be drawn between humans and chimpanzees, where humans sleep on





**Fig. 8.** Graph plotting the relationship between body mass ( $Mb$ , in kilograms,  $kg$ ) and total sleep time ( $TST$ , in hours,  $h$ ) for herbivores, including the blue wildebeest. While the regression indicates a lower  $TST$  with increased body mass, this regression only approaches significance for the herbivores included for analysis. Note that in relative terms the total sleep time for the blue wildebeest recorded in the current study appears to correlate with the data obtained from other herbivores. The data used in this plot is derived mainly from Siegel (2005), with data for the African elephant from Gravett et al. (2017) and the Arabian oryx from Davimes et al. (2018).

average for 8 h per day, with 23.75% of total sleep time being REM (Meddis, 1983), while chimpanzees sleep for an average of 10.8 h per day, with 15% of this sleep being REM (McNew et al., 1971; Balzamo et al., 1972). Thus, even though the chimpanzee sleeps for 2.8 h more per day than the human, the chimpanzee experiences only around 97 min per day of REM sleep, while the human experiences 114 min of REM sleep. Do humans living in the industrialized world show aspects of “the domesticated phenotype” regarding REM sleep? Further studies on REM sleep in humans leading a non-industrialized lifestyle (e.g. Yetish et al. (2015)) may provide an answer to this intriguing possibility.

#### 4.2. Comparison of actigraphy and polysomnography as measures of total sleep time and patterns in the blue wildebeest

Polysomnography (PSG) is considered the gold-standard of sleep measurement as it provides the ability to distinguish the various global neuronal states of a mammal, namely Wake, non-REM and REM sleep, but PSG is usually performed in a controlled laboratory setting. As mentioned, there are discrepancies regarding the total sleep time measured in the laboratory compared to a more natural setting (Rattenborg et al., 2008). Obtaining high-quality EEG recordings from free-roaming animals under naturalistic settings can be challenging. PSG was recorded in dairy cows using non-invasive surface-attached electrodes to measure different vigilance states (Ternman et al., 2012). Unfortunately, this method is not suitable for most animals, especially those in naturalistic settings. One difficulty with this method is that the animals may simply remove the electrodes, as was seen in the case of the dairy cows (Ternman et al., 2012), and this is likely to be more exaggerated in non-domestic species. Another drawback of the surface-attached electrodes is that they are susceptible to artifacts from muscle activity and movements being recorded (Rattenborg et al., 2017). Stable EEG recordings can be obtained from electrodes placed on the dura mater overlying the brain, as done in the current study; however, this then becomes an invasive procedure. Although the EEG provides a direct measure of sleep-related brain activity, by itself it provides an incomplete view of sleep (Rattenborg et al., 2017). It gives us insight to the vigilance states of the animal, but not necessarily how the animal structures its sleep under different ecological factors especially for extended periods of time.

Accelerometers have been used to study a wide range of behavioural and physiological aspects of animals in their natural habitat, including foraging, reproduction, activity, energy budgets, and locomotion (Brown et al., 2013; Wang et al., 2015). In a study on free-ranging pumas (*Puma concolor*), accelerometer measurements were validated successfully against behavioural observations (Wang et al., 2015), being able to accurately predict periods of no movement, low acceleration (i.e. stalking, walking) and high acceleration movements (i.e., trotting and running) in unobserved wild animals. Actigraphy (ACT) has also been shown to be a minimally-invasive method to objectively measure sleep/inactivity during prolonged naturalistic investigations in mammals (Davimes et al., 2016; Gravett et al., 2017). The accuracy of sleep measurement using accelerometers in free-roaming large mammals still needs to be validated against polysomnographical measures of sleep. In humans, there are a number of studies that have evaluated the ability of actigraphy to discriminate between sleep and wake as defined by PSG criteria (de Souza et al., 2003; Paquet et al., 2007; Sadeh, 2011; Shambroom et al., 2012). To the author’s knowledge the current study is the first attempt at a detailed comparison of the accuracy of actigraphic vs polysomnographic measures of sleep in a large non-human non-domestic mammal recorded from in a naturalistic setting.

While validation studies of ACT with PSG in humans, have shown that the concordance in sleep scoring between the two methods ranges from 83 to more than 90%, in the current study of the blue wildebeest, it was found that the concordance between ACT and PSG revealed only a fair agreement of approximately 45% (when looking at corresponding epochs scoring sleep and inactivity) and differences were noted in the different animals and on different days (Tables 1 and 2). In addition, on average the ACT method led to an approximately 15 min/day overscore of total sleep time, which would likely be considered quiet wake from PSG recordings. One crucial difference between the current study and those performed in humans, is the manner in which inactivity, or sleep, is assigned on the basis of the ACT recordings. In the current study each animal was implanted with two actiwatches, one in the neck and one in the leg. Each 1 min epoch that showed no activity in both actiwatches in one animal was scored as “Inactive” (any activity was scored as “Active”), and from the one minute epoch data the modal state for five minute epochs was calculated and assigned as either “Inactive” or “Active”. This was then compared to the PSG five minute modal data to

determine the extent of agreement. In contrast, studies in humans have developed complex algorithms to improve the concordance between PSG and ACT in human studies (de Souza et al., 2003; Paquet et al., 2007; Sadeh, 2011). Despite these methodological differences, it appears that, as a first attempt to determine whether ACT can be used as a successful replacement for PSG for the study of sleep of large mammals in their natural habitat, ACT does appear to be a useful method in this endeavour for aspects such as total sleep time and sleep phasing; however, at this stage further studies across more individuals of the current species and a broader range of species is required in order to determine to what extent ACT recordings can be regarded as true sleep as opposed to the less proscriptive terms used here such as “Inactive” and “Active” due to the only 45% agreement in the two scoring techniques when analysing corresponding epochs. It appears likely that with further studies and increased stringency on the analysis of the ACT recordings, that ACT could be used successfully to determine what happens to total sleep times and sleep architecture in large, free-roaming mammals for extended periods of time (although these recordings could not determine what happens to specific sleep states, such as non-REM and REM). It should also be noted that the development of potential algorithms to increase the accuracy of ACT when recorded in parallel with PSG, are likely to be species or phylogenetic group specific. Such validation of ACT methodology for the accurate assessment of sleep would expand the opportunities for studying sleep in various large mammalian species in their natural habitat, providing accurate quantifiable information that can be related to a variety of environmental conditions, including, for example, changes in weather patterns (seasonally, daily, or with specific weather phenomenon), levels of predation risk, mating and birthing schedules, and long-distance migrations. Thus, properly validated ACT has the potential to provide a method to broaden our understanding of mammalian sleep and how this relates to the natural habitat and life histories of mammals, but further refinement of the technique is required.

### Conflicts of Interest

The authors have no conflicts of interest to declare.

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### Statement of ethics

All animals were treated and used according to the guidelines of the University of the Witwatersrand Animal Ethics Committee (AESC number: 2014/53D), which parallel those of the National Institutes of Health (NIH) for the care and use of animals in scientific experimentation. Permits from the Gauteng Provincial Government were obtained for the capture and transport of the animals from the wild.

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### Author Contributions

This study was conceived by IBM, NG and PRM. The recording and analysis of the data was undertaken by all authors, with the first draft of the manuscript written by IBM, which was subsequently edited by all authors.

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