

Sleep and wake in rhythmic vs arrhythmic chronotypes of a microphthalmic species of African mole rat (*Cryptomys mechowii*)

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Abstract

The giant Zambian mole rat (*Cryptomys mechowii*) is a subterranean African rodent noted for its regressed visual system and unusual patterns of circadian rhythmicity – within this species some individuals exhibit distinct regular circadian patterns while others have arrhythmic circadian patterns. The current study was aimed at understanding whether differences in circadian chronotypes in this species affects the patterns and proportions of the different phases of the sleep-wake cycle. Physiological parameters of sleep (EEG and EMG) and behaviour (video recording) were recorded continuously for 72 h from six mole rats (three rhythmic and three arrhythmic) using a telemetric system and a low light CCTV camera connected to a DVD recorder. The telemetric data was scored (in both 5 s and 1 min

epochs) as wake, nonREM sleep (NREM) and rapid eye movement (REM) stages subject to the correlation between EEG, EMG and behaviour. Spectral power was calculated for EEG in each implanted individual, which assisted in understanding the sleep phases and the intensity of NREM between the chronotypes. In addition REM periodicity was calculated from which sleep cycle length was inferred. The results indicate that the arrhythmic individuals spend more time in waking with a longer average duration of a waking episode, less time in NREM with a shorter average duration of a NREM episode though a greater nonREM sleep intensity, and similar sleep cycle lengths. The time spent in REM and the average duration of a REM episode was similar between the chronotypes.

1. Introduction

The reversible, homeostatically controlled state of reduced responsiveness, reduced motor ability and reduced metabolism, commonly defines the physiological phenomenon of sleep (Siegel, 2008). In mammals, this state is often separated into rapid eye movement (REM) and non-REM stages that are differentiated by changes in the electroencephalogram (EEG) and electromyogram (EMG) amongst other features (Siegel, 2009). Within mammals, sleep has been studied by visual observation or physiological recording in 17 orders, 47 families and 127 species (McNamara et al., 2008), but the exact function of sleep remains unclear.

Most mammals studied meet the standard criteria for sleep in that they have clearly distinguishable NREM and REM states (the marine mammals being unusual in this sense, Lyamin et al., 2008). Tobler (1995) proposed that sleep is not fundamentally different in mammalian species, but Siegel (2004) suggests that adaptive mechanisms rather than phylogenetic relationships provide a better explanation of the variation in sleep time and other parameters across species. In contrast to this, Capellini et al. (2008) indicate that they have detected a 'phylogenetic signal' in mammalian sleep, perhaps indicating some predictability of this state across species. Detailed sleep studies in rodents have examined the rat (Alfoldi et al., 1990), mouse (Richardson et al., 1985; Ayala-Guerrero et al., 1998; Tang and Sanford, 2002), Djungarian hamster (Deboer et al., 1994), Syrian hamster (Tobler and Jaggi, 1987), chipmunk (Dijk and Daan, 1989; Estep et al., 1978), gerbil (Kastaniotis and Kaplan, 1976; Susic and Masirevic, 1986; Ambrosini et al., 1994), guinea pig (Tobler et al., 1993; Tobler and Franken, 1994), squirrel (Folk, 1963; Chepkasov, 1980), *Octodon degu* (Kas and Edgar, 1998) and the blind mole rat (Tobler and Deboer, 2001).

In the current study sleep was physiologically and behaviourally recorded in the giant Zambian mole rat (*Cryptomys mechowii*) which is well known for its subterranean lifestyle, regressed visual system (Cooper et al., 1993; Hart et al., 2004; Nemeč et al., 2004; McMullen et al., 2010) and unusual patterns of circadian rhythmicity (Lovegrove and Papenfus, 1995; Lovegrove and Muir, 1996; Oosthuizen et al., 2003). It has been shown that the two main retinorecipient structures of the circadian system, the suprachiasmatic nucleus and the intergeniculate nucleus/leaflet, have different neuropeptide populations and afferents and are reduced in size in mole rats compared to other rodents (Negroni et al., 2003; Bhagwandin et al., 2010). Other approaches directed at understanding the unusual circadian rhythmicity in mole rats have shown that these subterranean rodents possess an endogenous melatonin rhythm that has a circadian pattern (Gutjah et al., 2004) and that body temperature is not a reliable indicator of endogenous circadian activity in mole rats (Lovegrove and Muir, 1996). Furthermore, locomotor activity studies have shown that within a species of mole rat there are individuals that have a rhythmic chronotype and others that have a distinctly arrhythmic chronotype, and this is seen in both social and solitary species of mole rat (Oosthuizen et al., 2003).

The giant Zambian mole rat leads a solitary lifestyle, inhabiting diverse soil types in the tropical woodland and savannah regions of northern Zambia, southern Democratic Republic of Congo and central Angola (De Graaf, 1971). This species is typically pale brown in colour with a maximum body mass of 600g for males and 350g for females (Burda and Kawalika, 1993). The current study was aimed at determining: (1) whether measurable sleep parameters vary predictably with individuals that possess rhythmic chronotypes as opposed to individuals that are distinguishably arrhythmic; and (2) if dramatic changes in phenotype, such as the regressed visual system, affect sleep parameters in comparison to other rodents.

2. Materials and Methods

In the present study, physiological measures of EEG and EMG were telemetrically recorded from six individual wild caught adult male giant Zambian mole rats (*Cryptomys mechowii*) (Table 1). At the Mammal Research Institute, University of Pretoria these animals were identified as either rhythmic or arrhythmic (Fig. 1). Prior to the commencement of sleep recording, the animals were allowed a month of acclimatisation in an isolated (to minimise noise and other disturbances) and environmentally controlled room (12h light/dark cycle with

Animal ID	M3	M8	M9	Weighted mean rhythmic	M1	M5	M6	Weighted mean arrhythmic
Circadian chronotype	Rhythmic	Rhythmic	Rhythmic		Arrhythmic	Arrhythmic	Arrhythmic	
Body mass (g)	389	254	350	331	208	220	310	246
Brain Mass (g)	2.2	2.2	2.4	2.3	2.2	2.2	2.2	2.2
Sex	Male	Male	Male		Male	Male	Male	
TWT (% 24 h)	68	68	72	69	78	74	77	76
TST (% 24 h)	32	30	26	29	20	23	23	22
TNREM (% 24 h)	27	25	22	25	16	18	18	17
TREM (% 24 h)	5	5	4	5	4	5	5	5
TWT-Light period (% 12 h)	68	77	74	73	82	74	79	78
TST-Light period (% 12 h)	31	23	23	26	15	22	21	19
TNREM-Light period (% 12 h)	27	20	19	22	11	17	17	15
TREM-Light period (% 12 h)	5	4	3	4	4	5	4	4
TWT- Dark period (% 12 h)	67	60	71	66	74	75	75	75
TST- Dark period (% 12 h)	33	37	28	33	26	25	25	25
TNREM- Dark period (% 12 h)	28	30	24	27	21	19	19	20
TREM- Dark period(% 12 h)	6	7	5	6	4	5	6	5

Table 1: Table presenting 5 s epoch scored data for rhythmic and arrhythmic mole rats for parameters measured in the current study. TWT – Total waking time; TST – Total sleep time; TNREM – Total slow wave sleep/non REM sleep; TREM – Total REM.

a constant ambient temperature of 25°C). A single mole rat was housed in an enclosure (60 x

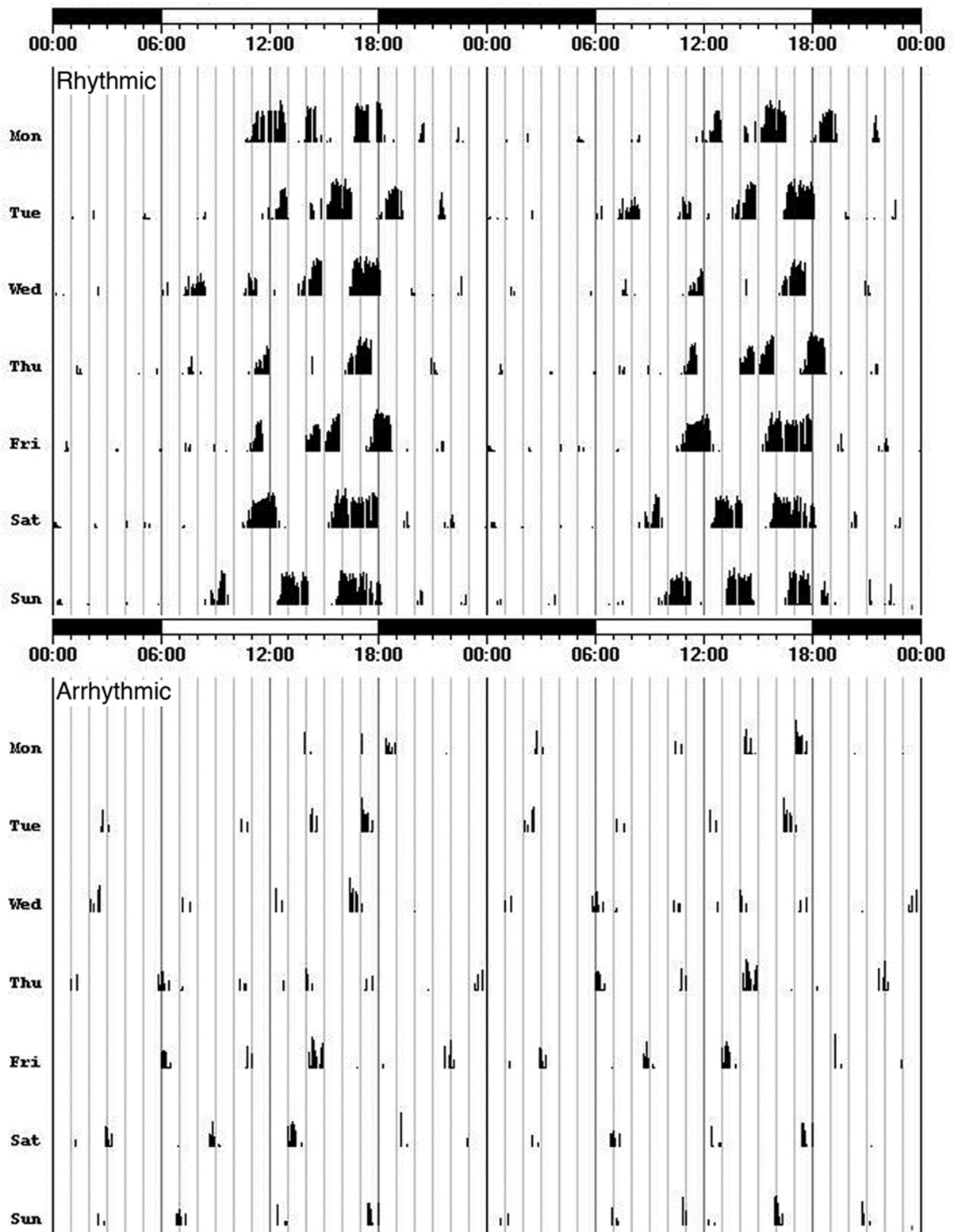


Figure 1: Actigrams illustrating circadian patterns of locomotor activity for one week in a rhythmic and arrhythmic giant Zambian mole rat (*Cryptomys mechowii*). These actigrams indicate that the rhythmic mole rats have a predictable period of activity during the light period whereas locomotor activity in the arrhythmic animals is irregular.

50 cm) in which a transparent perspex tube (20 cm long with a diameter of 6.5 cm) was secured to the floor of the enclosure. Wood shavings were used to line the floor, but not enough was placed to hinder visual observation of the animals. Each animal was fed a mixture of sweet potatoes, apples and carrots once a day. All animals were treated and used according to the guidelines of the University of the Witwatersrand Animal Ethics Committee, which parallel those of the NIH for the care and use of animals in scientific experimentation.

2.1 Determination of rhythmicity patterns

A system using combinations of emitters, running wheels and magnetic switches and/or, infrared detectors, was used to detect activity patterns in the mole rats. The mole rats were exposed to differing light conditions that included, light (L) and dark (D) cycles, DD cycles and LL cycles. Initially the experimental protocol started with a 12L:12D cycle and once entrainment was achieved the light cycle was switched to constant darkness for 1 month. Once this was completed the light cycle was reversed to 12L:12D. For details regarding the specifics of circadian rhythm determination see Oosthuizen et al. (2003).

2.2 Surgery

Once the animals were acclimatised, they were administered a weight dependant mixture of ketamine and xylazine (2:1, 0.01ml/100g, Bayer HealthCare). Throughout surgery, anaesthesia was maintained using Isoflurane (at a concentration of 1% - 2.5% in an oxygen/70% nitrous oxide mixture, Safe Line Pharmaceuticals) fed from a mask placed directly over the nostrils that was supplied by a respirator. After shaving the scalp and left abdomen, a chemical disinfectant (CHX Chlorhexidine, 0.5% chlorhexidine in 75% alcohol, Kyron Laboratories) was applied to the skin using sterile swabs. Two incisions were made for implantation of the transmitter and electrodes, one over the midline of the skull and one over the left abdomen. At the abdominal incision a subcutaneous pocket was created to house the transmitter. The lead wires of the transmitter (two for EEG and one for EMG) were then fed subcutaneously from the abdominal incision to the cranial incision. Once the skin had been reflected at the cranial incision, the temporalis muscle over the left hemisphere was retracted to reveal the dorsal cranial surface. Cotton swabs immersed in H₂O₂ were used to clean the surface of the cranial vault and a small dental drill was used to bore two holes over the motor cortex of the left hemisphere without penetrating the dura mater. Thirty six hours prior to surgical implantation, the transmitter and electrode wires were sterilised for 18 h using Cidex (a non-irritant chemical sterilant, Johnson & Johnson) and then placed in sterile normal saline

for a further 18 h. Using a surgical microscope, the EEG electrodes (the leads of which were made out of stainless steel, have an outer diameter of 0.3 mm and a lead diameter of 0.2 mm) were placed into each hole to rest above the dura mater and secured in place using dental acrylic (Profident). The EMG electrodes were then sutured into the nuchal musculature. Once the electrodes were secure, the transmitter was carefully inserted into the subcutaneous pocket and both incisions sutured. Anaesthesia was reversed by switching off the flow of Isoflurane and the analgesic Temgesic (0.3mg/100g i.m., Reckitt Benckisser Healthcare) was administered. The animals were then returned to the enclosure and monitored for seven days prior to recording.

2.3 Recording

EEG and EMG were recorded using a Data Sciences International (DSI) telemetric system that included a transmitter (PhysioTel Telemeric Systems, TL11 M2 F40-EET-Implant, the leads of which were made out of stainless steel, have an outer diameter of 0.3 mm and a lead diameter of 0.2 mm; weight: 7 g; volume: 4.5 cc; height: 13.8 mm) and a receiver (placed directly under the enclosure) connected to a DEM matrix that was connected to a computer. EEG and EMG were recorded continuously for 72 h in each animal and stored as a DSI file. Once recording was completed, the DSI files were converted to text files (containing information for the frequency bands 1.2 - 4 Hz; as well as information relating to muscle movements) that were subsequently converted into Spike files using the Spike 2 software (version 4.2, Cambridge Electronic Design). The Spike files generated presented the EEG and EMG data in a fashion that facilitated visual scoring of defined physiological states. Spectral power for the EEG was also calculated for each individual.

In addition to EEG and EMG, behaviour was recorded using the output of a low light CCTV camera connected to the hard disk drive of a commercially available DVD recorder. At the cessation of recording, this information was copied to DVD-R discs and analysed in conjunction with the physiological data. Red dark room lights were used to illuminate the enclosure throughout the 72 h period.

2.4 Analysis

EEG and EMG allowed the scoring of 3 states: waking (W) which was characterised by low amplitude, high frequency EEG activity and irregular high amplitude EMG activity; NREM which was defined by high amplitude, low frequency EEG activity and stable low amplitude EMG activity; and REM/paradoxical (R) which was defined by low amplitude,

high frequency EEG activity (similar to that of waking) and low amplitude EMG activity that displayed large, high amplitude infrequent spikes that were correlated with observed behavioural muscle twitches and jerks. EEG and EMG were scored in 5 s epochs and the results entered into a Microsoft Excel generated template. Subsequently, this data was rescored at 1 min intervals by employing a mode formula (using Microsoft Excel) on the 5 s scored data, whereby the modal state for each minute was recorded.

Behavioural data was scored at 1 min intervals (where a state was recorded only if it persisted for 50% or more of the scoring interval) as eating (1), active wake (2), grooming (3), repositioning (4), immobile (5) and sleep posture (6), the latter of which was assigned when the animal assumed a curled 'ball-like' position where the head was noticeably tucked underneath the body. The time spent in wake and sleep states (represented as a % of 24 h) and distribution of wake and sleep states between light and dark periods (% of 12 h) for both physiological and behavioural data were calculated in each individual.

The average number of episodes of wake and sleep states and the average duration of an episode of wake and sleep states were calculated for a 24 h cycle and for light and dark periods. In four individuals the average spectral power of slow wave activity (SWA, 1.2 – 4.0 Hz) during NREM for consecutive 2 h periods was plotted for the 72 h recording. Total spectral power of SWA during NREM was calculated in rhythmic and arrhythmic groups and compared between light and dark periods. The length of a sleep cycle was calculated as the difference in time between the onsets of consecutive REM episodes. This calculation eliminated episodes of waking with an uninterrupted duration that was greater than 10 min.

Data collected for the circadian chronotypes of the current study was analysed for statistically significant differences in the time spent in wake and sleep states, the average number of episodes of wake and sleep states and average duration of an episode of wake and sleep states, within and between chronotypes and within and between days and light and dark periods. Statistical tests used in the current study employed the PAleontological STatistics (PAST, version 2.02) software program (Hammer et al., 2001). A normality test was employed on each dataset and if the dataset was normally distributed ($p > 0.05$), a one-way ANOVA was employed; but if the dataset was not normal in its distribution ($p < 0.05$), a Mann-Whitney test was performed. Statistical significance was noted when the $p < 0.05$ for both ANOVA and Mann-Whitney tests. To determine the root of the statistical significance, univariate analyses were carried out for the datasets in question.

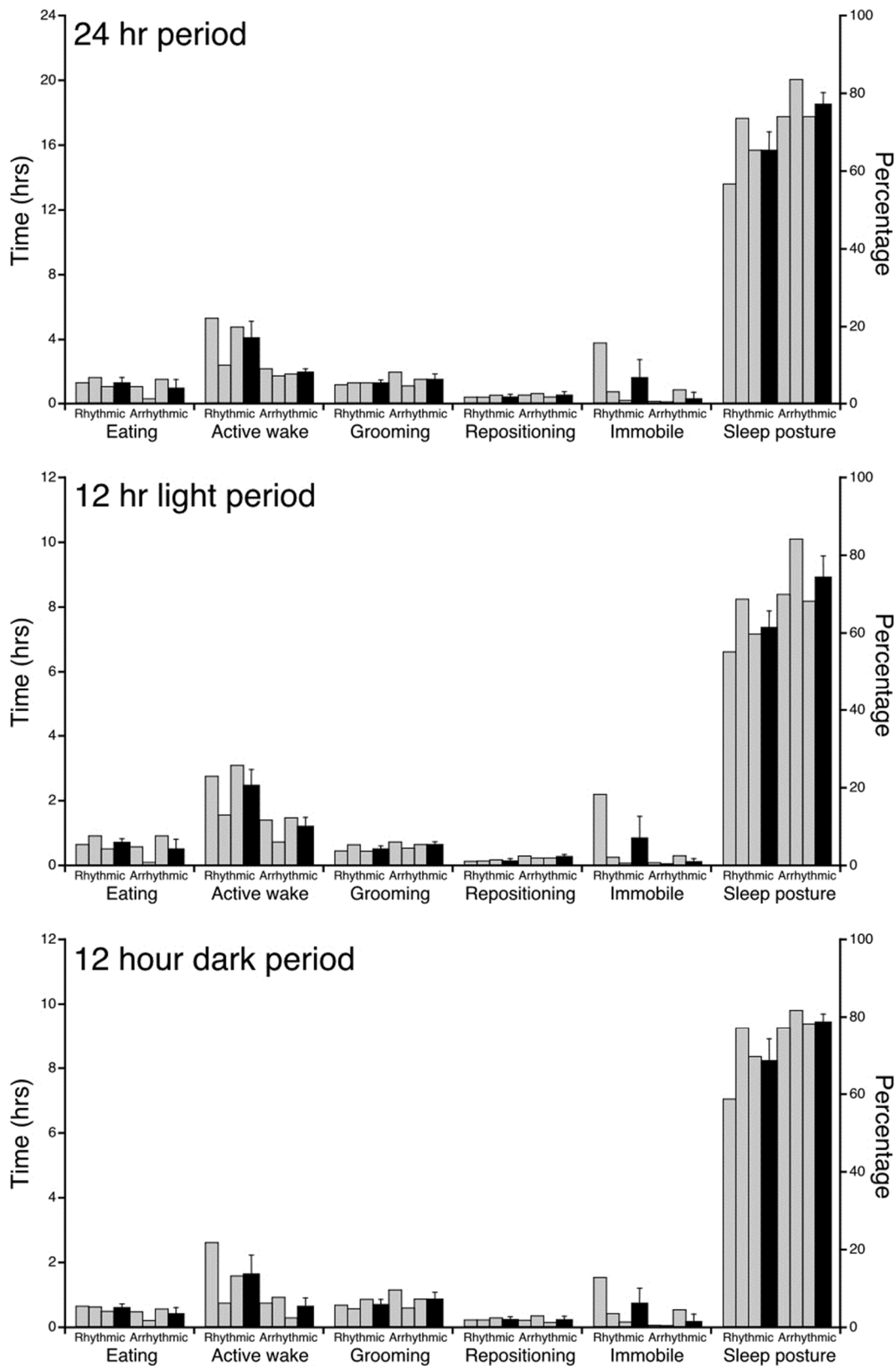


Figure 2: Graphs showing data for the various behavioural states in rhythmic and arrhythmic individuals (grey bars, same individuals from left to right in each graph and in all subsequent similar graphs) and the respective weighted means (black bars). The graphs represent the percentage and average amount of time spent in the various behavioural states for a 24h cycle (from 72 h of recording) and for the 12 h light and dark period periods (from 36 h of recording).

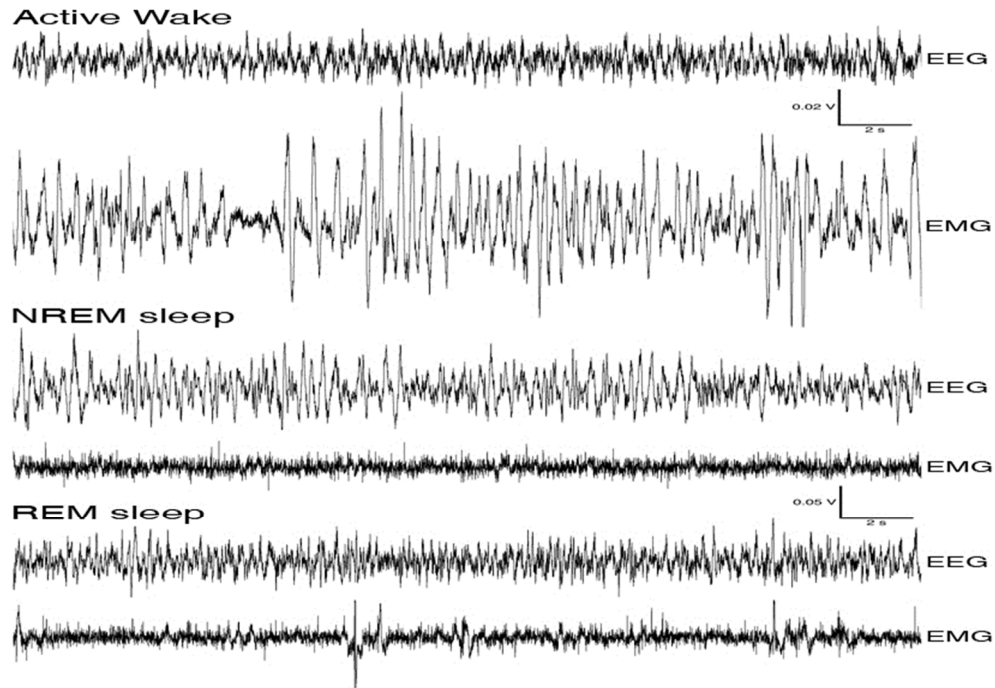


Figure 3: Polygraphic traces illustrating examples of the EEG and EMG recorded during sleep and wake states in the giant Zambian mole rat (*Cryptomys mehowi*). Active wake was characterised by low amplitude, high frequency EEG and high amplitude EMG. NREM was characterised by high amplitude, low frequency EEG and low amplitude EMG. REM sleep was characterised by low amplitude, high frequency EEG (similar to that seen in waking) and low amplitude EMG that contained large, high amplitude infrequent spikes (indicative of muscle twitches and jerks).

3. Results

In the present study, electroencephalographic waking, NREM and REM, through the recording of EEG and EMG, were telemetrically recorded continuously over a period of 72 hours, in conjunction with behavioural recordings, from six individual giant Zambian mole rats (*Cryptomys mehowi*). Three of the mole rats displayed a rhythmic chronotype, while three displayed an arrhythmic chronotype (Fig. 1). Behaviourally scored waking involved the animal moving around the enclosure, eating, grooming and re-posturing (Fig. 2). These behavioural states were identified physiologically by low amplitude, high frequency EEG activity and irregular high amplitude EMG activity. NREM was behaviourally identified when the animal presented itself in either a curled ‘ball-like’ position, where the head was

noticeably tucked under the body, or a state of immobility with the head resting on the floor of the enclosure. NREM was characterised by high amplitude, low frequency EEG activity and stable low amplitude EMG activity. REM sleep was behaviourally scored when body twitches or muscle jerks were observed while the animal was in a curled ‘ball-like’ posture or other immobile postures such as lying prone. This state was physiologically identified by low amplitude, high frequency EEG waves (similar to that seen in waking) and when the EMG had a very low muscle tone (lower than waking and NREM) and when the EMG displayed large, high amplitude infrequent spikes (that correlated with behavioural twitches and jerks upon *post hoc* analysis) (Figs. 3, 4). The results of the present study showed that the rhythmic chronotype spent more time in a state of NREM and had a longer average duration of an episode of NREM, while the arrhythmic chronotype spent more time in a state of waking and had a longer average episode of waking. The time spent in REM sleep, the average number of episodes of REM sleep and the average duration of an episode of REM was similar between the rhythmic and arrhythmic groups.

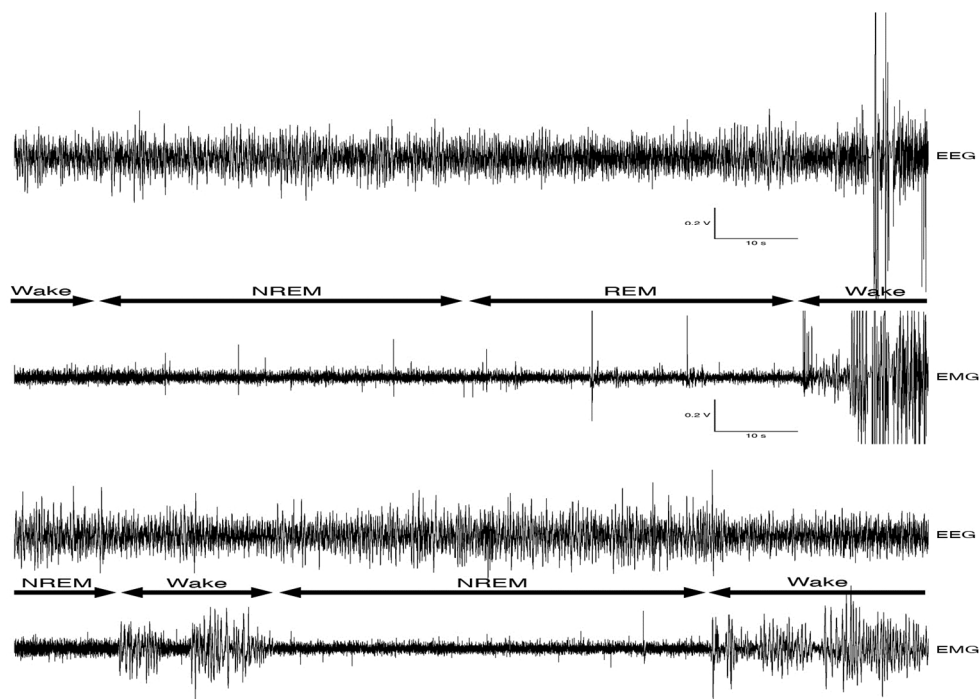


Figure 4: Polygraphic traces illustrating examples of the EEG and EMG during transitions from wake to NREM to REM to wake between wake and NREM.

3.1 Behavioural analysis

Behaviour was recorded continuously over 72 h in each animal (n=6) and in 1 min epochs as: eating, active wake, grooming, repositioning, immobility and sleep posture (curled 'ball-like' position). Behavioural analysis showed that, on average per 24 h, the rhythmic mole rats spent 1.3 h eating, 4.1 h in a state of active wake, 1.3 h grooming, 0.4 h repositioning, 1.6 h immobile and, 15.6 h in sleep postures. The arrhythmic animals spent 1 h eating, 1.9 h in a state of active wake, 1.6 h grooming, 0.5 h repositioning, 0.3 h immobile and, 18.5 h in sleep postures (Fig. 2) (also see attached video files 1 – 8 showing the behavioural states). When the distribution of the various behavioural states between light and dark periods were compared it was observed that both rhythmic and arrhythmic animals spent, on average, similar times during both light and dark periods in the various behavioural states. The rhythmic animals were in a state of active wake 1.5 times more during the light than the dark periods, whereas the arrhythmic individuals were actively awake almost twice the time in light compared to dark periods (Fig. 2). A comparison of the total times spent in various behavioural states between the rhythmic and arrhythmic groups yielded no significant statistical differences across the 24 h periods or between the 12 h light and dark periods.

3.2 Physiological Analysis

3.2.1 State Definitions

The state of wake was determined by low amplitude, high frequency EEG activity and irregular high amplitude EMG activity and this was behaviourally correlated with activities that included moving around the enclosure (often in a clockwise direction), attempted burrowing which was identified in one of two ways: (1) when the animal was observed gnawing the inside of the perspex tube using their incisors; and (2) when the animal would flick the wood shavings backwards using their hind legs while moving in a backward direction), eating, grooming and repositioning (see video files 1 - 4, 8). The state of NREM was identified by high amplitude, low frequency EEG activity and stable low amplitude EMG activity and this was behaviourally associated with either the curled 'ball-like' position defined when the head was tucked beneath the abdomen of the animal (behaviourally scored as sleep posture), or when the head was rested on the floor of the enclosure (behaviourally scored as immobile) (see video files 5, 6). REM sleep was identified by low amplitude, high frequency EEG waves (similar to that seen in waking) and when the EMG displayed large, high amplitude infrequent spikes and this was behaviourally identified as varying combinations of neck jerks, leg jerks and body jerks that correlated with the large, high

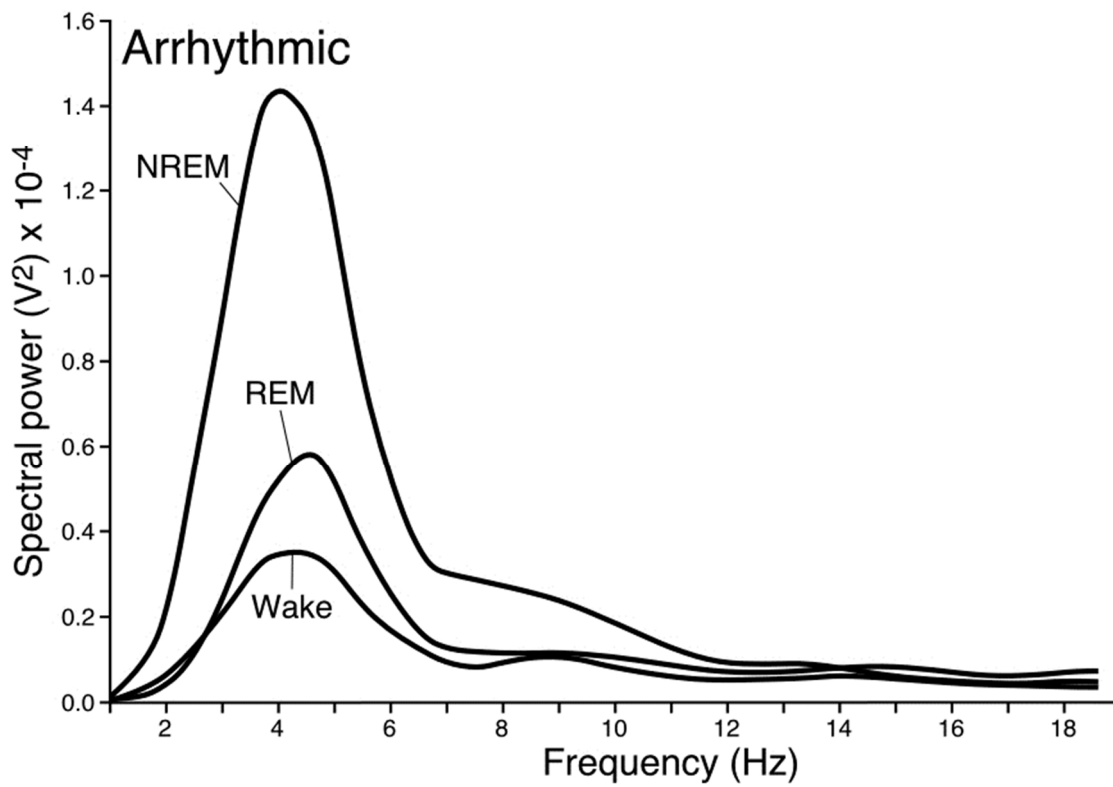
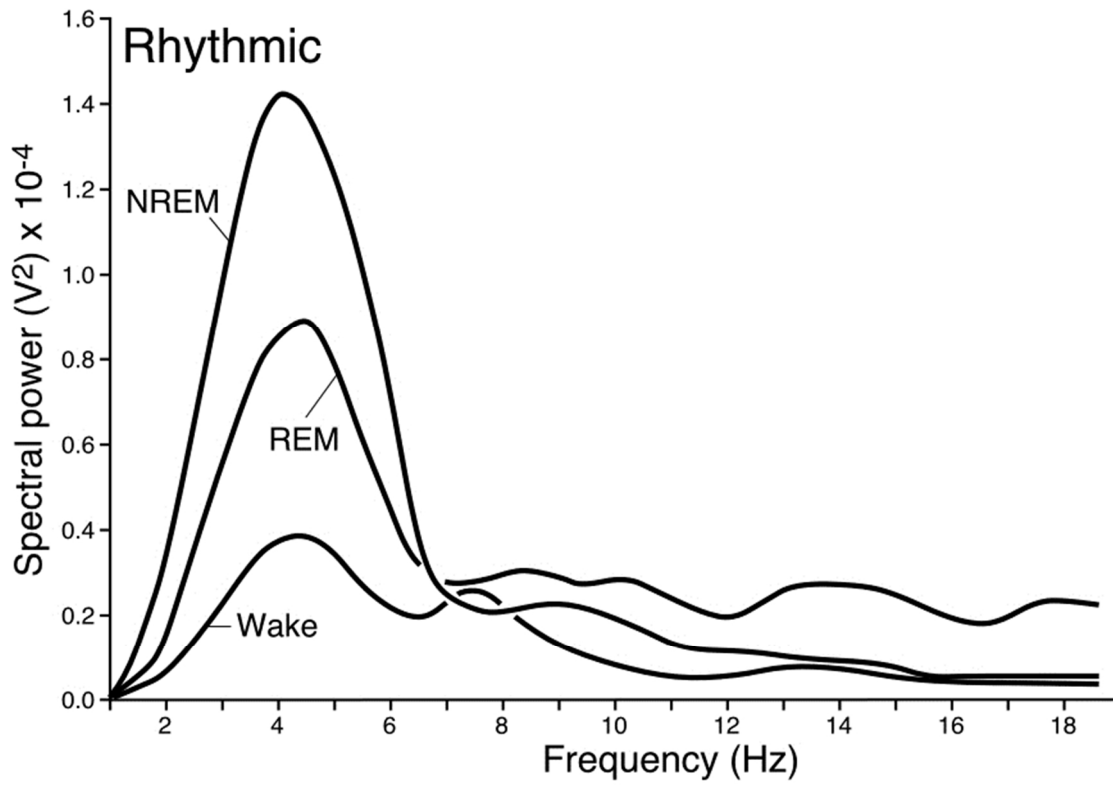


Figure 5: Graphs indicating the spectral power range during wake and sleep states for rhythmic and arrhythmic mole rats.

amplitude spikes observed in the EMG during sleep posture or immobility (Figs. 2, 3) (see video file 7). The peak amplitude for spectral power calculated within the rhythmic group during the 72 h recording was: $0.4 \times 10^{-4} \text{V}^2$ during waking, $1.4 \times 10^{-4} \text{V}^2$ during NREM and $0.9 \times 10^{-4} \text{V}^2$ during REM sleep. The peak amplitude for spectral power calculated within the arrhythmic group during the 72 h recording was: $0.35 \times 10^{-4} \text{V}^2$ during waking, $1.4 \times 10^{-4} \text{V}^2$ during NREM and $0.6 \times 10^{-4} \text{V}^2$ during REM sleep. It was observed that the frequency range for spectral power in the rhythmic group was 0 – 7 Hz for the physiologically defined wake and sleep states but the peak amplitude of spectral power occurred at: 4.5 Hz during waking, 4 Hz during NREM and 4.4 Hz during REM sleep. The arrhythmic group also displayed the 0 – 7 Hz range for spectral power, but it was noticed that the peak amplitude of spectral power occurred at: 4.5 Hz during waking, 3.9 Hz for NREM and 4.6 Hz during REM sleep in this group (Fig. 5).

3.2.2 Time spent in wake and sleep states

Upon analysis of the five second (5 s) epoch scored physiological data, it was observed that the rhythmic animals, on average for a 24 h cycle, spent 16.7 h (70%) in a state of wake (the distribution of which was higher in the light period than the dark period), 5.9 h (26%) in NREM (the distribution of which was higher in the dark period than the light period) and 1.2 h (5.0 %) in REM sleep (where the distribution was higher in the dark period than the light period). Total sleep time (TST) measured 29 % (~7.0 h) and the percentage of REM of TST was 16 % (~1.2 h) (Tables 1, 3). The arrhythmic individuals generally spent 18.3 h (77%) in a state of wake (the distribution of which was higher in the light period than the dark period), 4.2 h (19%) in NREM (the distribution of which was higher in the dark period than the light period) and 1.1 h (4.6 %) in REM sleep (where the distribution remained similar between light and dark times) (Figs. 6, 7, 8). Total sleep time (TST) measured 22 % (~5.3 h) and the percentage of REM of TST was 21 % (~1.1 h) (Tables 1, 3)

When the physiological data was scored at 1 min intervals, the results revealed that the rhythmic group, on average for a 24 h cycle, spent 17.1 h (71%) in a state of wake (the distribution of which was higher in the light period compared to the dark period), 5.1 h (21%) in NREM (the distribution of which was higher in the dark period compared to the light period) and 1.2 h (5.0 %) in REM sleep (where the distribution was slightly higher in the dark period compared to the light period). Total sleep time (TST) measured 26 % (~6.2 h) and the percentage of REM of TST was 19 % (~1.2 h) (Tables 2, 3). Similarly, the arrhythmic individuals spent 19.4 h (81%) in a state of wake (the distribution of which was higher in the

Animal ID	M3	M8	M9	Weighted mean rhythmic	M1	M5	M6	Weighted mean arrhythmic
Circadian chronotype	Rhythmic	Rhythmic	Rhythmic		Arrhythmic	Arrhythmic	Arrhythmic	
Body mass (g)	389	254	350	331	208	220	310	246
Brain Mass (g)	2.2	2.2	2.4	2.3	2.2	2.2	2.2	2.2
Sex	Male	Male	Male		Male	Male	Male	
TWT (% 24 h)	66	71	77	72	72	81	78	80
TST (% 24 h)	30	28	21	26	18	22	15	18
TNREM (% 24 h)	25	22	17	21	14	17	12	14
TREM (% 24 h)	5	6	4	5	4	5	3	4
TWT-Light period (% 12 h)	66	80	78	75	85	75	87	82
TST-Light period (% 12 h)	29	20	19	23	12	24	13	16
TNREM-Light period (% 12 h)	24	16	15	19	8	19	11	13
TREM-Light period (% 12 h)	4	4	4	4	4	5	3	4
TWT- Dark period (% 12 h)	67	63	76	68	77	80	82	80
TST- Dark period (% 12 h)	31	35	24	30	23	19	17	20
TNREM- Dark period (% 12 h)	25	28	19	24	19	14	13	15
TREM- Dark period(% 12 h)	6	8	5	6	4	5	4	5

Table 2: Table presenting 1 min epoch scored data for rhythmic and arrhythmic mole rats for parameters measured in the current study. TWT – Total waking time; TST – Total sleep time; TNREM – Total slow wave sleep/non REM sleep; TREM – Total REM.

	TST (h)	% REM of TST	Duration NREM episode (min)	Duration REM episode (min)	Source
<i>C. mechowii</i> Rhythmic 5s epoch scored data	7	16	0.5	2.5	Current study
<i>C. mechowii</i> Arrhythmic 5s epoch scored data	5.3	21	0.4	2.4	Current study
<i>C. mechowii</i> Rhythmic 1 min epoch scored data	6.2	19	2.5	3.2	Current study
<i>C. mechowii</i> Arrhythmic 1 min epoch scored data	4.3	23	2.0	3.3	Current study
<i>(R.norwegicus)</i> Long-Evans rat	13.2	18	5.2	1.7	1, 2, 6, 7
<i>(R.norwegicus)</i> Sprague Dawley rat	13.7				
<i>(M.musculus)</i> Laboratory mouse	12.8	13 - 20	4.7 – 7.1	0.7 – 0.9	3, 9
<i>(M.auratus)</i> Golden hamster	14.4	-	-	-	2
<i>(M. unguiculatus)</i> Mongolian gerbil	15.3	-	-	-	4
<i>(C. laniger)</i> Chinchilla	12.5	-	-	-	2
<i>(S. ehrenbergi)</i> Blind mole rat	12.4	15.1	9.7	2.5	5
Djungarian hamster	-	15	7	1.8	8

Table 3: Table presenting results from rhythmic and arrhythmic mole rats of the current study for: Total sleep time (TST), percentage REM sleep of TST, average duration of an episode of NREM, and average duration of episode of REM; compared to other rodents. Source references: (1) Mistlberger et al., 1983; (2) Van Twyver, 1969; (3) Valtax and Bugat, 1974; (4) Kastaniotis and Kaplan, 1976; (5) Tobler and Deboer, 2001; (6) Borbély and Neuhaus, 1979; (7) Franken et al., 1992; (8) Deboer et al., 1994; (9) Huber et al., 2000.

light period compared to the dark period), 3.4 h (14%) in NREM (the distribution of which was slightly higher in the dark period compared to the light period) and 1 h (4.2 %) in REM sleep (where the distribution was slightly higher in the dark period than the light period) (Figs. 6, 7, 8). Total sleep time (TST) measured 18 % (~4.3 h) and the percentage of REM of TST was 23 % (~1 h) (Tables 2, 3).

The time spent in waking, NREM and REM were then statistically compared (using both scoring techniques) between the groups for the 72 h recording and the light and dark periods. With regard to the 5 s epoch scored data, statistical significance was noted between the groups in: (1) the time spent in waking ($p < 0.05$, where the arrhythmic group showed a higher mean amount of time spent awake); (2) the time spent in waking during the light period ($p < 0.05$, where the arrhythmic group showed a higher mean amount of time spent awake); (3) the time spent in waking during the dark ($p < 0.05$, where the arrhythmic group showed a higher mean amount of time spent awake); (4) the time spent in NREM ($p < 0.05$, where the rhythmic group showed a higher mean amount of time spent in NREM); (5) the time spent in NREM during the light period between days ($p < 0.05$, where the rhythmic group showed a higher mean amount of time spent in NREM); and (6) the time spent in NREM during the dark period between days ($p < 0.05$, where the rhythmic group showed a higher mean amount of time spent in NREM). No statistically significant differences were noted for REM sleep times in the 5 s epoch data. When the 1 min epoch data was compared no statistically significant differences between the groups were observed.

3.2.3 Number of episodes of wake and sleep states

The number of episodes of waking, NREM and REM sleep were calculated for both 5 s epoch data and 1 min epoch data, in both rhythmic and arrhythmic groups. The results of the 5 s epoch data indicates that within the rhythmic group, the average number of episodes in 24 h was: 940 for waking (the number of which was higher in the dark period than in the light period), 779 for NREM (the number of which was higher during the dark period compared to the light period) and 28 for REM sleep (where the number was higher in the dark period than in the light period). The average number of episodes for the arrhythmic group for 24 h was: 966 for the waking state (the number of which was higher during the dark period compared to the light period), 669 for NREM (the number of which was higher in the dark period than the light period) and 28 for REM sleep (where the number was higher in the dark period compared to the light period) (Figs. 6, 7, 8). Analysis of the 1 min epoch data showed that the average number of episodes during a 24 h cycle within the rhythmic group

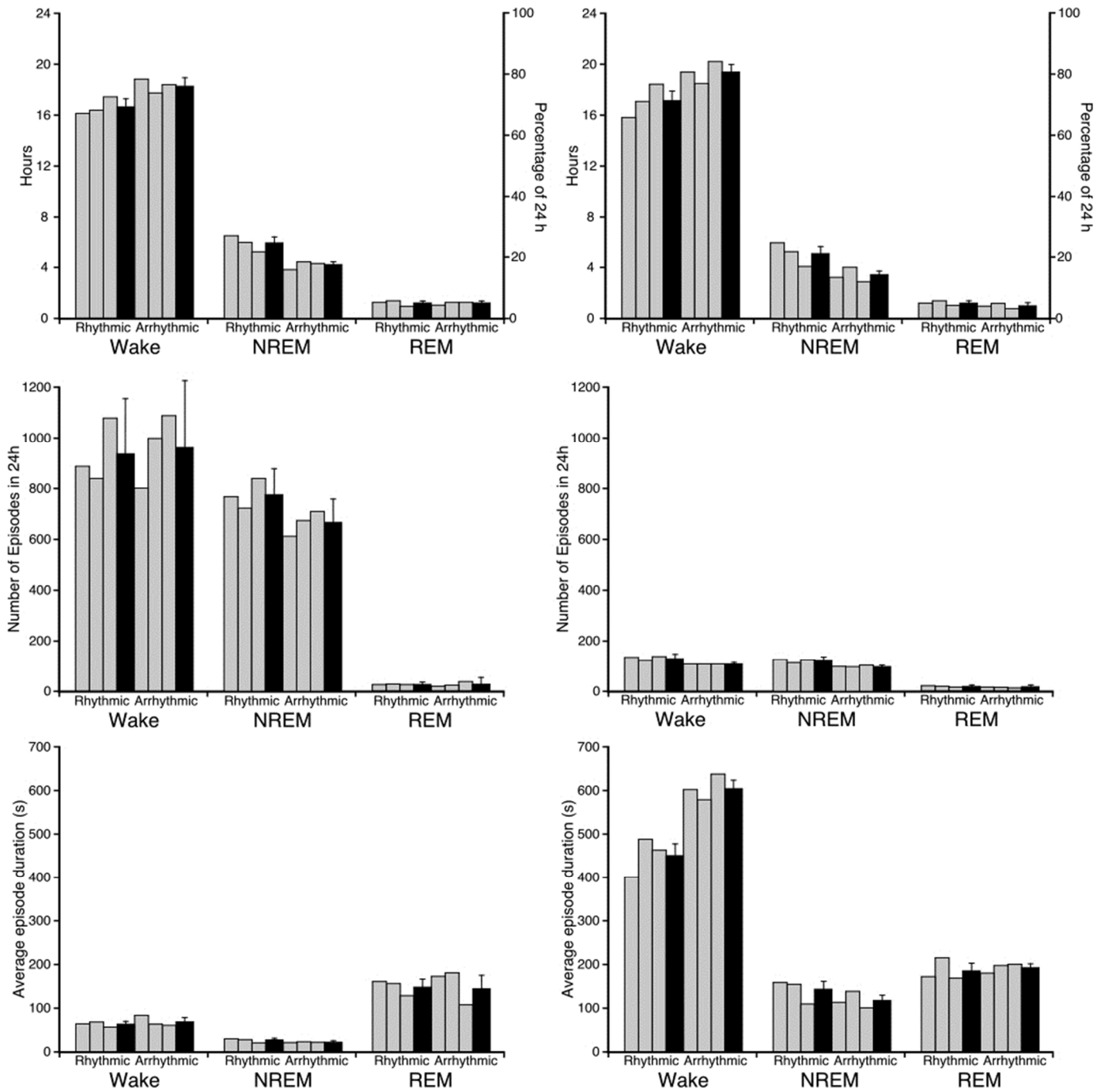


Figure 6: Graphs illustrating physiological data (5 s epoch scoring, left column of graphs, and 1 min epoch scoring, right column of graphs) for sleep and wake states during a 24 h cycle in rhythmic and arrhythmic individuals (grey bars) and the respective weighted means (black bars). The graphs indicate percentage and the average amount of time spent in wake and sleep states, the average number of episodes for wake and sleep states and the average duration of an episode in wake and sleep states.

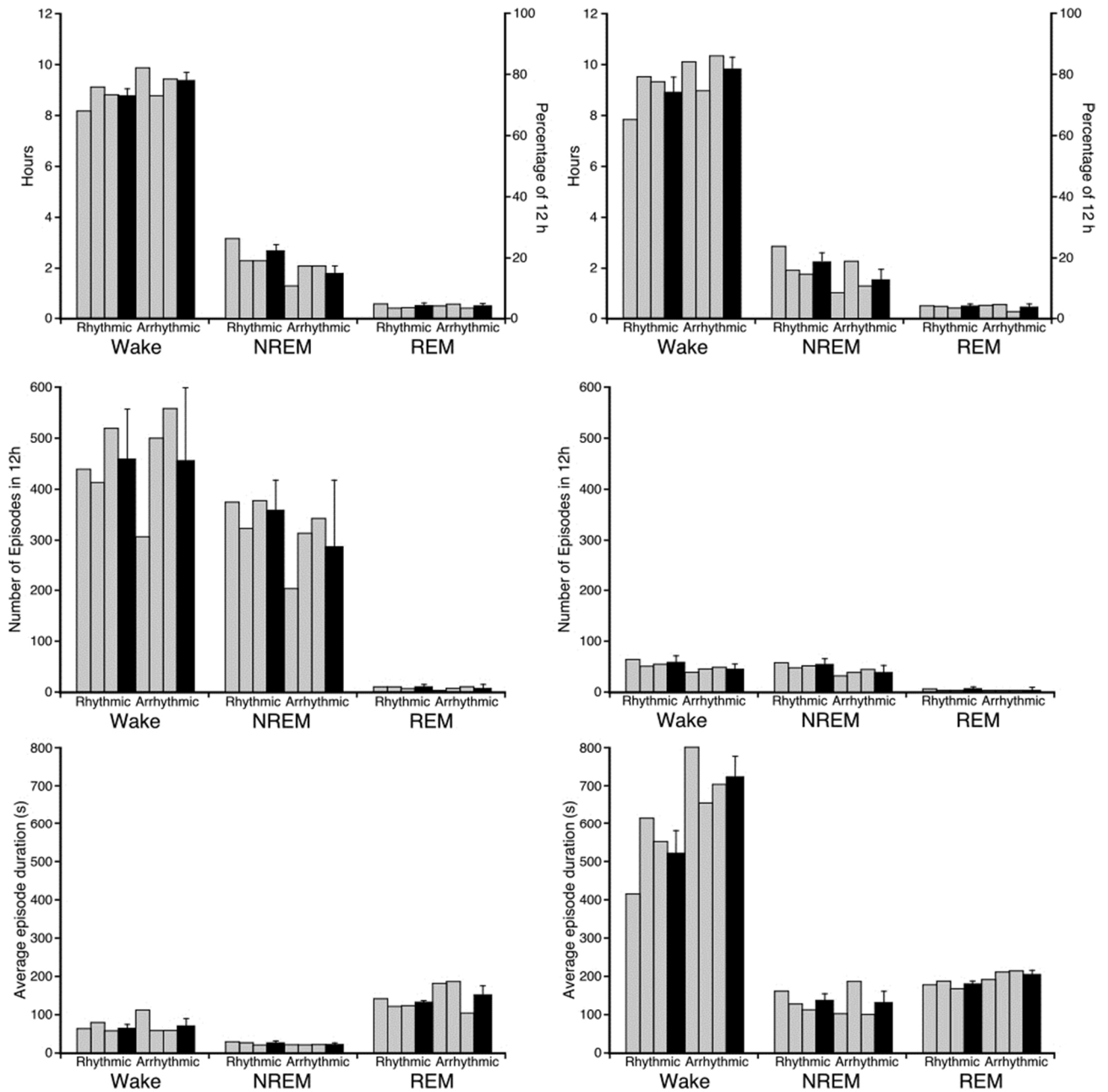


Figure 7: Graphs illustrating physiological data (5 s epoch scoring, left column of graphs, and 1 min epoch scoring, right column of graphs) for sleep and wake states during the 12 h light period in rhythmic and arrhythmic individuals (grey bars) and the respective weighted means (black bars). The graphs indicate percentage and the average amount of time spent in wake and sleep states, the average number of episodes for wake and sleep states and the average duration of an episode in wake and sleep states.

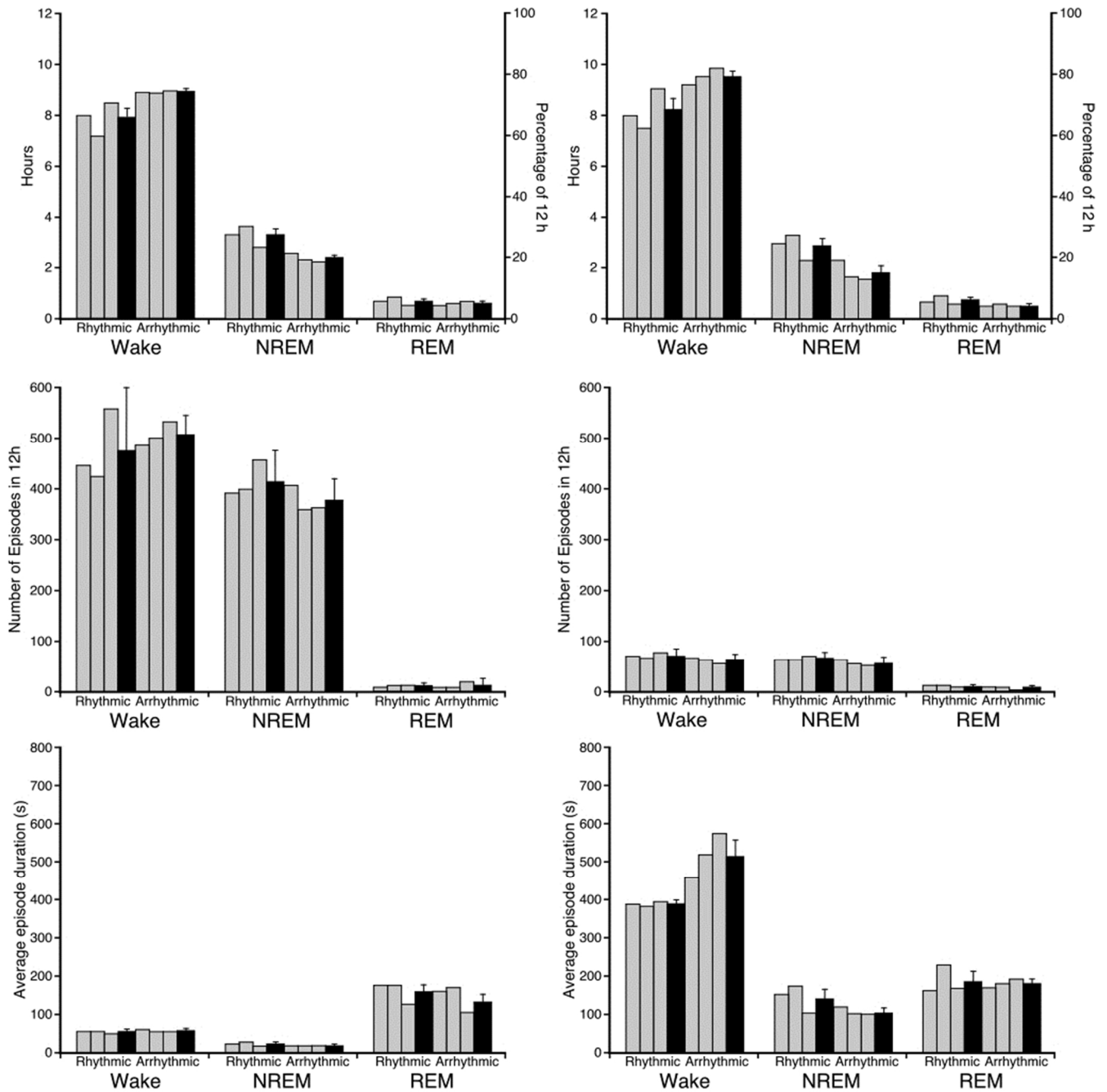


Figure 8: Graphs illustrating physiological data (5 s epoch scoring, left column of graphs, and 1 min epoch scoring, right column of graphs) for sleep and wake states during the 12 h dark period in rhythmic and arrhythmic individuals (grey bars) and the respective weighted means (black bars). The graphs indicate percentage and the average amount of time spent in wake and sleep states, the average number of episodes for wake and sleep states and the average duration of an episode in wake and sleep states.

was: 136 for waking (the number of which was higher during the dark period compared to the light period), 127 for NREM (the number of which was higher in the dark period compared to

the light period) and 23 for REM sleep (where the number was slightly higher in the dark period compared to the light). The arrhythmic average episodic composition for the 1 min epoch data was: 115 for waking (the number of which was higher in the dark period than the light period), 103 for NREM (the number of which was higher in the dark period compared to the light period) and 18 for REM (where the number was slightly higher in the dark period compared to the light period) (Figs. 6, 7, 8).

The number of episodes for waking, NREM and REM were statistically compared (using both scoring techniques) between the groups for the 72 h recording and the light and dark periods. With regard to the 5 s epoch data, statistical significance was noted between the groups in: (1) the number of NREM episodes ($p < 0.05$, where the rhythmic group showed a higher mean number of episodes); and (2) the number of NREM episodes during the light period ($p < 0.05$, where the rhythmic group showed a higher mean number of episodes). No statistically significant differences between the groups were observed when the 1 min epoch data was compared.

3.2.4 Duration of wake and sleep states

The average duration of an episode of waking, NREM and REM sleep were calculated for both 5 s epoch and 1 min epoch data in both rhythmic and arrhythmic groups. The results of the 5 s epoch data indicates that within the rhythmic group, the average duration of an episode was 64 s for waking (the duration of which was longer during the light period than the dark period), 27 s for NREM (where the duration was longer in the dark period than the light period) and 150 s for REM sleep (where the duration was longer in the light period than in the dark period). The arrhythmic group revealed an average duration of an episode of 68 s for waking (where the duration was longer in the light period than the dark period), 23 s for NREM (where the duration was similar between light and dark period) and 146 s for REM sleep (where the duration was longer in the dark period than the light period) (Figs. 6, 7, 8). Analysis of the 1 min epoch data showed that the average duration of an episode of waking was 453 s (where the duration was longer in the dark period than in the light period), 147 s for NREM (where the duration was longer in the dark period than in the light period) and 189 s for REM sleep (where the duration was longer in the dark period than in the light period). The arrhythmic group revealed an average duration for an episode of waking of 609 s (where the duration was longer in the light period than the dark period), 119 s for NREM (where the duration was longer in the light period than in the dark period) and

195 s for REM sleep (where the distribution was longer in the light period than the dark period) (Figs. 6, 7, 8).

The average duration of an episode of waking, NREM and REM were then statistically compared (using both scoring techniques) between the groups for the 72 h recording and the light and dark periods. With regard to the 5 s epoch data, statistical significance was noted between the groups in: (1) the average duration of a waking episode ($p < 0.05$, where the arrhythmic group showed a higher mean average duration); (2) the average duration of a waking episode during the dark period ($p < 0.05$, where the arrhythmic group showed a higher mean average duration); (3) the average duration of a NREM episode ($p < 0.05$, where the rhythmic group showed a higher mean average duration); and (4) the average duration of a NREM episode during the light period ($p < 0.05$, where the rhythmic group showed a higher mean average duration). No statistically significant differences between the groups were observed when 1 min epoch data was compared.

3.2.5 Slow wave activity (SWA) and spectral power

Statistical analysis of spectral power during sleep and wake states of both chronotypes of mole rat showed statistical significance in: (1) waking episodes compared to NREM episodes ($p < 0.05$, where NREM episodes had a higher mean); and (2) NREM episodes compared to REM episodes ($p < 0.05$, where NREM episodes had a higher mean) (Fig. 5). Comparisons of spectral power during wake and sleep states between the chronotypes of mole rat showed no statistical differences, though this result may be influenced by small sample sizes of the respective chronotypes. Spectral power (from the frequency band 1.2 – 4 Hz) is used to indicate the intensity of the SWA. The distribution of spectral power was calculated as an average of 2 h intervals. In the present study, SWA (calculated as a measure of spectral power) was compared between the groups during waking, NREM and REM sleep. It was observed that SWA was highest during NREM sleep and lowest during REM sleep in both groups of mole rats. The arrhythmic group showed a higher SWA during NREM sleep compared to the rhythmic group. When SWA was analysed between the groups during the light and dark period, it was observed that the arrhythmic group showed a higher SWA during NREM in both light and dark period compared to the rhythmic group (Fig. 9).

3.2.6 State transitions and REM periodicity

Transitions between the physiologically identified states were counted in both groups for both the 5 s and 1 min scored data. The 5 s data showed that from the waking state the

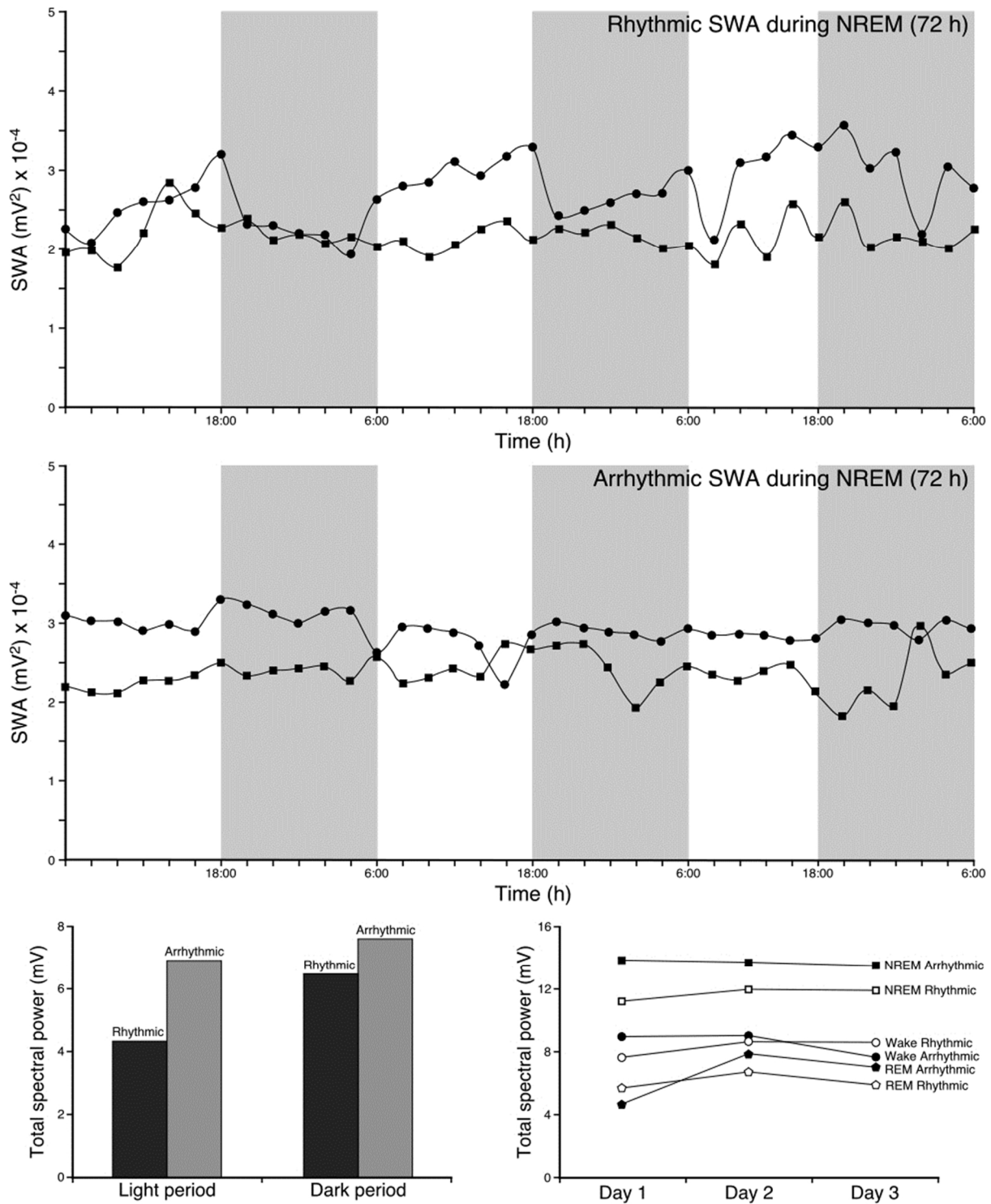


Figure 9: Graphs representing the variations of spectral power of slow wave activity (SWA) in NREM for 72 h between rhythmic and arrhythmic groups; the total spectral power of SWA in NREM for 72 h during the light and dark phases between rhythmic and arrhythmic groups; and the total spectral power in SWA during sleep and wake states for 72 h between rhythmic and arrhythmic groups for each day.

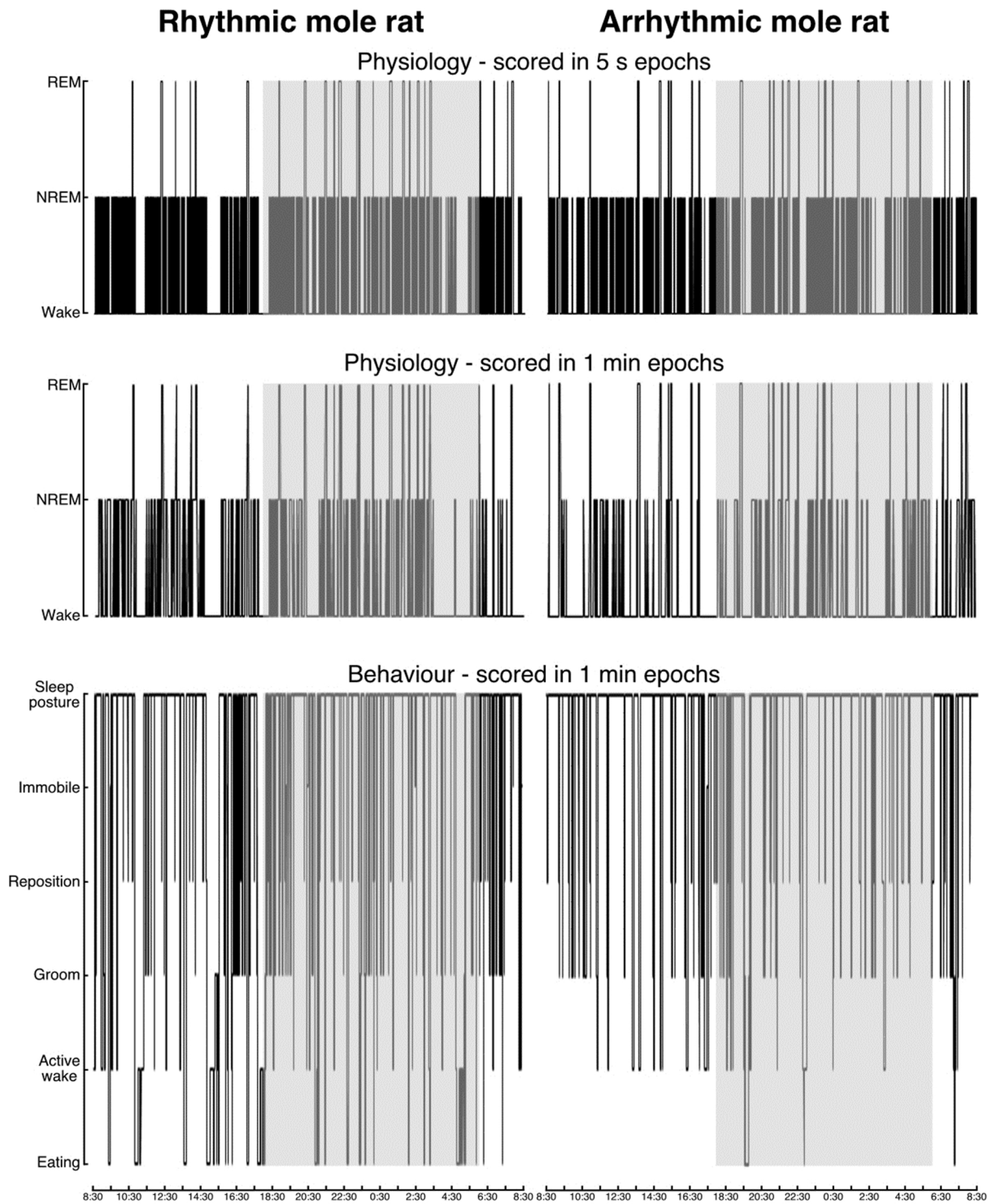


Figure 10: Hypnograms depicting physiological (5 s epoch scoring and 1 min epoch scoring) and behavioural data (1 min epoch scoring) in 24 h from a rhythmic and an arrhythmic individual. These hypnograms illustrate the compatibility of visually scored transitions between defined physiological wake and sleep states and the various behavioural states. Grey shaded areas indicate the dark period of a 24 h cycle.

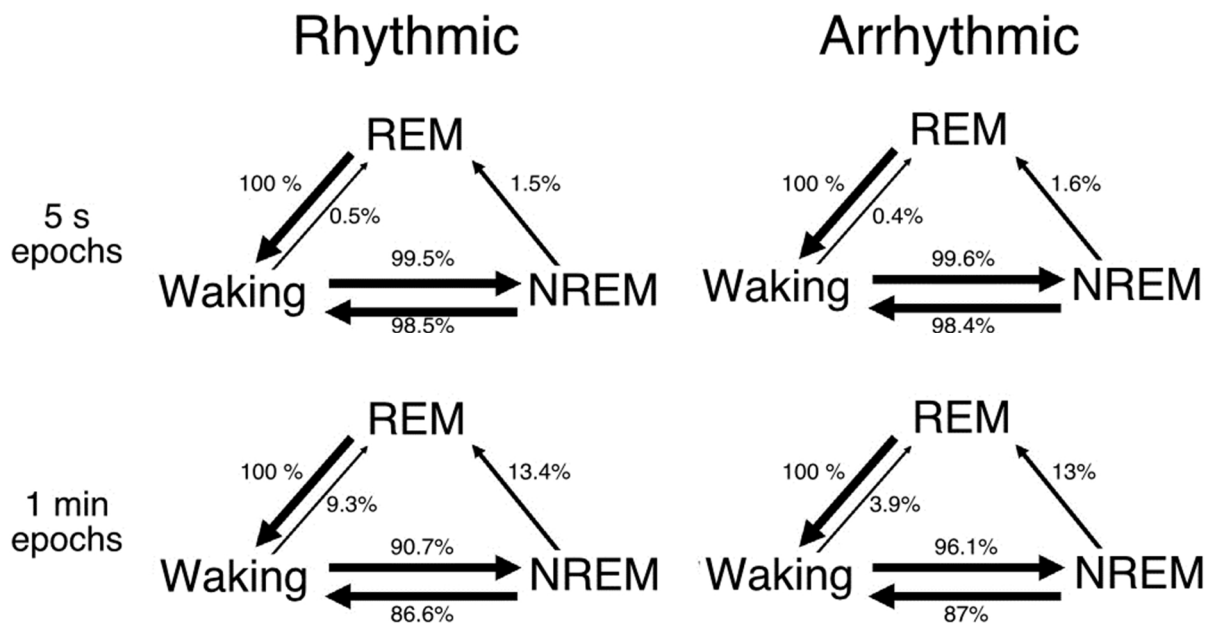


Figure 11: Flow diagrams illustrating transitions (represented as a percentage) between physiological wake and sleep states in 24 h for 5 s epoch scoring and 1 min epoch scoring data from rhythmic and arrhythmic individuals.

rhythmic individuals transitioned with greater frequency to NREM (99.5%) and only 0.5% into REM, whilst the arrhythmic group transitioned 99.6% and 0.4% respectively. From a state of NREM the rhythmic group transitioned significantly more often to waking (98.5%) than to REM (1.5%) while the arrhythmic group transitioned 98.4% and 1.6% respectively. In both groups, the state of REM transitioned only to waking (Figs. 10, 11). The 1 min scored data showed that from the state of waking the rhythmic group transitioned with greater frequency to NREM (90.7%) than into REM (9.3%) while the arrhythmic group transitioned 96.1% and 3.9% respectively. From a state of NREM the rhythmic group transitioned significantly more often to waking (86.6%) than to REM (13.4%) while the arrhythmic group transitioned into these states 87% and 13% respectively. Transitions from a state of REM were limited to waking only in both groups (Figs. 10, 11).

REM periodicity was calculated as the difference in time (1 min data only) between the onsets of consecutive REM episodes, and also when episodes of waking with a duration greater than 10 min were eliminated (Fig. 12). The results revealed that the rhythmic group exhibited a higher frequency of REM onset within an hour period indicating that the peak sleep cycle duration was 25-30 min in length. A similar scenario was noted when waking episodes greater than 10 min were eliminated, but peak cycle lengths were observed in the range of 15 -25 min. The arrhythmic group showed a relative variation in the frequency of

REM onsets within an hour bracket with a peak cycle length that occurred in the range of 10-15 min and 20-25 min, suggesting a more a variable cycle duration in this group (Fig. 12).

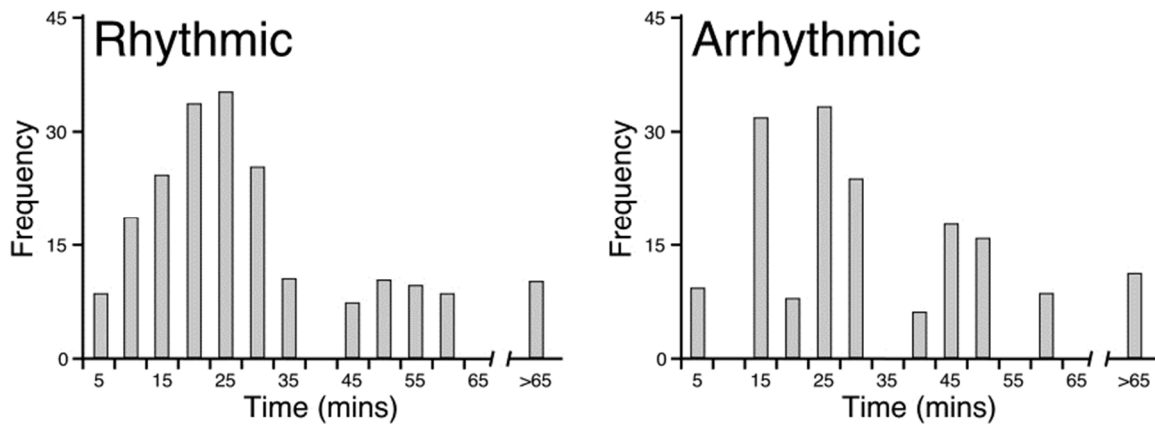


Figure 12: Graphs representing the time lapse (5 min periods) between onsets of consecutive physiologically identified REM episodes (REM periodicity) for 1 min epoch scoring data from rhythmic and arrhythmic individuals including and excluding episodes of waking greater than 10 min.

4. Discussion

The present study was undertaken to examine whether differences in wake and sleep states were readily observable in a species of mole rat from which individuals with a rhythmic or arrhythmic chronotype were identified and if a regressed visual system affected these parameters in comparison to other rodents. The current study used established techniques for identifying the chronotype of the individual molerats (Oosthuizen et al., 2003) and then employed telemetric recording of physiological EEG and EMG combined with behavioural recording. In assigning physiological states the EEG and EMG enabled identification of wake, NREM and REM sleep states in this species and these assignments correlated closely with behavioural recordings. We used both a 5 s epoch scoring method and a 1 min epoch scoring method to probe for potential differences, both methods having advantages and disadvantages in revealing similarities and differences, of which several were noted. The results indicate that the arrhythmic individuals spent more time in waking with a longer average duration of a waking episode, and less time in NREM with a shorter average duration of a NREM episode, though with a greater SWA intensity in NREM. Total time spent in sleep (TST) for both chronotypes of this rodent species was shorter than that seen in other rodents. This was a result of the species studied spending less time in NREM compared

to other rodents (see Table 3), as the amount of time spent in REM sleep was within the range, though at the upper end, reported previously for other rodents.

4.1 State definitions

In the present study wake and sleep states were telemetrically recorded and subsequently scored for both the 5 s epoch scoring and the 1 min epoch scoring. The state of wake was polygraphically identified by low amplitude, high frequency cortical EEG activity and irregular high amplitude nuchal EMG activity. The state of NREM was polygraphically identified by high amplitude, low frequency cortical EEG activity and stable low amplitude nuchal EMG activity. REM sleep was polygraphically identified by low amplitude, high frequency cortical EEG waves (similar to that seen in waking) and when the nuchal EMG was lower in amplitude (without reaching complete atonia) compared to waking and NREM and displayed large, high amplitude infrequent spikes (that correlated with behavioural twitches and jerks). These criteria used for the polygraphic identification of wake and sleep states in the mole rats of the current study are congruent with those previously utilised in the physiological identification of wake and sleep states in a different species of mole rat (Tobler and Deboer, 2001) and in other rodents and terrestrial mammals (see review by Campbell and Tobler, 1984; Tobler, 1995; McNamara et al., 2010).

4.2 5 s epoch scoring vs 1 min epoch scoring

The scoring techniques employed in the current study yielded some similarities and differences in the results. It was observed that between the rhythmic and arrhythmic mole rats for both methods of scoring (5 s and 1 min epochs) the time spent in waking, NREM and REM sleep remained similar. This is of importance as it reveals that the determination of the time spent in the various wake and sleep states is not altered by scoring method. The 5 s epoch scored data produced more statistically significant differences for the average number of episodes of wake and sleep states and average duration of an episode of wake and sleep states, within and between chronotypes and within and between days and light and dark periods when compared to the 1 min epoch scored data. The average duration of an episode of waking and NREM generated by the 5 s epoch scoring data seems unusually short, but if the average number of episodes is factored into this equation then the results indicate that both the rhythmic and arrhythmic individuals may exhibit a form of sleep fragmentation, as determined by the occurrence in brief arousals during NREM (Franken et al., 1991). The differences between the methods of scoring are a direct result of a decrease in sampling size

from the 5 s epoch scoring to the 1 min epoch scoring, but the 1 min epoch scoring data generates a more credible biological scenario for waking and NREM without significantly changing the times spent in these physiological states. Interestingly, despite the differences between the two methods for waking and NREM, the average number of episodes and average duration of an episode of REM sleep remained similar for both scoring methods. In light of the above, both the 5 s and 1 min scoring data appear to be a reasonable methods to draw conclusions regarding the total time spent in wake and sleep states, while the 1 min scoring data provides a more biologically relevant method, as it correlated more closely with the behaviour, to determine the average number of episodes of wake and sleep states and the average duration of an episode of wake and sleep states. In the text that follows the values generated from the 1 min scoring data are used for comparisons and discussion.

4.3 Rhythmic vs Arrhythmic mole rats

The results of the present study indicated that the arrhythmic mole rats spent more time in a state of wake (19.4 h vs 17.1 h, with a peak during the dark period), and had a longer average duration for a waking episode (609 s vs 453 s) than the rhythmic group. In contrast, the rhythmic mole rats spent more time in NREM (5.1 h vs 3.4 h, especially during the light period), had a higher average number of NREM episodes (122 vs 103) and had a longer average duration of a NREM episode (147 s vs 119 s) than the arrhythmic mole rats. Both groups spent a similar amount of time in REM sleep (1.2 h for rhythmic vs 1 h for arrhythmic), had a similar average number of REM sleep episodes (23 for rhythmic vs 18 for arrhythmic) and had a similar average duration of a REM episode (189 s for rhythmic vs 194 s for arrhythmic). The homeostatic nature of sleep dictates that sleep propensity is related to the history of prior waking i.e. the longer the vigilance state of wake is maintained, the greater the drive for sleep becomes (Tucci and Nolan, 2010). Given that the arrhythmic group spends more time awake than the rhythmic group, it is tempting to suggest that sleep propensity in the arrhythmic group should be higher. Interestingly, in potential mitigation of this apparent paradox, it was observed that SWA during NREM was consistently higher in the arrhythmic group compared with the rhythmic group, indicating that the arrhythmic individuals display a greater intensity of NREM compared to the rhythmic individuals. As SWA in NREM can be used as an indicator of NREM intensity, it is possible that the decrease in total NREM time observed in the arrhythmic mole rats is compensated for by increasing SWA. The SWA activity during NREM of the arrhythmic mole rats of the current study is congruent with previous studies of sleep deprivation in different mammals that have

consistently shown an increase in SWA during NREM following sleep deprivation (Tobler, 1995).

4.4 Comparison to other rodents

In the present study it was found that the total sleep time (TST) in the rhythmic individuals was 6.5 h while for the arrhythmic group it was 4.3 h. These values are lower than those noted (under similar recording conditions) for other rodents (Table 3). The amount of time spent in REM sleep in both groups of mole rats falls within the range that has been observed in other rodents (0.7 – 1.9 h, Table 3); however, the proportion of REM sleep relative to the total sleep time in the rhythmic group is 19 % while that of the arrhythmic group is 23 %, which while within the range seen for other rodents are on the higher end of the range (Table 3). The average duration of an episode of NREM in the rhythmic and arrhythmic mole rats (147 s and 119 s respectively) are shorter than that seen in other rodents (Table 3), but the average duration of a REM episode in the rhythmic and arrhythmic mole rats (189 s and 195 s respectively) are longer than those reported in other rodents (Table 3).

The consistency in the average duration of an episode of REM sleep between rhythmic and arrhythmic groups suggests a crucial baseline function for REM sleep in the mole rats. It has been previously noted that subterranean mole rats have a lower body temperature than surface dwelling mammals (Bennett and Faulkes, 2000) though it is unknown whether body temperature differences exist between different circadian chronotypes. It has been shown that REM sleep reaches maximal activity when body temperature is at its lowest (Dijk and Franken, 2005), therefore it could be possible that REM sleep is specifically regulated in these mole rats irrespective of their chronotype in relation to their body temperature, and this may explain the slightly higher duration of REM in the mole rats of the current study compared to other rodents (Table 3). A possible explanation for the discrepancy observed between the mole rats of the current study and other rodents could lie in the genetic variation of these rodents, as some of the classic sleep disorders have been associated with single gene mutations (Kimura and Winkelmann, 2007) and several studies have identified genomic regions that contain allelic variations affecting quantifiable sleep parameters commonly referred to as quantitative trait loci (QTL) (Tafti et al., 1997; Tafti, 2007). It is known in humans and mice that genetics are responsible for the phenomenological architecture of sleep (Lindowski et al., 1991; Franken et al., 1998, 1999; Tafti et al., 1997). It would appear that a series of loci on chromosomes 5, 7, 12 and 17 are associated with the vigilance phenotype in mice (Tafti et al., 1997), and this was further

assessed by characterising differences in EEG parameters in inbred mice strains where a number of differences between sleep states but also significant genotype-specific variations were reported, thereby indicating that EEG parameters are under genetic control (Franken et al., 1998). It would be of interest to examine the mole rats for specific QLTs on the chromosomes mentioned to determine if specific differences to other rodents that may explain the differences in sleep could be found.

4.5 Circadian rhythmicity and sleep

One of the aims of the current study was to determine whether baseline physiological sleep parameters would differ in individuals of the same species with distinct circadian chronotypes. The results of the present study indicate that while there were some differences between the rhythmic and arrhythmic groups, for the most part the measured sleep parameters were not significantly different. It is known that circadian rhythmicity and sleep homeostasis both contribute to the sleep-wake cycle and the structure of sleep in animals, yet it is apparent that sleep homeostasis is driven by sleep-wake behaviour, whilst the circadian clock is influenced by light and might be affected by the feedback of the sleep-wake cycle (Dijk and Franken, 2005). Several studies have shown that the phase relationship between the sleep-wake cycle and circadian rhythms may change and that the duration and structure of sleep may depend on the circadian phase at which sleep occurs (e.g. Czeisler et al., 1980; Zulley et al., 1981). Furthermore, sleep deprivation studies in species with an ablated SCN demonstrated an increase in SWA and sleep duration that suggests a neuroanatomical separation of the circadian and homeostatic regulation of sleep (Dijk and Franken, 2005). The results of the present study support the notion of elevated SWA as it was observed that SWA in NREM of the arrhythmic group was consistently higher when compared to the rhythmic group and this was seen in both light and dark periods; however, the results disagree with notion of an increase in sleep duration as it was observed that the average duration of an episode of NREM in the arrhythmic group was significantly lower when compared to the rhythmic group.

Circadian rhythms can be altered through genetic mutation of clock genes. Studies have shown that ablation of the clock genes *per1* and *per2*, which results in the absence of circadian rhythms in mice, do not affect sleep homeostasis and are not associated with a major change in the sleep deprivation induced increase in SWA (Kopp et al., 2002; Shiromani et al., 2004). Wisor et al (2002) showed that mice lacking the cryptochrome genes, *cry1* and *cry2*, also had an absence of circadian rhythmicity. This study concluded that the

cryptochrome genes affect sleep homeostasis, where NREM sleep time is increased and SWA is higher compared to wild types and sleep deprivation produces no change to NREM sleep time yet attenuates SWA compared to wild types. These genetic mutation studies indicate that the separation of circadian rhythmicity and sleep homeostasis does not extend to all components of circadian rhythm generation (Dijk and Franken, 2005). In the present study, the results are congruent with the theory postulating independence of circadian rhythmicity and sleep homeostasis, since no statistical significance could be identified between the rhythmic and arrhythmic groups in the amount of waking, amount of NREM sleep, percentage of total sleep time, amount of REM sleep and the percentage of REM sleep relative to the total sleep time; however, changes in the clock genes within the arrhythmic group may explain the statistically longer duration of waking and shorter duration of NREM sleep compared to the rhythmic group.

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Video Legends

Video file 1: Video recording of a mole rat showing 20 s of the behaviourally scored state of eating.

Video file 2: Video recording showing 20 s of a mole rat actively moving around the recording enclosure.

Video file 3: Video recording showing 20 s of typical grooming behaviour.

Video file 4: Video recording showing 25 s of typical repositioning behaviour while in the curled 'ball-like' position.

Video file 5: Video recording showing a 20 s example of the behaviourally scored state of immobility. In this case the head is rested on the floor of the perspex tube.

Video file 6: Video recording showing 20 s of a mole rat in a curled 'ball-like' position that was behaviourally scored as sleep posture. In this example the head of the mole rat is tucked under the body.

Video file 7: Video recording showing 20 s of behavioural REM sleep where muscle jerks on the head and body typically associated with this state are visible.

Video file 8: Video recording showing a 25 s example of attempted burrowing behaviour.