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Sleep in the Arabian oryx, *Oryx leucoryx*

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319626



Original published work submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree

of

Doctor of Philosophy

Johannesburg, 2018

Sleep in the Arabian Oryx...



DECLARATION

I, Joshua Gabriel Davimes declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Signature:

Date: 20 February 2018

DEDICATION

"For the pursuit of eternal knowledge and ineffable understanding, one must only dream..."

Josh Davimes

This work is dedicated to the important people in my life. My parents Claudia and Stephen, my brother Dominic, my Daschunds, Luigi and Franchesca and lastly, my most wondrous companion Camilla.

ABSTRACT

The evolution and function of sleep remains an enigma in modern science. Significant variation can be observed within species and across taxa. Little is understood about how sleep exists, or presents itself in species surviving in extreme conditions. For example, harsh desert ecosystems with notably lower survival rates for resident species. It has been hypothesized that specialized sleep-related behaviours impact thermoregulation to promote survival advantage. The threatened Arabian oryx (*Oryx leucoryx*), a member of the Cetartiodactyl superorder, is a large desert dwelling ungulate species successfully residing in the arid zones of the Middle East. Arabian Oryx exhibit numerous morphophysiological adaptations and capabilities to cope with their harsh environment. The current thesis provides an examination of the neuroanatomy, physiology and behavioural patterns related to sleep in wild, free-roaming Arabian oryx under natural conditions. It was our objective to determine whether the extreme conditions and climate of the Arabian Desert has led to novelties within the organization of the oryx's sleep related neuronal architecture, seasonal behaviour patterns and sleep physiology. Using immunohistochemistry and stereology our results indicate the sleep related nuclei in the brain of the oryx exhibit a typical mammalian organizational plan with additional order-specific and novel, species-specific features. Actigraphy revealed that oryx exhibit temporal niche switching patterns seasonally presenting with winter diurnal- and summer nocturnal activity and intermittent patterns during the transitional periods of spring and autumn. Polysomnography results indicate novel sleep patterns between seasons and physiologically confirm activity-based temporal niche switching at a seasonal level. Our work covers the first examination of the Arabian oryx brain sleep centers as well as its year-long activity patterns. Importantly, it is the first examination of physiological sleep in wild mammals within a desert environment. Our results suggest that ambient temperature acts a dominant driving force for the adaptive behavioural and physiological features described. The Arabian oryx is a remarkable species, well adapted to such an extreme environment and its highly plastic survival mechanisms appear unique. Considering the continual desertification of our planet and the predicted consequences of climate change, knowledge of such species and their continued conservation is of vital importance.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THE WORKS IN THIS THESIS

Publications

Davimes, J., Alagaili, A., Bennet, N., Mohammed, O., Manger, P., Gravett, N., 2015. Nuclear organization and morphology of the sleep related nuclei in the brain of the Arabian oryx, Oryx leucoryx. *Journal of Neurochemistry, 134*, p149. Conference proceeding: 25th ISN-APSN-ANS Biennial Meeting, Cairns, Australia, August 23-27th, 2015. ISSN Print: 0022-3042.

> *Author contributions: Conceived and designed the experiments:* ***DJ,*** *MP, and GN. Assisted Surgeries: AA, BN, MO, and MP. Performed the experiments:* ***DJ,*** *MP, and GN. Analyzed the data:* ***DJ,*** *MP, and GN. Contributed reagents/materials/analysis tools: AA, BN, MO, MPR, and GN. Wrote Conference Abstract:* ***DJ****. Designed Poster:* ***DJ****. Presented at Conference:* ***DJ.***

Davimes, J.G., Alagaili, A.N., Gravett, N., Bertelsen, M.F., Mohammed, O.B., Ismail, K., Bennett, N.C. and Manger, P.R., 2016. Arabian oryx (Oryx leucoryx) respond to increased ambient temperatures with a seasonal shift in the timing of their daily inactivity patterns. *Journal of biological rhythms*, *31*(4), pp.365-374. https://doi.org/10.1177/0748730416645729

> *Author contributions: Conceived and designed the experiments:* ***DJG,*** *MPR, and GN. Assisted Surgeries:* ***DJG,*** *GN, AAN, BMF, BNC, MOB, IK, and MPR. Performed the experiments:* ***DJG,*** *GN, AAN, BMF, BNC, MOB, IK, and MPR. Analyzed the data:* ***DJG,*** *MPR, and GN. Contributed reagents/materials/analysis tools: AAN, BMF, BNC, MOB, IK, MPR, and GN. Wrote the paper:* ***DJG,*** *MPR, and GN. Provided significant comments and editing on the paper:* ***DJG,*** *MPR and GN. All authors reviewed the final manuscript prior to journal submission. Rebuttal and Revisions of Manuscript:* ***DJG,*** *MPR, and GN.*

Davimes, J.G., Alagaili, A.N., Gravett, N., Bertelsen, M.F., Mohammed, O.B., Ismail, K., Bennett, N., Manger, P.R., 2016. A possible non-photic primary zeitgeber seasonally entraining dual-phasing in the Arabian oryx. *Journal of Sleep and Sleep Disorders Research.* Conference

proceeding: Sleep 2016, Denver, Colorado, United States of America, June 2016. ISSN Print: 0161-8105.

Author contributions: Conceived and designed the experiments: ***DJG,*** *MPR, and GN. Assisted Surgeries:* ***DJG,*** *GN, AAN, BMF, BNC, MOB, IK, and MPR. Performed the experiments:* ***DJG,*** *GN, AAN, BMF, BNC, MOB, IK, and MPR. Analyzed the data:* ***DJG,*** *MPR, and GN. Contributed reagents/materials/analysis tools: AAN, BMF, BNC, MOB, IK, MPR, and GN. Wrote Conference Abstract:* ***DJG****. Designed Poster:* ***DJG****. Presented at Conference:* ***DJG***

Davimes, J.G., Alagaili, A.N., Bennett, N.C., Mohammed, O.B., Bhagwandin, A., Manger, P.R. and Gravett, N., 2017. Neurochemical organization and morphology of the sleep related nuclei in the brain of the Arabian oryx, Oryx leucoryx. *Journal of Chemical Neuroanatomy*, *81*, pp.53-70. https://doi.org/10.1016/j.jchemneu.2017.02.002

Author contributions: Conceived and designed the experiments: ***DJG,*** *MPR, and GN. Assisted Surgeries: AAN, BNC, MOB, and MPR. Performed the experiments:* ***DJG,*** *BA, MPR, and GN. Analyzed the data:* ***DJG,*** *BA, MPR, and GN. Contributed reagents/materials/analysis tools: AAN, BNC, MOB, BA, MPR, and NG. Wrote the paper:* ***DJG,*** *MPR, and GN. Provided significant comments and editing on the paper:* ***DJG,*** *MPR and GN. All authors reviewed the final manuscript prior to journal submission. Rebuttal and Revisions of Manuscript:* ***DJG,*** *MPR, and GN.*

Davimes, J.G., Alagaili, A.N., Bertelsen, M.F., Mohammed, O.B., Hemingway, J., Bennett, N.C., Manger, P.R. and Gravett, N., 2017. Temporal niche switching in Arabian oryx (Oryx leucoryx): Seasonal plasticity of 24h activity patterns in a large desert mammal. *Physiology and Behavior*, *177*, pp.148-154. https://doi.org/10.1016/j.physbeh.2017.04.014

Author contributions: Conceived and designed the experiments: ***DJG,*** *MPR, and GN. Assisted Surgeries: AAN, BNC, MOB, and MPR. Performed the experiments: AAN, BNC, MOB, MPR, and GN. Analyzed the data:* ***DJG,*** *HJ, MPR, and GN. Contributed reagents/materials/analysis tools: AAN, BNC, HJ, MOB, MPR, and GN. Wrote the paper:* ***DJG,*** *MPR, and GN. Provided significant comments and editing on the paper:* ***DJG,*** *HJ, MPR, and GN. All authors reviewed the final manuscript prior to journal submission. Rebuttal and Revisions of Manuscript:* ***DJG,*** *MPR, and GN.*

Davimes, J.G., Alagaili, A.N., Bhagwandin, A., Bertelsen, M.F., Mohammed, O.B., Bennett, N.C., Manger, P.R. and Gravett, N., 2018. Seasonal variations in sleep of free-ranging Arabian oryx (Oryx leucoryx) under natural hyper-arid conditions. *Sleep.* In Press.

> *Author contributions: Conceived and designed the experiments:* ***DJG,*** *MPR, and GN. Assisted Surgeries: AAN, BNC, MOB, BA, and MPR. Performed the experiments: AAN, BNC, MOB, MPR, BA, and GN. Analyzed the data:* ***DJG,*** *MPR, AB, and GN. Contributed reagents/materials/analysis tools: AAN, BNC, MOB, MPR, AB, and GN. Wrote the paper:* ***DJG,*** *MPR, and GN. Provided significant comments and editing on the paper:* ***DJG,*** *MPR, and GN. All authors reviewed the final manuscript prior to journal submission. Rebuttal and Revisions of Manuscript:* ***DJG,*** *MPR, and GN.*

Conference Presentations

Joshua G. Davimes, Abdulaziz N. Alagaili, Mads F Bertelsen, Nigel C. Bennett, Osama B. Mohammed, Paul R. Manger, Nadine Gravett. Title: Seasonal effects on sleep architecture by means of actigraphy in free-roaming Arabian Oryx, *Oryx leucoryx. 12th International Society of Neuroscientists of Africa (SONA) Conference*- Durban, South Africa. March 26th - 30th 2015.

Joshua G. Davimes, Abdulaziz N. Alagaili, Nigel C. Bennett, Osama B. Mohammed, Paul R. Manger, Nadine Gravett. Title: Nuclear organization and morphology of the sleep related nuclei in the brain of the Arabian Oryx, *Oryx leucoryx. 43rd Annual Conference of the Anatomical Society of Southern Africa*- Parys, South Africa. May 9th - 13th 2015.

Joshua G. Davimes, Aziz N. Alagaili, Nigel C. Bennett, Osama B. Mohammed, Paul R. Manger, Nadine Gravett. Title: Nuclear organization and morphology of the sleep related nuclei in the brain of the Arabian Oryx, *Oryx leucoryx. 9th IBRO world congress*- Rio de Janeiro, Brazil. July 7th - 11th 2015.

Joshua G. Davimes, Abdulaziz N. Alagaili, Nigel C. Bennett, Osama B. Mohammed, Paul R. Manger, Nadine Gravett. Title: Nuclear organization and morphology of the sleep related nuclei in the brain of the Arabian Oryx, *Oryx leucoryx. 25th Meeting for the International Society for Neurochemistry*- Cairns, Australia. August 23rd - 27th 2015.

Joshua G. Davimes, Abdulaziz N. Alagaili, Nigel C. Bennett, Osama B. Mohammed, Paul R. Manger, Nadine Gravett. Title: Nuclear organization and morphology of the sleep related nuclei in the brain of the Arabian Oryx, *Oryx leucoryx*. University of the Witwatersrand *7th Cross-Faculty Postgraduate Symposium*. March 1st – 2nd 2016.

J.G Davimes, A.N Alagaili, N Gravett, M.F Bertelsen, O.B Mohammed, K Ismail, N.C Bennett and P.R Manger. Title: A Possible Non-Photic Primary Zeitgeber Seasonally Entraining Dual-Phasing In The Arabian Oryx. *SLEEP 2016* – Denver, Colorado, United States of America. June 11th - 15th. Additionally: *SLEEP 2016 data blitz session* at club Hypnos for Sleep Research Society (SRS) - Same data.

J.G Davimes, A.N Alagaili, N Gravett, M.F Bertelsen, O.B Mohammed, K Ismail, N.C Bennett and P.R Manger. Title: A Possible Non-Photic Primary Zeitgeber Seasonally Entraining Dual-Phasing In The Arabian Oryx. *13th International Society of Neuroscientists of Africa* (SONA) Conference – Entebbe, Uganda. June 11th - 14th 2017.

Joshua G. Davimes, Abdulaziz N. Alagaili, Nigel C. Bennett, Osama B. Mohammed, Paul R. Manger, Nadine Gravett. Title: Nuclear organization and morphology of the sleep related nuclei in the brain of the Arabian Oryx, *Oryx leucoryx*. *13th International Society of Neuroscientists of Africa* (SONA) Conference – Entebbe, Uganda. June 11th - 14th 2017.

Joshua G. Davimes, Alagaili Abdulaziz, Bennet Nigel, Bertelsen Mads, Bhagwandin Adhil, Gravett Nadine, Hemingway Jason, Ismail Khairy, Manger Paul, Mohammed Osama. Title: Sleep in the Arabian oryx. University of the Witwatersrand *8th Cross-Faculty Postgraduate Symposium*. October 25th – 26th 2017.

ACKNOWLEDGEMENTS

I would firstly like to acknowledge and thank all the funding bodies that made the research within this thesis possible: the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award Number: 11-ENV1918-02; my Innovation Doctoral Scholarship Programme and additional support from the South African National Research Foundation (NRF) (94233, IFR150115112130, 64956, 87888); my In-country Masters Scholarship Programme within the NRF and German Academic Exchange Service (DAAD); the Deanship of Scientific Research Chairs at the King Saud University through the research group project number RGP_020; Prince

Bander bin Saud Al-Saud, former President of the Saudi Wildlife Authority (SWA); the Vice Deanship of Research Chairs at the King Saud University; and the South Africa Research Chair for Mammal Behavioural Ecology.

I am also grateful for the tremendous help provided by our colleagues at the National Wildlife Research Center in Taif, Saudi Arabia, especially Mr. Ahmed Boug, Dr. Saud Anagariyah, Mr. Khairy Ismail, Mr. Ghazi Raziman, Mr. Raed Aljuaid and Mr. Ali Aljuaid. I also extend my sincerest thanks to Mr. Jan Pickard at Ratelfontein, South Africa, who allowed us to use one of his Arabian oryx in the Karoo, South Africa. To all of my co-authors, I would like to acknowledge all your work towards the information accumulated and contained within this thesis and thank you for making our research successful so far. A big thanks to Mr. Martin Haupt from African Wildlife Tracking for his assistance in assembling, and Dr. Alexei Vyssotski for his technical assistance with, the Neurologger® devises used in Article IV.

To my two supervisors, Dr. Nadine Gravett and Prof. Paul Manger, I would like to acknowledge your continued and long-term support of my academic and research growth since my undergraduate days at the University of the Witwatersrand. It was your teaching and enthusiasm that lead me to focus on the neurosciences. Thank you for always pushing me hard and also for leaving me to my own devices so that I could grow as an independent researcher, student and scientist. Lastly, thank you for providing the opportunity to travel the world for field work, conferences and teaching schools, it has truly made my postgraduate studies an absolute pleasure!

To all my fellow colleagues within the Comparative Neurobiology Laboratory and within the School of Anatomical Sciences at the University of the Witwatersrand, thank you for the support, friendship and interesting debates and discussion over the years. To Adhil Bhagwandin and Brendon Billings, thank you both for your invaluable and instrumental mentorship and backing over the years as both colleagues and friends and for all the wonderful experiences during conference and field work travel. To Nicholas Bacci, for all the local conference camaraderie and wonderful debate and friendship over the years. To Illke Philander, for our shared adventure and anguish since masters and through the entirety of our PhDs. Thank you for all your help and for our shared undertakings related to Wits University. To Jason Hemingway,

for all the statistical support and guidance in the research and for the shared interests in music and film. A big thanks to all my colleagues at the University of Johannesburg, especially Dr. Elaine Swanepoel for her flexibility with my research and teaching responsibilities. To all my colleagues and fellow table doctors in the dissection hall at Wits University. It has been a fun and fantastic learning and teaching experience over the last few years.

I must thank all of my friends outside of University and the ones I met at Wits. It has been an absolute pleasure to share science with you and have a constant outlook to the world from people outside of science. A huge thank you to my family, for their continued support over the last ten years of my university studies. For putting up with my frustrations, outbursts and the general pandemonium associated with a PhD degree. For encouraging me always and celebrating all my accomplishments, large and small. My mom and dad, Claudia and Stephen Davimes, thank you very much from the bottom of my heart, it would not be possible without you both. My brother Dominic, for providing endless comedy and distraction from my work when I really needed it. To my gran, Anne Koseff, for showing interest in all my academic endeavors and my photography career and for always giving great advice and loving support. To my late gran, Vera Davimes, for her financial and loving support during my undergraduate studies. My two Daschunds, Luigi and Franchesca for their companionship and presence over the years. Lastly, to my darling Camilla Lamb, my long-standing companion and partner. Thank you for always supporting me in every way possible. For nudging and pushing me in the right direction, especially the difficult ones so far encountered. For all the wonderful travel we have experienced together and all the scientific debate, argument and interest we share and outright disagree over. I look forward to the exciting times ahead!

Last, but not least, I would like to mention my appreciation for the Arabian oryx used in my research, for whom none of this research would exist otherwise. May the species continue to thrive in such an unforgiving environment. I hope for the continued success of the conservation and preservation programmes that exist in supporting the Arabian oryx across the deserts of the world.

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ABBREVIATIONS & SYMBOLS

Δ	Change
Ø	Diameter
®	Registered
4V	Fourth Ventricle
5-HT	Serotonin
A6d	Diffuse portion of the locus coeruleus nuclei

A7d	Diffuse portion of the subcoeruleus nuclei
A12	Tuberal cell group
A15d	Anterior hypothalamic group, dorsal division
ACT	Actigraphy
ARC	Arcuate nucleus
CB	Calbindin
cd	Conservation dependent
ChAT	Choline acetyltransferase
CHX	Chlorhexidine
CR	Calretinin
Cu	Copper
DAAD	German Academic Exchange Service
DBP	Diastolic blood pressure
DD	Constant darkness
DMH	Dorsomedial hypothalamus
DRd	Dorsal subdivisions of the dorsal raphe nuclear complex
DRl	Lateral subdivisions of the dorsal raphe nuclear complex
dSPZ	Dorsal subparaventricular zone
DT	Dorsal thalamus
ECG	Electrocardiography
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
f	Fornix
FFT	Fast Fourier transform

FMR	Field metabolic rate
GABA	ɣ-aminobutyric acid
GC	Nucleus gigantocellularis
GP	Globus pallidus
Hcrt	Hypocretin
Hz	Hertz
ic	Inferior colliculus
IO	Inferior olive
IUCN	International Union for the Conservation of Nature
LC	Locus coeruleus
LD	Light-dark
LDT	Laterodorsal tegmental nucleus
LHA	Lateral hypothalamus
LL	Constant light
MAARIFAH	National Plan for Science, Technology and Innovation, King Abdulaziz City for Science and Technology
Mc	Orexinergic main cluster
MC	Nucleus magnocellularis
MPO	Medial preoptic nucleus
MS	Mahazat as-Sayd Protected Area
NCWCD	National Commission for Wildlife Conservation and Development
NRF	South African National Research Foundation
NREM	Non-rapid eye movement sleep
NRT	Non-reversed Ta cycle
NWRC	National Wildlife Research Center

nt	Near threatened
OT	Optic tract
Otc	Orexinergic optic tract cluster
Ox-A	Orexin-A
PIA	Pontine inhibitory area
PM	nucleus paramedianus
PPN	Pedunculopontine nucleus
PPT	Pedunculopontine tegmental nucleus
PSG	Polysomnography
Pvc	Parvocellular cluster
PVC	Polymer of vinyl chloride
PVH	Paraventricular hypothalamus
R	Reticular thalamus
RAS	Rostral reticular formation system
REM	Rapid eye movement sleep
RHT	Retinohypothalamic tract
RT	Reversed Ta cycle
SBP	Systolic blood pressure
SCN	Suprachiasmatic nucleus
SPZ	Subparaventricular zone
SWA	Slow wave activity
SWA	Saudi Wildlife Authority
SWS	Slow wave sleep
Ta	Ambient air temperature
Tb	Body temperature

Tc	Core body temperature
Tg	Ground temperature
Tsc	Subcutaneous temperature
TEWL	Lower total evaporative water loss
TH	Tyrosine hydroxylase
UBM	Uruq Bani Ma'arid Protected Area
VLPO	ventrolateral preoptic nucleus
zi	Zona incerta
Zic	Orexinergic zona incerta cluster
V	Volts
VIP	Vasoactive intestinal peptide
VMH	Ventromedial hypothalamus
VP	Vasopressin
vSPZ	Ventral subparaventricular zone

PART I

Integrated Narrative

Literature Review

1. The Arabian oryx

1.1 An Introduction to the Species of Interest: Characteristics, Conservation, Ecology, and Taxonomy



Figure 1 - Arabian oryx in the Arabian Desert. *Copied from http://christindim.com/wp-content/uploads/2015/10/06-Arabian-Oryx.jpg. Accessed 20/05/2017.*

The Arabian oryx, *Oryx leucoryx*, is the smallest member of the genus *Oryx*, and is grouped within the Artiodactyla order. The oryx, an even-toed ungulate, is native to the harsh deserts and steppes of the Arabian Peninsula. The Arabian oryx has an average body mass of 90 kg for males and 80 kg for females; and has an average body length of 1.60 m, with a shoulder height between 81 and 102 cm. They possess white coats, black blazes and black markings on the legs and underside of the abdomen (Fig. 1). Arabian oryx breed opportunistically and without seasonal preference in wild populations (Stanley Price, 1989; Greth and Schwede, 1993; Vié 1996; Ancrenaz et al., 1998; Strauss, 2006; Wronski et al., 2011). Females give birth, on average, to one calf per year. The average life span for oryx is 8.8 – 20.8 years, depending on environmental locations or captivity status (Treydte et al., 2001; Weigl, 2005; Harding et al., 2007; Strauss, 2008; Wronski et al., 2011). The reproductive behaviours of Arabian oryx appear to be dictated by food and water availability as well as surrounding climate (Sempéré et al.,

1996; Ancrenaz et al., 1998). Socially, Arabian oryx exhibit various dynamics and herd behaviours. Both large mixed-sex herds and smaller herds have been observed. Population numbers and herd structure are, like reproduction, heavily dependent on climate and are affected most severely by drought (Tear and Ables, 1999; Ismail et al., 2011). Their ruminant diets comprise primarily of bushy grasses; however, they are reported to digest various vegetation - types when available, which include trees, fruit, tubers and roots (Spalton et al., 1999; Ostrowski et al., 2002).

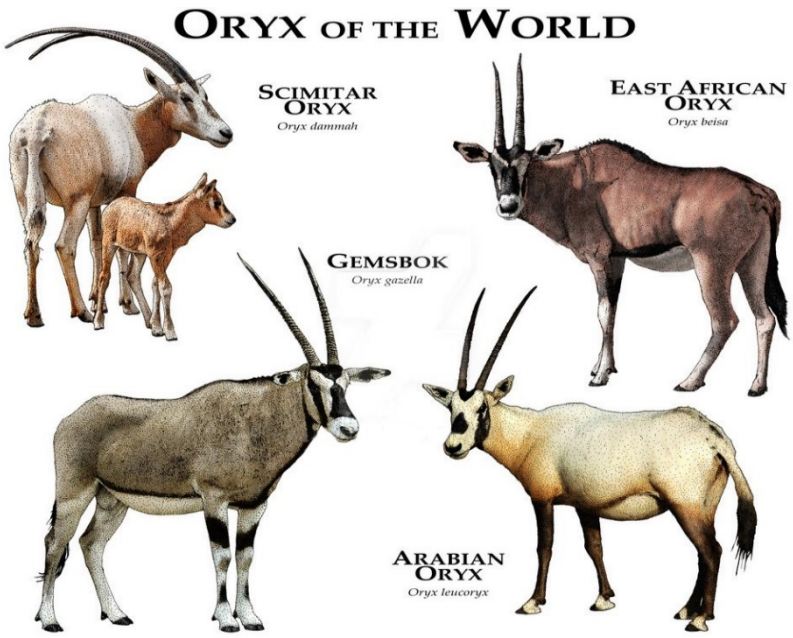


Figure 2 - Species of the genus *Oryx. Modified and copied from* http://img10.deviantart.net/8dca/i/2015/354/8/a/oryx_of_the_world_by_rogerdhall-d9kujix.jpg. *Accessed 20/05/2017*

Recent lineage-based studies have grouped the two orders Artiodactyla (e.g. even toed ungulates) and Cetacea (e.g. whales and dolphins) via phylogenetic, paraphyletic and morphological tracings into a new superorder, Cetartiodactyla, consisting of 290 extant species (Shimamura et al., 1997; O'Leary, 1999; Price et al., 2005). The Arabian oryx is within the genus *Oryx* and sister species include: *Oryx beisa* (Beisa oryx); *Oryx dammah* (Scimitar-horned oryx); and *Oryx gazelle* (Gemsbok) (Fig. 2) (http://www.iucn.org/). The Oryx is grouped within the family Bovidae, order Artiodactyla (Pallas, 1777) and superorder Cetartiodactyla within the Mammalia class. The Artiodactyla order is vastly successful on an evolutionary basis and comprises 240 species, of which 90 include large extant ungulates. The Artiodactyla order evolved during the Eocene and began to speciate during a warming period in the Mid-Miocene Climatic Optimum (Barnosky et al., 2003). During these periods, environmental conditions were

much hotter and more arid than at present and may have led the Artiodactyla to be pre-adaptive to desertification and climate change (Barnosky et al., 2003; Mitchell and Lust, 2008).



Figure 3 - Sand seas and dunes of the Arabian Desert. *Copied from https://s-media-cache-ak0.pinimg.com/originals/61/96/b3/6196b3d3ca84dc9f941c7b4e156f457a.jpg. Accessed on 20/05/2017*

Arabian oryx survive in one of the most food-depauperate environments in the world - the deserts of the Arabian Peninsula (Fig. 3). The natural environment of the Arabian oryx, the Arabian Desert, covers 2,300,000 km^2 and undergoes extreme climatic change during the different seasons of the year. During the summer months, the ambient air temperature (Ta) can reach 54 ºC and drops as low as -12 ºC in the winter (Fig. 4), with the chance of a rare snowfall in some areas. While some of the coastal areas maintain some humidity through rainfall, most of the interior has very little rainfall with a yearly average of less than 100 mm. Seasonal sandstorms and winds reaching up to 100 km per hour shift tonnes of sand and reshape the desert each year (Holm et al., 2013). The survival rate of free-roaming mammals within such extreme environments is very low. These low success rates result from a highly varying range of ambient temperatures, minimal to zero rainfall, intense solar radiation and low primary productivity (Noy-Meir, 1973; Degen, 1997; Schmidt-Nielsen, 1997). These extreme environmental characteristics dictate that the mammals found here are prone to desiccation and must regulate their energy and water balance strictly via appropriate specialized adaptations.

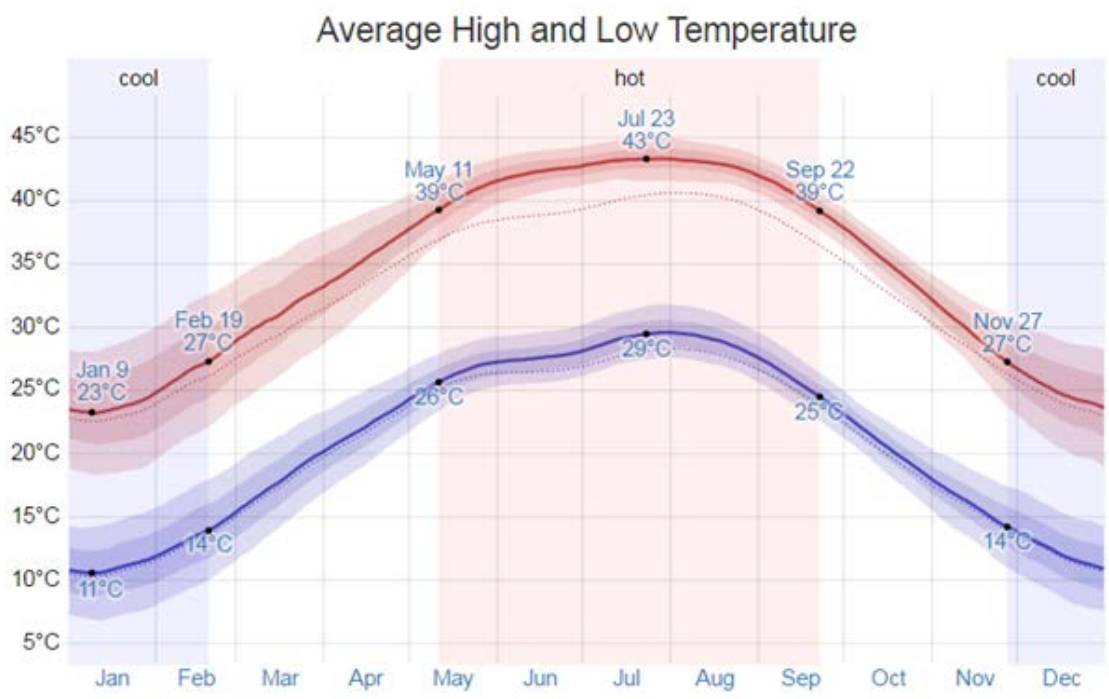


Figure 4 - Average high and low ambient temperatures from As Sulayyil, near the Rub al Khali desert (Arabian Desert) from 2016. Daily average high (red line) and low (blue line) temperature, with 25th to 75th and 10th to 90th percentile bands. Thin dotted lines are the corresponding average perceived temperatures. *Modified and copied from Weather Spark. https://weatherspark.com/y/103712/Average-Weather-in-As-Sulayyil-SaudiArabia, Accessed 20/05/2017.*

The Arabian oryx was formerly widespread throughout the Arabian Peninsula as far north as Kuwait and Iraq (Fig. 5 demonstrates distribution across the Middle East and Fig. 6 historical distribution across Saudi Arabia). Their distributions were mostly split into two populations; one northern group across the Nafud desert and a southern group across the Rub al Khali desert (Arabian Desert) and the plains of Oman (http://www.iucn.org/; Stanley Price, 1989). Wild, free-roaming Arabian oryx were brought to extinction with the last populations being eliminated in the Nafud desert and the Rub al Khali desert in the Kingdom of Saudi Arabia during the 1970's (Henderson, 1974). The overall endangerment of the Arabian oryx has been attributed to unregulated hunting although it was also related to oil industry activity, habitat loss from domestic livestock and illegal capture (Gilad et al., 2008). Since the late 1970's populations have been reestablished as a result of introductions through zoos and private collections and today the Arabian oryx is officially classified as "vulnerable" on the International Union for the Conservation of Nature (IUCN) Red List (http://www.iucnredlist.org/). The countries currently populated by the Arabian oryx include Saudi Arabia, Oman, Israel, United Arab Emirates and Jordan. The global population of Arabian oryx is approximately 1,000 free roaming and around 6,000-7,000 within captivity worldwide, although these figures may require update (http://www.iucnredlist.org/). There are major habitat and population conservation efforts worldwide dedicated to the Arabian oryx; however, they are at present still under major threat

from illegal live capture for sale, poaching, habitat reduction, drought and overgrazing (Mallon and Kingswood, 2001; Seddon and Ismail, 2002; Mésochina et al., 2003; Gilad et al., 2008). Forty-six percent of antelope species are currently included in the IUCN Red List of threatened species. Of the included species 65% are of arid environment origin and 92% of the 65% are living under the extreme conditions of the Arabian deserts and the Sahara deserts (Table 1) (Hilton-Taylor, 2000; Mésochina et al., 2003).

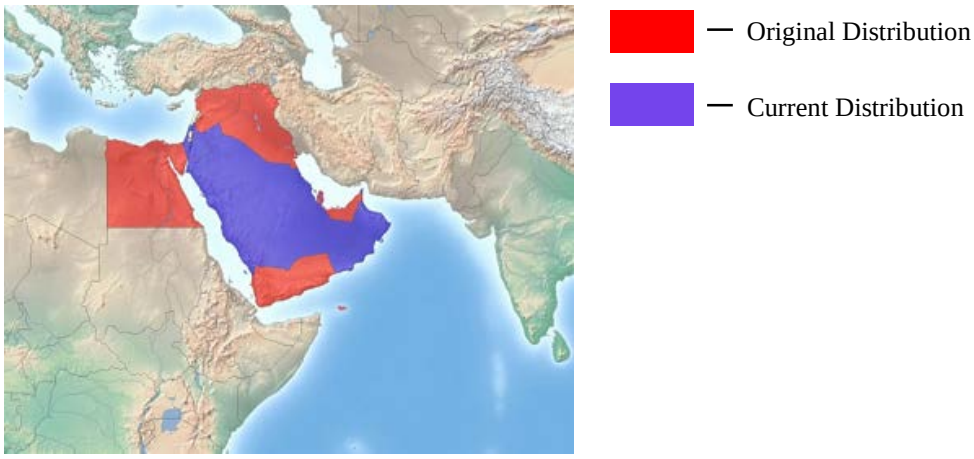


Figure 5 - Distribution of Arabian oryx across the Middle East. *Modified and copied from - http://www.ourendangeredworld.com/wp-content/uploads/2013/03/Arabian-Oryx-Range-Map.png. Accessed 20/05/2017.*

Table 1 - IUCN conservation status of wild antelope species that occur in the Sahara and Arabian deserts. nt = near threatened; cd = conservation dependent. *Reproduced and modified from Mésochina et al. (2003) with permissions (see Appendix 4).*

Species	IUCN Status 1996	IUCN Status 2000	IUCN Status 2016
Gazella dama, dama gazelle	Endangered	Endangered	Critically Endangered
Gazella leptoceros, slender-horned gazelle	Endangered	Endangered	Endangered
Gazella cuvieri, Cuvier's gazelle	Endangered	Endangered	Vulnerable
Gazella dorcas, dorcas gazelle	Lower Risk – nt	Vulnerable	Vulnerable (2008)
Gazella subgutturosa marica, Arabian sand gazelle	Lower Risk – nt	Vulnerable	Vulnerable (2008)
Gazella gazella cora, Arabian mountain gazelle	Lower Risk – cd	Lower Risk – cd	Vulnerable (2008)
Gazella rufifrons, red-fronted gazelle	Vulnerable	Vulnerable	Vulnerable (2008)
Oryx dammah, scimitar-horned oryx	Critically Endangered	Extinct in the Wild	Extinct in the Wild
Addax nasomaculatus, addax	Endangered	Critically Endangered	Critically Endangered
Arabitragus jayakari, Arabian Tahr	Endangered	Endangered	Endangered (2008)
Capra nubiana, Nubian Ibex	Endangered	Endangered	Vulnerable (2008)
Ammotragus lervia, Barbary sheep	Vulnerable	Vulnerable	Vulnerable (2008)
Oryx leucoryx, Arabian oryx	Endangered	Endangered	Vulnerable (2011)

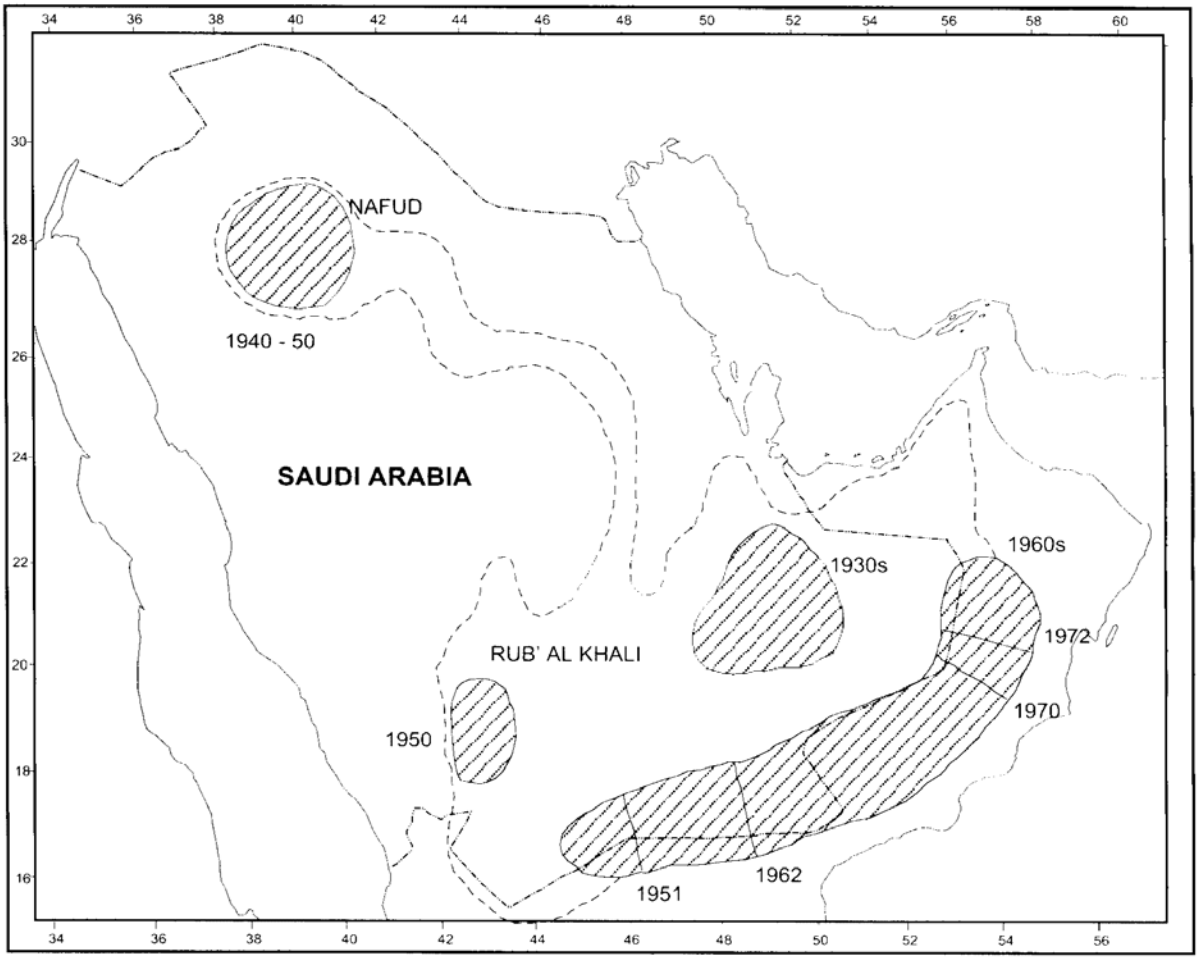


Figure 6 - Historical distribution of the Arabian oryx across Saudi Arabia indicating the split distribution between North and South. Dates next to regions indicate approximate dates of extirpation of the oryx populations. Dotted lines delimit the Arabian sand seas. *Reproduced from Stanley Price (1989) and Seddon and Ismail (2002) with permissions (see appendix 4).*

1.2 Adaptations and Specializations of the Arabian oryx

The Arabian oryx is one of very few large mammals that inhabit a truly extreme terrestrial environment. The conditions of such an environment are extremely hot and arid for most of the year and often surpass the typical mammalian thermo-neutral zone for prolonged periods. Arid-adapted species are thus thought to reduce their energy demands, conserve bodily water content and produce less heat to survive (MacMillen, 1983; Bennett, 1988; Williams et al., 1997, 2001). These demands are met through specialized adaptations which, outside of autonomic adaptation (panting, sweating, shivering), include cooler microclimate selection, body orientation and restriction of activity to cooler periods to maintain body temperature (Tb) within natural limits (Berry et al., 1984; Cain et al., 2006, 2008). For the Arabian oryx to cope and successfully attain food and water as well as breed and manage offspring, they employ a suite of behavioural and morpho-physiological responses to high temperature and other climatic challenges.

At a behavioural level the oryx have been reported to increase the time spent in the shade during the hot season, where they to dig shallow depressions to rest in at the expense of the time spent foraging (Stanley Price, 1989; Seddon and Ismail, 2002). These depressions expose a cooler microclimate and are suggested to aid in the dissipation of body heat during the hottest parts of the day (Jungius, 1978; Ostrowski et al., 2003). Cooler microclimate selection behaviour has previously been reported to occur year-round and additionally appears to become more pronounced and with enhanced efficiency during the hotter months of the year (Hetem et al., 2012a). They track fresh plant growth following rains and seek refuge under *Maerua crassifolia* trees in the wadis (ephemeral valleys) during the hot summer (Ostrowski et al., 2002). On a morphological level, the white pelt of the Arabian oryx, similar to the white springbok (*Antidorcas marsupialis)* (Hetem et al., 2009), may provide additional thermoregulatory protection from solar radiation by reflecting ultraviolet rays, an association established through extensive study (Finch, 1972; Millien et al., 2006; Hetem et al., 2009), However, no such examination has been conducted for the Arabian oryx.

There is also a suite of more specific behavioural and morpho-physiological features that appears common to all oryx species residing in arid environments (Taylor, 1968a, b, 1969, 1970a, b; Taylor and Lyman, 1972; King et al., 1975; King, 1979; Stanley Price, 1985). Some of these features include reduced fasting metabolic rates, lower total evaporative water loss (TEWL) rates and lowered water influx/turnover rates (Williams et al., 2001) compared to other artiodactyls of a similar size. Water availability in desert ecosystems is scarce throughout most of the year and besides for small ephemeral pools after brief rainfall, no free-standing water is available for drinking. Instead oryx rely mostly on their diet, namely grasses, for the preformed water intake in addition to satisfy their energy requirements (Williams et al., 2001). The Arabian oryx exhibits a remarkably low TEWL rate of 870.0 ml day^{-1} (Williams et al., 2001), which appears lower than the camel (*Camelus dromedarius*). TEWL was not directly measured in the camel; however, and data values were approximated from reports of water balance (Schmidt-Nielsen et al., 1956). The oryx exhibit vastly varying field metabolic rates (FMR) between two periods of the year; one in which grasses are low on water content and Ta is high (June – September) and the other occurring when grasses are green and possess high water content (March – April). The difference in FMR reported between the two periods by Williams et al. (2001) represents the greatest disparity for any eutherian mammals to date. During the dry period, the FMR was 11076 KJ day^{-1} for Arabian oryx weighing 81.5 kg and increased substantially to 22081 KJ day^{-1} for oryx weighing 89.0 kg after the rains. These reports on TEWL and FMR suggest that the Arabian oryx are able to markedly reduce their energy

expenditure through variations in behaviour and physiology during the summer season in which Ta is very high and rainfall is non-existent (Williams et al., 2001).

Of the numerous adaptive abilities of the Arabian oryx, one of the most remarkable is the ability to utilize shifts in Tb for bodily water retention, referred to as adaptive heterothermy. Adaptive heterothermy allows Tb to rise throughout the day and the excess heat to dissipate throughout the cooler night thereby avoiding the need to utilize evaporative cooling mechanisms (i.e. sweating) (Ostrowski et al., 2003; Hetem et al., 2010; Bertelsen et al., 2017; Streicher et al., 2017). This feature has been observed for several desert dwelling species (Schmidt-Nielsen et al., 1956; Taylor, 1969, 1970a; Ostrowski et al., 2003). In the Arabian oryx, adaptive heterothermy putatively relates to limitations in water availability rather than the high temperatures of the desert (Hetem et al., 2010). Endothermic species normally employ homeothermy to maintain their core body temperature (Tc) within narrow limits, such as ± 2 ˚C, even under wide environmental temperature ranges (Ostrowski et al., 2003).

Adaptive heterothermy is common to all oryx species residing in arid conditions and has previously been reported for the fringe-eared oryx (*Oryx beisa callotis*) (Taylor, 1969, 1970a) as well as for several other artiodactyl species including the dromedary camel (*Camelus dromedarius*), Grant's gazelle (*Gazella granti*) and Thomson's gazelle (*Gazella thomsonii*) (Schmidt-Nielsen et al., 1956) (see Hetem et al., 2016 for body temperature review in unrestrained artiodactyls). In the aforementioned studies, animals in captivity were used and were examined under controlled conditions. There exists a gap in the understanding of this adaptation in free-roaming animals under natural conditions (Parker and Robbins, 1985).

The study performed by Ostrowski et al. (2003) was the first to document adaptive heterothermy in a free-roaming species, namely the Arabian oryx. They report a mean daily Tb variation of 4.1 ± 1.7˚C during the summer season and a variation of 1.5 ± 0.6 ˚C during winter. In a later study by Hetem et al. (2012b) they report an even greater mean daily Tb variation of 5.0 ± 0.5 ˚C during the hot month of June and 2.9 ± 0.2 ˚C during the cooler wetter months (April - May). They further report a 7.7 ˚C maximum daily amplitude of Tb in two of the oryx included in their study during the hot-dry period (June) (Fig. 7). Hetem et al. (2010) attribute the employment of adaptive heterothermy by Arabian oryx in relation to water limitations and not high Ta.

In a later article, Hetem et al. (2012c) compared Tb variations between the Arabian oryx and the smaller (±15 kg) Arabian sand gazelle (*Gazella subgutturosa marica*), both inhabitants of the Saudi Arabian desert. They demonstrated that both species respond similarly to variations in environmental conditions and do exhibit adaptive heterothermy. When compared with the oryx,

the sand gazelle exhibited higher mean daily Tb and higher minimum and maximum Tb. The higher thermoregulatory set-point of Tb seen in the smaller sand gazelle is potentially related to body mass. The larger Arabian oryx appeared to be more sensitive to external environmental temperature. Hetem et al. (2012c) thus showed contrast to a previous proposition (Taylor, 1970a; Mitchell et al., 2002) that species with a greater thermal inertia like that of the Arabian oryx are more likely to employ heterothermy or enhanced heterothermy than a species with a lesser thermal inertia like the sand gazelle.

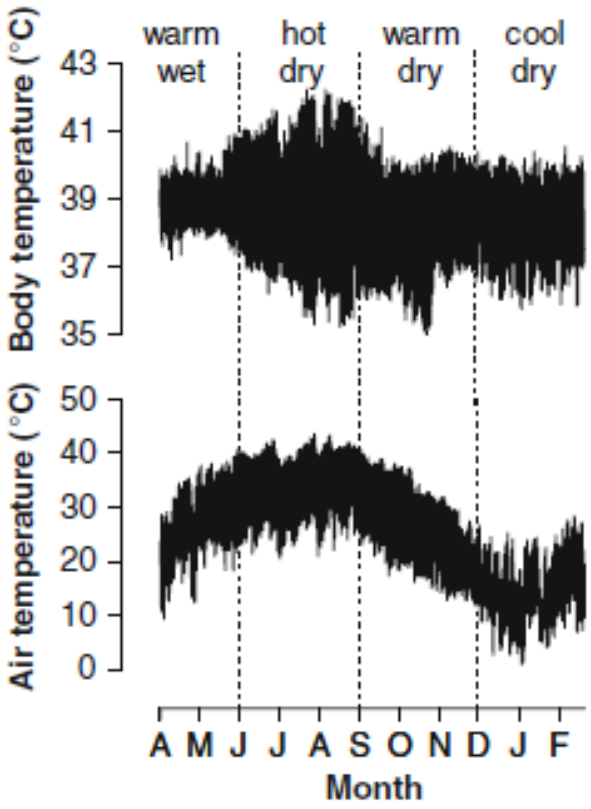


Figure 7 - Time courses of body and air temperatures. Upper panels show 15-min recordings of body temperature from a single free-living male oryx over the 11-month study period (April 2006 to February 2007). Lower panel shows air temperature recorded at nearby weather stations, over the same period. The dotted lines indicate the four seasonal periods analysed, namely warm-wet, hot-dry, warm-dry and cool-dry. Note the large Tb variations during the hot dry season. *Reproduced from Hetem et al. (2010) with permissions (see appendix 4).*

While Tb may vary significantly in the oryx the brain temperature needs to remain relatively stable to perform optimally. It has been reported that the Arabian oryx has enhanced capacities to selectively cool the brain, especially during the hotter months of the year (Hetem et al., 2012b). The selective brain cooling allows for conservation of body water by reducing thermal drive for evaporative cooling. The evolution of the carotid rete and subsequent use of selective brain cooling has been widely exemplified as a potential contributor to the successful adaptation of ungulates and specifically arid-environment dwelling Artiodactyls (Fuller et al., 2008;

Mitchell and Lust, 2008; Hetem et al., 2012b). The carotid rete acts as a network of warm arterioles and cool venous sinuses interacting to allow for brain temperature to be below arterial blood temperature and thus generating selective brain cooling (Fig. 8) (Baker, 1982; Mitchell et al., 1987; Hetem et al., 2012b). Compared with another species of oryx, the gemsbok (*Oryx gazella gazella)* (Maloney et al., 2002), the Arabian oryx exhibited a lower threshold for selective brain cooling with a higher frequency and higher maximum magnitude overall (Hetem et al., 2012b). The gemsbok also inhabits a hot and dry environment (Southern Africa), albeit with substantially higher yearly rainfall and lower Ta extremes. Therefore, selective brain cooling, as noted in both Arabian oryx and gemsbok is deemed necessary for survival.

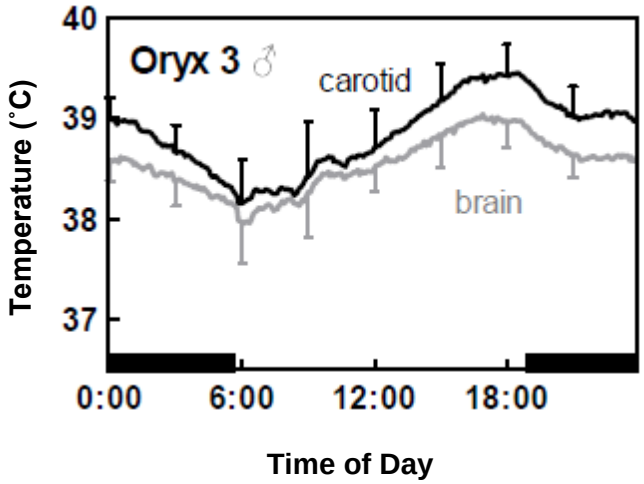


Figure 8 - Average 24-hour rhythm of brain (grey line) and carotid blood (black line) temperatures for a male Arabian oryx in April. Horizontal bars indicate night-time. *Modified and reproduced from Hetem et al. (2012b) with permissions (see appendix 4).*

1.3 Varied Behavioural Patterns in the Arabian oryx

As previously mentioned Arabian oryx exhibit numerous specialized adaptations for controlling their metabolism and body water, Tb, Tc and brain temperature in relation to high environmental temperature, drought and periods of low biomass availability. In conjunction with these remarkable capabilities, Arabian oryx demonstrate interesting behaviours to reduce their energy expenditure which include the temporal shift of activity patterns and the variation in amount of total activity. Arabian oryx were first suggested to show altered daily activity patterns by Stanley Price (1989) followed by Seddon and Ismail (2002), Ostrowski et al. (2003) and later by Hetem et al. (2012a). During the study of Arabian oryx in Oman, Stanley Price (1989) described how during high maximum Ta, oryx would respond through reduced activity and shade-seeking. He proposed that the higher the Ta, the longer time spent in shade, and further suggested that the relationship may be positively linear above a threshold of 27 ˚C (Stanley Price, 1989). Lastly, it was suggested that Arabian oryx compensate for the reduced daytime feeding during the hot periods by engaging in nocturnal foraging (Stanley Price, 1989; Seddon and Ismail, 2002).

In 2002, Seddon and Ismail indicated, using visual observations, that Arabian oryx residing within the Mahazat as-Sayd Protected Area were less active on hot days due to an increased amount of time shade seeking or sitting under the shade. They show that an inverse relationship exists between temperatures and feeding, and that oryx were spending more time in the shade, during the day, at the expense of time spent feeding. A significant decrease in the amount of time spent feeding and in all other types of activity (feeding, walking, grazing or browsing, courtship, mating, fighting etc.) was noted on warm days compared to cool days. They describe a marked diurnal pattern for feeding with morning and late afternoon peaks (crepuscular) when Ta was cooler. This pattern was more apparent during the warmer periods of the year (Fig. 9). The relationship between Ta and shading behaviour shown by Seddon and Ismail (2002) was similar to the relationship described by Stanley Price (1989). At this point it was apparent that Arabian oryx exhibit some form of altered activity patterns in relation to extreme Ta; however no prior study has examined the behaviour of the oryx during the nighttime or using continuous recording techniques in a quantitative manner. Additionally, no study performed to date had examined oryx continuously for long periods at a time including across several seasons.

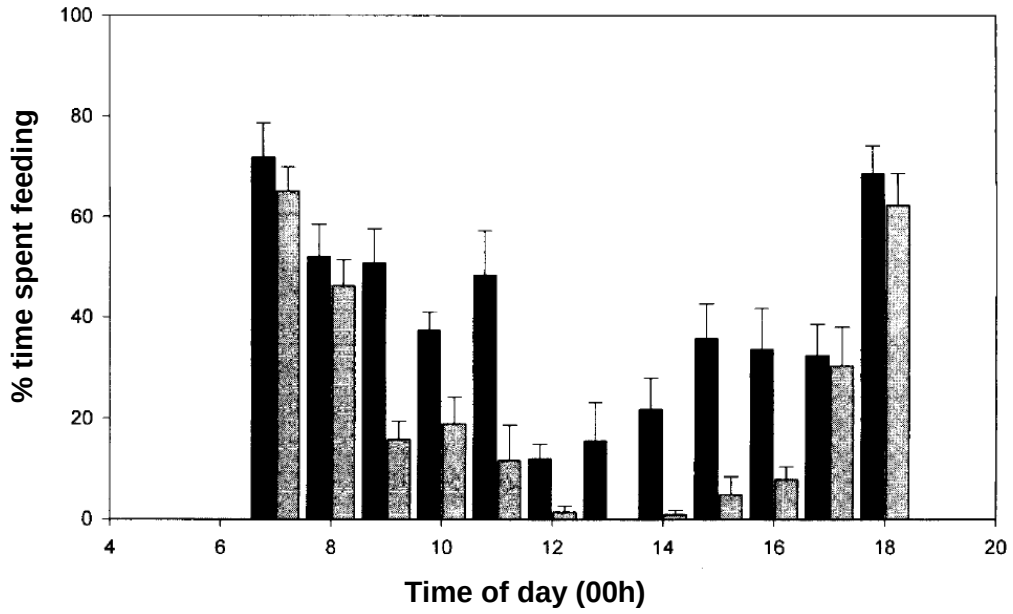


Figure 9 - Percentage of time spent feeding by Arabian oryx in central Saudi Arabia. Means + 1 SE are shown for males and females combined on 'cool' days (dark bars; n=11) and 'warm' days (pale bars, n=12). *Reproduced from Seddon and Ismail (2002) with permissions (see appendix 4).*

A study by Ostrowski et al. (2003) examining heterothermy on free-living Arabian oryx in the Mahazat as-Sayd included in their study observations of activity patterns during daytime and nighttime in order to document shading behaviour. They utilised visual observations during the daytime and pulse intervals from radio-collar signals to classify behaviour during the night

(night time observations were likely limited and not continuous over the recording period). They documented Tb data from May to September over four years; however, they did not specify the length of activity recordings. Ostrowski et al. (2003) reported that oryx during summer were most active between 04h00 and 08h00 and more inactive and under shade when Tb was increasing (during the remainder of the day). During winter, they describe no relationship between activity and Tb (Fig. 10).

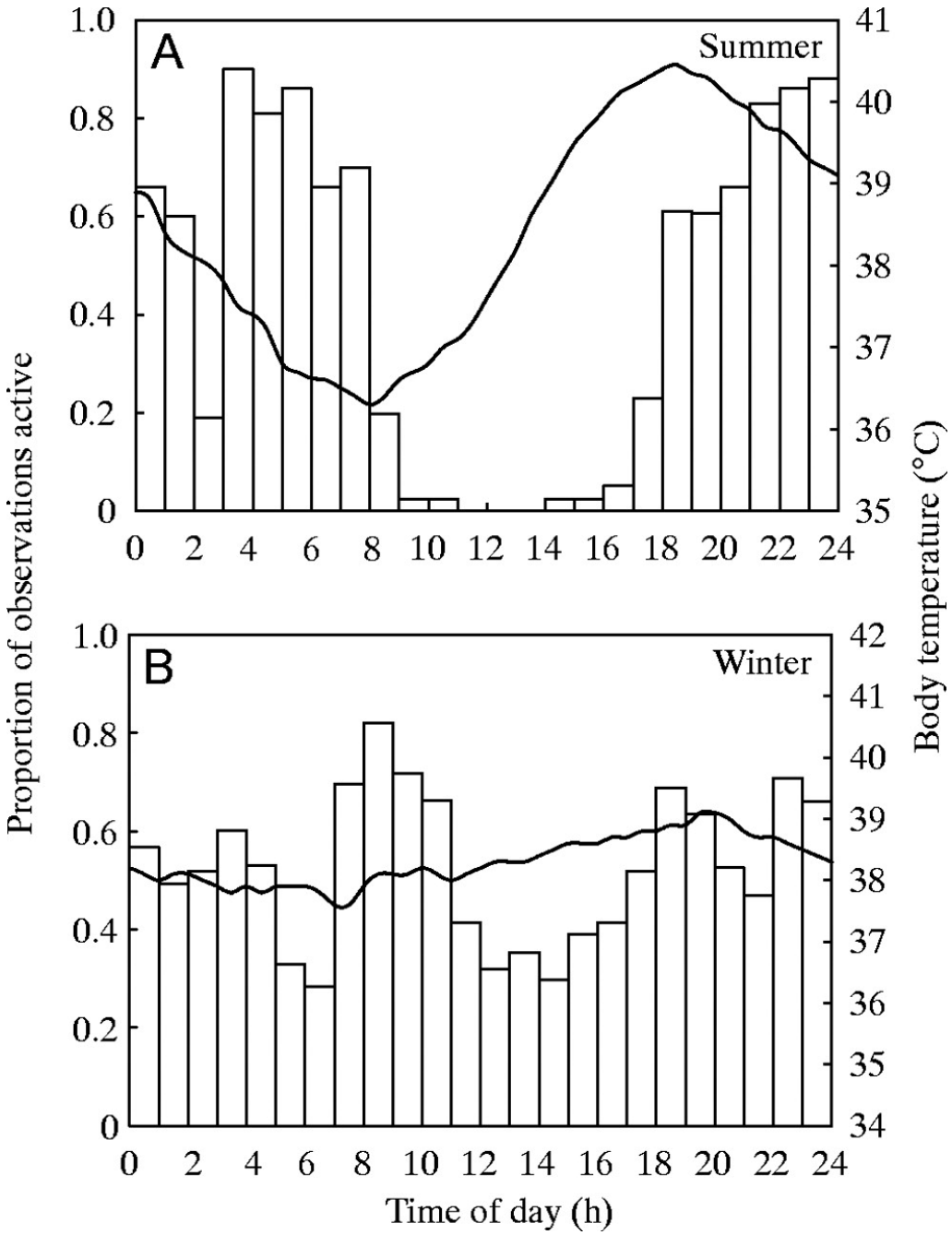


Figure 10 - Proportion of time spent active (bars) and mean body temperature (line) for Arabian oryx during summer (N=14 oryx) and winter (N=17 oryx) in Mahazat as-Sayd. *Reproduced from Ostrowski et al. (2003) with permissions (see appendix 4).*

Nine years later in 2012, Hetem et al. (2012a) showed that Arabian oryx exhibited seasonal flexibility in activity patterns. Through the use of biologging, they measured continuous locomotor activity over 8 months in free-roaming oryx and showed that during the cooler months (April – May and September – November), oryx exhibit a continuous 24 h activity pattern with crepuscular peaks shifting to a predominantly nocturnal activity pattern during the hotter months (June – August) (Fig. 11). A second paper by Hetem et al. (2012c) differently specifying the months of the year, showed similar activity patterns to those previously noted. Oryx exhibited biphasic or crepuscular activity rhythms during the warm wet period (April – May) and nocturnal patterns during the hot dry period (June – July) (Fig. 12). They additionally compared Arabian oryx with the smaller sand gazelle showing that both ungulates employ heterothermy, cathemerality (shifting the timing of activity depending on environmental conditions; Tattersall, 2006) and shade seeking during the hot seasons (Hetem et al., 2012c). During the shift into the hotter months, oryx did not however, show a significant reduction in the total 24 h activity levels. In unison with the measured activity patterns, Hetem et al. (2012a) also measured black globe temperature via collars worn by the oryx. They found that the decreased diurnal activity patterns during the hot months complemented the selection of cooler microclimates (via shade seeking, as discussed previously). The oryx were therefore reducing their exposure to daily solar radiation and partially offsetting increases in body temperature and possibly reducing the need for evaporative cooling (Seddon and Ismail, 2002; Ostrowski et al., 2003; Hetem et al., 2012a, c).

As climate changes and water limitations increase drastically in the coming years, xerocoles will face comprise and tradeoffs between thermoregulation, osmoregulation and the attainment of energy. Autonomic thermoregulation itself is an energetically costly process and it's likely that behavioural thermoregulation and adaptive activity patterns, like that observed for Arabian oryx, will be more prevalent in times ahead (Bustamante et al., 2002; Hetem, 2010). Behavioural flexibility exhibited seasonally by the Arabian oryx may act as a buffer to the adverse effects of the extreme desert climate and may further aid with the more extreme conditions predicted to occur with climate change (Hetem et al., 2012a).

The work of Hetem et al. (2012a, b, c) embodies the most recent literature on the Arabian oryx and represents the first remote and continuous measurement of brain and body temperature, activity and microclimate selection in free-roaming Arabian oryx in Saudi Arabia. Through the progression of technology and methodology, like that of actigraphy, biologging and long-term continuous recordings, our understanding of the behaviour of wild species will be advanced.

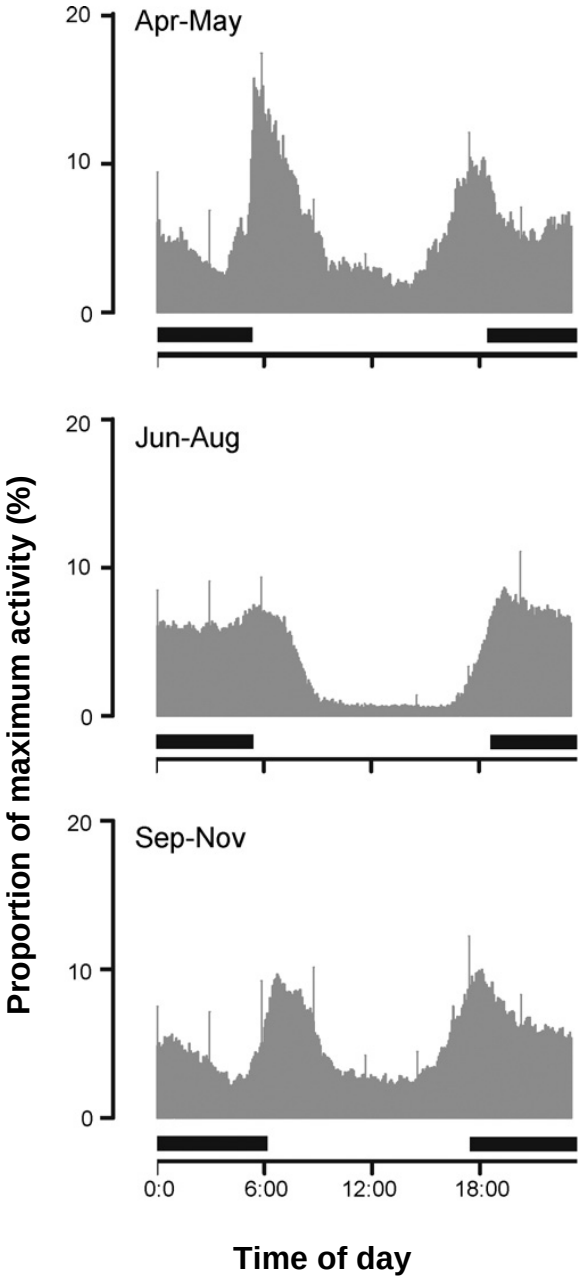


Figure 11 - Mean 24-h locomotor activity of five free-living oryx. Oryx exhibit a shift from a continuous activity pattern with crepuscular peaks during April – May and September – November to a nocturnal pattern during June – August. Activity was biologged over 5-min epochs, and is shown as the percentage of the maximum reading for each logger. Horizontal bars indicate night. *Reproduced from Hetem et al. (2012a) with permissions (see appendix 4).*

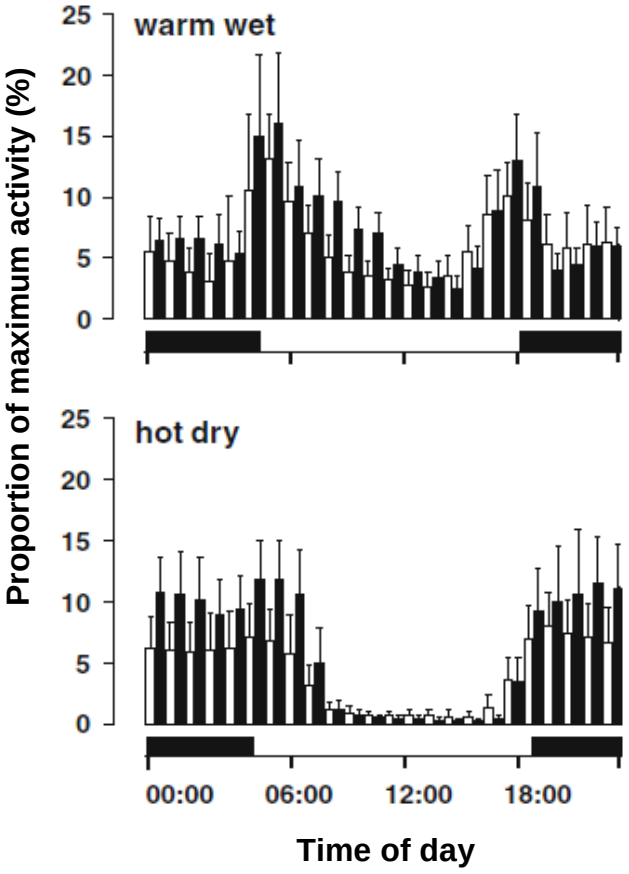


Figure 12 - Nychthemeral rhythm of activity for six sand gazelle (black bars) and five oryx (white bars) over both the warm wet (upper graph) and hot dry (lower graph) periods. Both species shifted from a continuous 24-h activity with crepuscular peaks during the warm wet period to nocturnal activity during the hot dry period. Activity was measured over the abdomen with a subcutaneous activity data logger, in activity counts expressed as a percentage of maximum counts for that animal. Black bars represent night periods. *Reproduced from Hetem et al. (2012c) with permissions (see appendix 4).*

Considering the vast number of species present within the Cetartiodactyl superorder and the remarkable diversity in body size, morphology, phenotype and lifestyles, very little research has been accumulated for the sleep phenomenology, sleep-related neuroanatomy and related circadian rhythms in general. An important step to understanding the evolution and function of sleep and its states is to examine the variation in sleep phenomenology, and the neuronal systems

regulating it, comparatively across species with various physiological phenotypes and ecologies. To date, approximately 1% of all mammalian species have been examined in terms of their sleep and thus a large void exists within the literature and our understanding. The majority of sleep-based research is conducted in the lab, in which conditions do not truly reflect natural ecology and may lead to erroneous conclusions. Very little is known regarding "wild" or natural sleep and this is especially prevalent with species sleeping under extreme conditions like that of the Arabian Desert. The ability to sleep under such hostile and harsh conditions is not well understood, and any variations to typical mammalian sleep in these settings are unknown. The unique features, specialized adaptations, and extreme habitat of the Arabian oryx sets it aside from other Cetartiodactyls in terms of survival success and research opportunities. Understanding the relationship between the extreme seasonal climatic characteristics of the Arabian Desert and possible influence on the sleep-related behaviour, brain architecture and physiology of this species may provide key information for the evolution and functions of sleep.

The work conducted, to date, on the Arabian oryx and its specialized adaptions to such extreme conditions have led to the conceptualization, design and conduction of the work within this thesis. The following review covers the relevant literature related to sleep and circadian rhythms prior to the publications arising from the current thesis. As such, no reference to the published works will be made wherever possible.

2. The Anatomy and Physiology of Sleep

2.1 What is Sleep?

Sleep can broadly be defined "… as a rapidly reversible state of immobility and greatly reduced sensory responsiveness" (Siegel, 2008). It can also be identified by "… sustained quiescence in a species-specific posture accompanied by reduced responsiveness to external stimuli" (Zepelin et al., 2005). Additionally, sleep exhibits physiological responses such as a reduction in metabolism and Tb (Flanigan, 1972; Siegel, 2005; Downs et al., 2015) in relation to homeostatic regulation (Tobler, 1995). Sleep appears to be ubiquitous throughout the animal kingdom (Cirelli and Tononi, 2008; Lesku et al., 2009a) and is a largely exclusive state that prohibits animals from conducting other behaviours or tasks (Roth et al., 2010). Sleep can be considered a behaviour, with some vital function, that often interferes or interrupts some other behaviour (Lima et al., 2005; Siegel, 2008; Roth et al., 2010).

The functions of sleep are currently an active topic of debate and have been for decades (Zepelin and Rechtschaffen, 1974; Rechtschaffen, 1998; Siegel, 2005; Stickgold and Walker, 2005; Lima and Rattenborg, 2007; Rattenborg et al., 2007, 2008; Diekelmann and Born, 2010). The potential functions of sleep have been primarily based on two variables, sleep duration (Table 2) and timing of sleep over a 24 h period (Campbell and Tobler, 1984). To date numerous studies have examined sleep duration and its possible relationship to certain variables, such as life expectancy, metabolic rates, brain mass, and body size (Table 3) (Zepelin and Rechtschaffen, 1974; Meddis, 1977; Campbell and Tobler, 1984; Elgar et al., 1988, 1990; Berger, 1990; Zepelin et al., 2005; Siegel, 2004a, 2008). Similarly, studies have examined sleep timing and environmental factors such as habitat, predation risk, light, food availability, and temperature (Van Twyver and Allison, 1970; Webb, 1974a, b; Allison and Cicchetti, 1976; Meddis, 1983; Campbell and Tobler, 1984; Siegel, 2005; Lesku et al., 2006, 2009a; Acerbi and Nunn, 2011; Voirin et al., 2014; Downs et al., 2015).

Table 2 - Daily sleep durations in a sample of Mammalian species. *Reproduced from Zepelin et al. (2005) with permissions (see appendix 4).*

Species	Total Daily Sleep Time* (hr)	Daily REM Time (hr)
Horse	3.0	0.5
Elephant	4.0	?
Giraffe	4.5	0.5
Seal	6.0	1.5
Rabbit	8.0	1.0
Humans	8.0	2.0
Echidna	8.5	?
Mole	8.5	2.0
Baboon	9.5	1.0
Guinea Pig	9.5	1.0
Dolphin	10.0	?
Cat	12.5	3.0
Rat	13.0	2.5
Hamster	14.0	3.0
Platypus	14.0	7.0
Koala	14.5	?
Ferret	14.5	6.0
Armadillo	17.0	3.0
Opossum	18.0	5.0
Bat	19.0	3.0

*Total daily sleep time includes daily REM time. ?, reported absence of REM sleep or uncertainty.

Table 3 - Correlations between sleep parameters and constitutional variables. *Reproduced from Zepelin et al. (2005) with permissions (see appendix 4).*

Constitutional Variables	Total Daily Sleep Time (hr)	Quiet Sleep Time (hr)	Paradoxical Sleep Time (hr)	Paradoxical Sleep (%)	Cycle Length
Body weight	-.53* (85)	-.53* (65)	-.45* (65)	-.12 (65)	.83* (33)
Brain weight	-.55* (71)	-.48* (56)	-.52* (54)	-.25 (56)	.89* (32)
Metabolic rate	33† (65)	.30‡ (51)	.13 (50)	-.09 (50)	-.82* (29)
Encephalization quotient	-.17 (69)	-.10 (55)	-.20† (53)	-.30‡ (55)	.52† (32)

Common logarithmic transformations were used for the constitutional variables. Log (1 + X) transformations were used for the paradoxical sleep values. Number of cases per coefficient is in parentheses. $^{*}P < .001$, $^{\dagger}P < .01$, $^{\ddagger}P < .05$.

Sleep is considered a homeostatically regulated physiological response and this property has been incorporated into the well-studied "two process model" of sleep regulation (Fig. 13) (Borbély, 1982; Daan et al., 1984; Schwartz and Kilduff, 2015). This model accounts for the timing of sleep in mammals and can be used to describe anatomical substrates and subsequent physiological patterns. "Process S" is related to homeostatic regulation of sleep, and the accumulation of hypnogenic chemicals in the brain generating a sleep drive, and "Process C" relates to the circadian regulation of sleep. In the two-process model, process S integrates input from process C in order to control or gate the occurrence of sleep and wakefulness throughout a 24 h period. Process S includes the neurochemical aspects of sleep, involving neuroanatomical correlates, neurotransmitters and specific physiological patterns. At the onset of waking, process S begins to escalate towards a threshold value for which sleep will then result occurring only if process C is within the appropriate circadian phase (described later) (Schwartz and Kilduff, 2015).

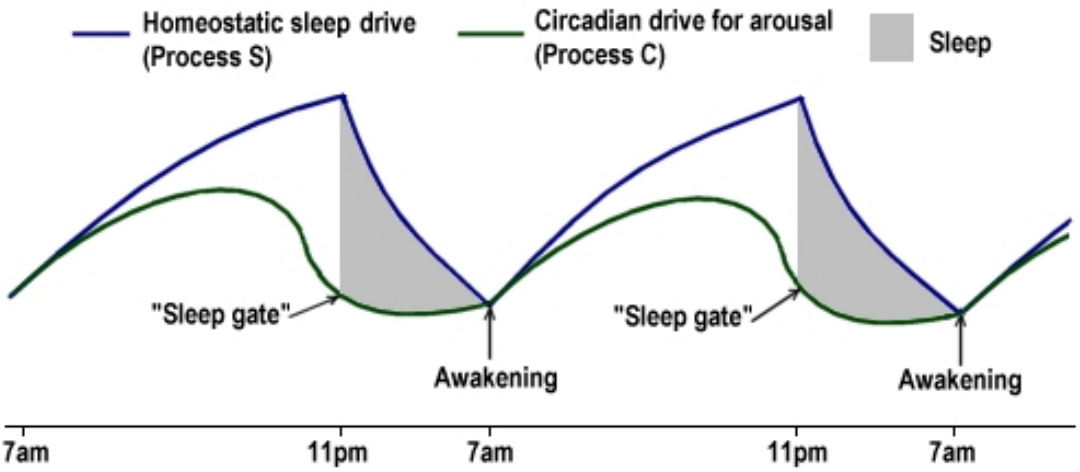


Figure 13 - Sleep-wake regulation: interaction between the homeostatic sleep drive (Process S) and the circadian drive for arousal (Process C) in humans. *(Image by Luke Mastin), copied from https://www.howsleepworks.com/how_twoprocess.html. Accessed on 21/05/2017.*

Sleep appears to play some vital and necessary role in mammals which generally spend a quarter to a third of their life sleeping (Van Twyver and Allison, 1970; Zepelin et al., 2005; Downs et al., 2015). The amount of sleep required and exhibited varies largely between individuals, species and across orders (Zepelin and Rechtschaffen, 1974; Meddis, 1977; Campbell and Tobler, 1984; Elgar et al., 1988, 1990; Ferrara and DeGennaro, 2001; Lesku et al., 2006; Steinmeyer et al., 2010). Any form of sleep deprivation, or excessive loss of sleep, deleteriously impacts on many aspects of an animal, such as: cognitive responses (Bonnet and Arand, 2003; Steinmeyer et al., 2010); vigilance (Bonnet and Arand, 2003); body temperature (Kräuchi et al., 2006); body mass (Knutson et al., 2007); immune function (Majde and Krueger, 2005; Imeri and Opp, 2009); adult neurogenesis (Guzman-Marin et al., 2005; Mueller et al., 2008); neural physiology (Rogers et al., 2001; McDermott et al., 2003); and can even indirectly result in death (Rechtschaffen and Bergmann, 2002).

Considering the clear necessity of sleep, many theories regarding its function have been proposed and are actively debated. Some of these proposed functions include: energy conservation (Zepelin and Rechtschaffen, 1974; Berger, 1975; Berger and Phillips, 1995); adaptive non-responding (Meddis, 1977; Allison and Cicchetti, 1976); memory consolidation (Stickgold and Walker, 2005; Mignot, 2008) immune function (Majde and Krueger, 2005; Imeri and Opp, 2009; Preston et al., 2009); brain metabolism (Benington and Heller, 1995) predation risk reduction (Meddis, 1975; Lima and Rattenborg, 2007; Lesku et al., 2008; Voirin et al., 2014) and neural maintenance (Kavanau, 1996; Krueger and Obal, 2003; Tononi and Cirelli, 2003, 2006; Cirelli et al., 2005). Sleep between avian and mammalian species share some similarities and may hint to the history and functions of sleep (Allison and Van Twyver, 1970; Vyazovskiy and Delogu, 2014; Tisdale et al., 2017). The appearance of the sleep cycle appears to be associated with endothermy, found only in these two orders. Endothermy allows for the occupancy of colder climates and allows for nocturnal activities. As no clear evidence for a defined sleep cycle has been demonstrated for reptiles to date, it appears that birds and mammals are the first to exhibit such behaviours. The evolution of the sleep cycle may thus have developed independently in the two orders or alternatively may originate in common ancestors (mammal-like reptiles) (Zepelin et al., 2005; Rattenborg et al., 2017; Tisdale et al., 2017).

2.2 The Physiology of Sleep

Mammalian sleep can be physiologically defined as two brain states, namely (i) non-rapid eye movement sleep (NREM), which primarily consists of slow wave sleep (SWS), and (ii) rapid eye

movement sleep (REM) (Nicolau et al., 2000; Zepelin et al., 2005; Cirelli and Tononi, 2008; Lesku et al., 2008; Siegel, 2008; Gravett et al., 2012). Human sleep can be further divided into several states: (i) NREM or N1; (ii) deeper stages of NREM, (iii) N2 and N3; and (iv) REM sleep or R (Carskadon and Dement, 2011). The electrical activity of the cerebral cortex is primarily used to discern various sleep states from wakefulness (Loomis et al., 1935; Schwartz and Kilduff, 2015). Sleep is measured physiologically by means of Polysomnography (PSG). PSG is a multi-parametric recording method viewed as the gold standard in the study of sleep; however, it has several limitations with regard to longitudinal and more naturalistic studies (de Souza et al., 2003). PSG is mostly used as a tool for diagnosis in sleep medicine in humans and laboratory-based animal model studies. PSG includes, *inter alia,* electroencephalography (EEG), electrooculography (EOG), electromyography (EMG) and electrocardiography (ECG). These types of recording measure brain activity, eye movements, skeletal muscle activation, and heart rhythms respectively.

Neurons within the cortex of the brain generally exhibit decreasing firing rates during NREM sleep compared to REM sleep and wakefulness (Szymusiak, 2010; Siegel, 2009). The patterns that are observed in EEG activity, measured via PSG, reflect the firing rates of large neuron groupings, which are referred to by bandwidths as per convention (Schwartz and Kilduff, 2015). In mammalian sleep patterns, the following approximate frequency bandwidths are normally described: delta (0-4 Hz); theta (4-8 Hz); alpha (8-12 Hz); beta (>12 Hz) and gamma (>30 Hz) (Schwartz and Kilduff, 2015). Slow wave activity (SWA) (0.5-4 Hz) appears to exhibit variations in its peak frequency across species and rhythmic theta activity is considered a strong indicator of REM sleep (also known as paradoxical sleep) (Zepelin et al., 2005). The PSG data in comparative sleep studies including EEG, EMG and EOG is normally scored in either 5 sec, 30 sec or 1-minute epochs (an instant in time chosen as the origin of a particular period) as: (1) wake, characterized by low-voltage, high-frequency EEG, high-voltage EMG and low voltage high frequency EOG; (2) NREM, characterized by high-voltage, low-frequency EEG and an EMG lower in amplitude than waking; (3) REM, characterized by low-voltage, high-frequency EEG, an almost atonic EMG and high voltage low frequency EOG with distinct bursts correlating with periods of rapid eye movements (Zepelin et al., 2005; Hänninen et al., 2008). Sleep can normally be observed following a predictable sequence of PSG patterns during the night or day depending on the species.

NREM and REM sleep normally alternate within a bout (a period of a specified state of sleep) during the night or day (depending on the species) in a cycle, termed the sleep cycle or sleep rhythm (Ball, 1992; Carskadon and Dement, 2011). The sleep cycle has been defined as the

duration of sleep between the onset of one REM sleep period and the next (Zepelin and Rechtschaffen, 1974; Zepelin et al., 1989; Ball, 1992). The average lengths of the NREM-REM sleep cycles usually increase during the sleep period with intermittent waking throughout. This is the general pattern for humans and most primates, who are considered monophasic sleepers, with a single consolidated or uninterrupted sleep bout (Tobler, 1989; Ball, 1992; Capellini et al., 2008a; Acerbi and Nunn, 2011). For biphasic sleeping species, sleep occurs in two separate, equally long or varying length bouts throughout 24 h and alters with two periods of activity (considered a type of polyphasic sleep) (Tobler, 1989; Ball, 1992). In polyphasic sleeping species, such as carnivores and most Artiodactyls, multiple short and frequent sleep bouts are spread out over 24 h. The timing of these bouts is species-dependent and appears driven by environmental factors (Tobler, 1989; Ball, 1992; Capellini et al., 2008a; Acerbi and Nunn, 2011). It appears that less than 14% of all mammals studied can be considered strictly monophasic, and that overall, most mammals are likely some form of polyphasic sleepers (Campbell and Tobler, 1984; Ball, 1992). Overall, the duration of sleep as well as the phasing of sleep in mammals appears to be constrained by ecological factors (Allison and Cicchetti, 1976; Tobler, 1989; Ball, 1992; Lesku et al., 2006; Capellini et al., 2008a; Voirin et al., 2014).

A distinct interrelationship exists between sleep regulation and thermoregulation. During NREM sleep, a decrease in brain and body temperature (passive heat loss) can be observed across most mammals whilst REM and waking states are associated with an increase in these rhythms (Kawamura and Sawyer, 1965; Hayward and Baker, 1969; Valatx et al., 1973; Parmeggiani et al., 1975; Obál et al., 1985; Franken et al., 1992; Tobler et al., 1993; Deboer et al., 1994; Tobler and Deboer, 2001; Kräuchi and Deboer, 2010). It also appears that body heat exhibits redistribution patterns as the vigilance states shift, for example at NREM sleep onset, brain and core body temperature decrease whereas skin and peripheral temperatures increase (Alföldi et al., 1990; Kräuchi and Deboer, 2010). Numerous studies have examined the physiological changes of thermoregulation induced by sleep; however, an unequal amount of literature exists for the reversed effects of thermoregulation and thermal environment on sleep (Schmidek et al., 1972; Gilbert et al., 2004). The anatomical substrates and correlates of sleep in the brain have been demonstrated to possess thermo-sensitive neurons and as such it is likely that the sleep-wake cycle is directly affected by thermoregulation and thermal shifts in the environment (Alam et al., 1996; Sherin et al., 1996; Gilbert et al., 2004). Such shifts may act either directly or indirectly to affect aspects of sleep like initiation, onset, total sleep time and termination, although it is not known whether this occurrence is causative or coincidental (Parmeggiani and Rabini, 1967; Parmeggiani, 1986; Fletcher et al., 1999; Gilbert et al., 2004). The EEG patterns

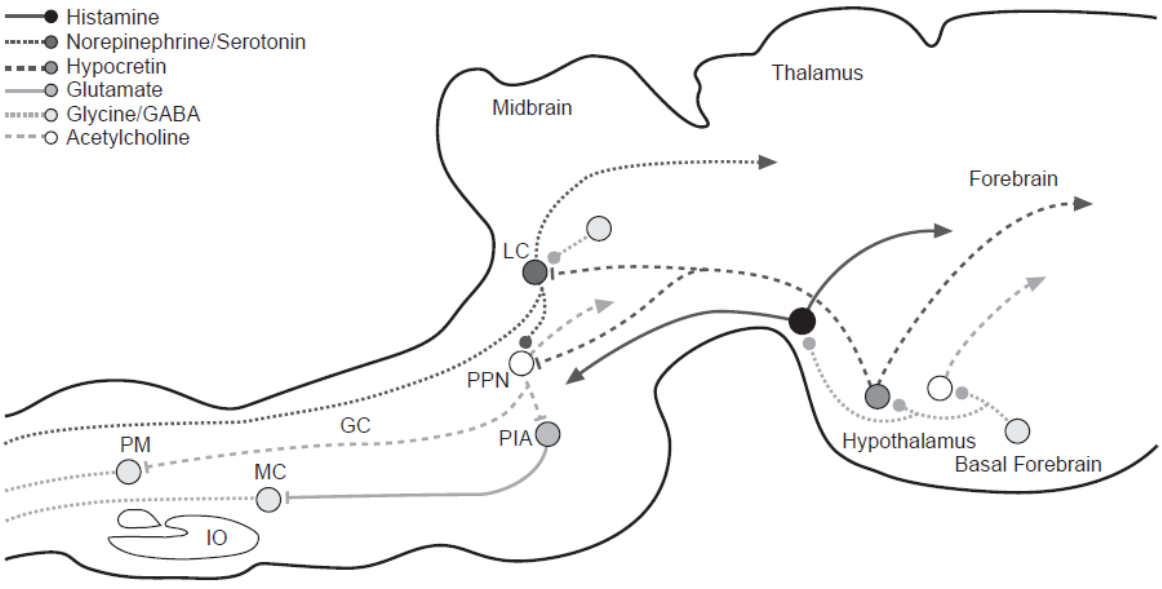
during the vigilance states have been shown to be effected during active cooling or warming of brain temperature. During active cooling of the brain in rats, cats and humans, the amplitude and frequency of EEG wave patterns decreases effecting sleep quality (Koella and Ballin, 1954; Weiss, 1964; Deboer and Tobler, 1995; Schmitt et al., 2002; Kräuchi and Deboer, 2010). Nakao et al. (1995) show that accumulated heat loads augment NREM sleep and that NREM sleep itself evokes heat loss processes which subsequently reduce such loads (Ta < Tb). In the case of Ta > Tb, heat may still be gained passively, yet at a slower rate than during waking. A major portion of the thermo-sensitive sleep-wake system neurons are located within the hypothalamus, the same brain region implemented in the control of Tb.

2.3 The Anatomy of Sleep

There are several essential brain regions associated with sleep in mammals. These regions are responsible for releasing the neurotransmitters that cause neurons to fire in specific ways thereby producing physiological sleep and subsequent EEG patterns. The major brain regions include the hypothalamus, brain stem and the basal forebrain (Siegel, 2004b; Kalia, 2006). These regions are all interconnected and project to one another and other areas of the brain to induce and control various states of sleep and wakefulness (Fig. 14) (Siegel, 2006; Kalia, 2006). The major neurotransmitters involved include: histamine, norepinephrine, dopamine, serotonin hypocretin/orexin, acetylcholine, glutamate, and γ-aminobutyric acid (GABA) (Siegel, 2004b; Kalia, 2006; Schwartz and Kilduff, 2015).

Several regions of the hypothalamus are heavily involved with sleep duration and regulation and this region is arguably the most important regarding these functions (Sigel, 2004b). Within the hypothalamus, areas related to circadian rhythms including the suprachiasmatic nucleus (SCN) and the pineal gland. The lateral hypothalamus is considered the exclusive source for hypocretin/orexin neurotransmitters, primarily involved with the promotion of arousal and the transition from sleep to wakefulness (Koyama et al., 2002; Kalia, 2006). The ventrolateral preoptic (VLPO) nucleus found within the anterior hypothalamus is considered a sleep generating area within the brain and has associations with NREM and REM sleep through GABAergic neuronal interactions (Lu et al., 2000, 2002; Kalia, 2006). Within the posterior hypothalamus (an area of arousing effect) lies the tuberomammillary nucleus (Saper et al., 2001; John et al., 2004; Siegel, 2004b; Kalia, 2006). This nucleus primarily utilizes histamines and projects to many areas within the central nervous system. It receives input from the orexinergic neurons of the lateral hypothalamus and GABAergic neurons of the VLPO which in

combination play a key role in sleep (Nitz and Siegel, 1996; Siegel, 2004b; Kalia, 2006). The brainstem includes a major area, the rostral reticular formation (RAS) and raphe nuclei, which project to the forebrain and regulate the sleep-wake cycle (Jasper, 1949; Steriade, 1981; Siegel, 2004b, Kalia, 2006). This area of the brainstem mostly utilizes serotoninergic neurons and consists of two pathways, the dorsal ascending and the ventral ascending pathways. These pathways project to different regions of the sleep related areas of the brain including the thalamus, caudal midbrain, rostral pons, locus coeruleus, medial septum and diagonal band of Broca (Jasper, 1949; Steriade, 1981; Kalia, 2006).



Abbreviations: GABA = γ-aminobutyric acid, GC = nucleus gigantocellularis, IO = inferior olive, LC = locus ceruleus, MC = nucleus magnocellularis, PIA = pontine inhibitory area, PM = nucleus paramedianus, PPN = pedunculopontine nucleus.

Figure 14 - Synaptic relationships underlying the loss of consciousness and correlated loss of muscle tone in normal sleep. Simplified drawing of a sagittal section of a cat's brain depicting some of the major connections playing a major role in sleep control. Also illustrated is the complexity of the synaptic relationships underlying the loss of consciousness and correlated loss of muscle tone that characterizes normal sleep. Lines ending in solid dots indicate inhibitory output. Lines ending in arrows indicate excitatory output. *Reproduced from Siegel (2004b) with permissions (see appendix 4).*

The neurotransmitters within the sleep related neuronal systems possess global functions. The cholinergic nuclei (acetylcholine) located throughout the pons and basal forebrain are mostly involved in cortical activation and fire rapidly during waking and REM sleep (Harris, 2005; Jones, 2005; Siegel, 2004b, 2006). The serotonergic nuclei of the midbrain and pons are primarily active during low arousal states with reduced activity during NREM sleep and no activity during REM sleep. The catecholaminergic nuclei (dopamine and norepinephrine) are primarily involved in waking, REM sleep and generalized sleep state homeostasis (Smeets and González, 2000; Jones, 2005). The orexinergic/hypocretinergic neurons within the hypothalamus are mainly involved in arousal; however, they also play a role in a variety of other behaviours

like copulation, aggression and appetite and are strongly linked to circadian rhythms (Taheri et al., 2000; Brown et al., 2001; Fujiki et al., 2001; Yoshida et al., 2001; Kukkonen et al., 2002; Matsuzaki et al., 2002; Tao et al., 2006). The GABAergic neurons, found throughout the sleep related brain regions, primarily promote NREM sleep by inhibiting arousal promoting nuclei and display various firing rates throughout the sleep cycle (Siegel, 2004b).

In 2005, Manger proposed that "…the subdivisions of a neural system exhibit the same complement within all species of a particular mammalian order, irrespective of the brain size, phenotype or life history". Manger's (2005) theory includes a vast empirical neuroanatomical database including monotremes, cetaceans, rodents, carnivores and primates and has been subsequently broadened by numerous studies on chiroptera (Kruger et al., 2010a), Afrotherians (Gravett et al., 2011; Calvey et al., 2013; Maseko et al., 2013), lagomorphs and scadentia, (Calvey et al., 2015), and Cetartiodactyla (Bux et al., 2010; Dell et al., 2012, 2016a, b, c; Mahady et al., 2017). In general a large void still exists in the Cetartiodactyl order for the examination of the sleep related neuroanatomy and incomplete descriptions are especially apparent for the Artiodactyls. It is also pivotal to the understanding of the evolution and possible function of sleep that the neuroanatomical systems are studied in parallel with other aspects (i.e. physiology and behaviour) as possible differences within these neuronal systems might provide important clues as to potentially unusual sleep patterns observed within a particular species.

2.4 Comparative Sleep and the Setbacks

An important step to understanding the evolution and function of sleep and its states is to examine the variation in sleep quotas comparatively across species with various physiological phenotypes and ecologies (Tobler, 2005; Zepelin et al., 2005; Rattenborg et al., 2008; Siegel, 2008; Horne, 2009; Lesku et al., 2009b; Rial et al., 2010; Gravett et al., 2012; Rattenborg et al., 2017; Tisdale et al., 2017). This method to understanding sleep and its value has been acknowledged for five decades (e.g. Weiss and Roldán, 1964; van Twyver, 1969; Lesku et al., 2009b) and has led to remarkable advances in the neurobiology of sleep. Comparative sleep research is a fascinating and rich subject (Lesku et al., 2009b); from the early work of Zepelin and Rechtschaffen (1974), who compiled electrophysiological and behavioural data on 53 species, to the first quantitative study to incorporate ecological factors (predation index) as predictors of mammalian sleep quotas (Allison and Cicchetti, 1976). There have been ±130 sleep recordings, to date, in mammalian species, providing a comprehensive database and rich array of varying phenotypes (McNamara et al., 2008; Phillips et al., 2013a). Such a seemingly large

database actually only accounts for approximately one percent of all mammals and a large void still exists in comparative sleep research (Allison and Cicchetti, 1976). One of the major drawbacks to previous comparative research, however, is the obvious lack of studies of an animal under natural conditions (i.e. in the wild). A need for more ecologically realistic studies has been emphasized from the early beginnings of comparative sleep research (Allison et al., 1972; Bert et al., 1975; Lesku et al., 2009b; Rattenborg et al., 2017).

Standard laboratory conditions are not reflective of what an animal may experience naturally in the wild. There are several factors that could attribute to sleep duration discrepancies seen in the laboratory compared to experimentation in a species' natural habitat for example, light intensity, *ad libitum* feeding protocols, social isolation, absence of predators, temperature variations and human disturbances (Capellini et al., 2008b; Rattenborg et al., 2008; Scriba et al., 2013). A study by Rattenborg et al. (2008) observed the sleep physiology and ecology of the brown-throated three-toed sloths (*Bradypus variegatus*) and was the first study to record PSG (EEG and EMG) in a wild species (Figs. 15 and 16). The study demonstrated a 6-hour discrepancy in sleep duration from that previously reported for sloths in captivity (de Moura Filho et al., 1983). The reasoning suggested by Rattenborg et al. (2008) for the reduced sleep periods (15h in captivity versus 9h hours in the wild), was that the need for foraging and the monitoring of predators only allows for an average of nine hours of sleep a day. The discrepancy is particularly large and demands a need for truly reflective sleep recordings from animals in their natural habitat to possibly answer the questions of species-specific essential sleep time durations and the function of sleep.

The general fundamental assumption of comparative studies, as pointed out by Rattenborg et al. (2008), is that "… the time spent sleeping in captivity reflects a largely inflexible species-specific need for sleep". As mentioned, factors such as foraging and predator vigilance appear to increase sleep quotas in captive conditions (Horne, 1988; Rattenborg et al., 2008). In a contrasting viewpoint, animals in a captive environment may consider their surroundings to be dangerous and therefore sleep less than in the wild (Scriba et al., 2013). In light of these findings, and with the advent of better recording techniques making wild-based studies possible, it now becomes of great importance to move sleep research to the animals' natural environment. These studies might provide novel insight to understanding the behaviour of animals in their natural environment as well as the evolution of sleep (Rattenborg et al., 2017).

Four wild based studies conducted thus far, using electrophysiology, include Rattenborg et al. (2008); Lesku et al. (2011); Scriba et al. (2013) and Voirin et al. (2014). A study by Lesku et al. (2011) demonstrated the first electrophysiological sleep recordings for ostrich (*Struthio camelus*)

in both an outdoor enclosure and a large reserve within the South African savannah. Their results suggest that ostrich REM sleep is similar to that of the platypus (*Ornithorhynchus anatinus*) (Siegel et al., 1999) with both species being considered basal animals in terms of the evolution of sleep.

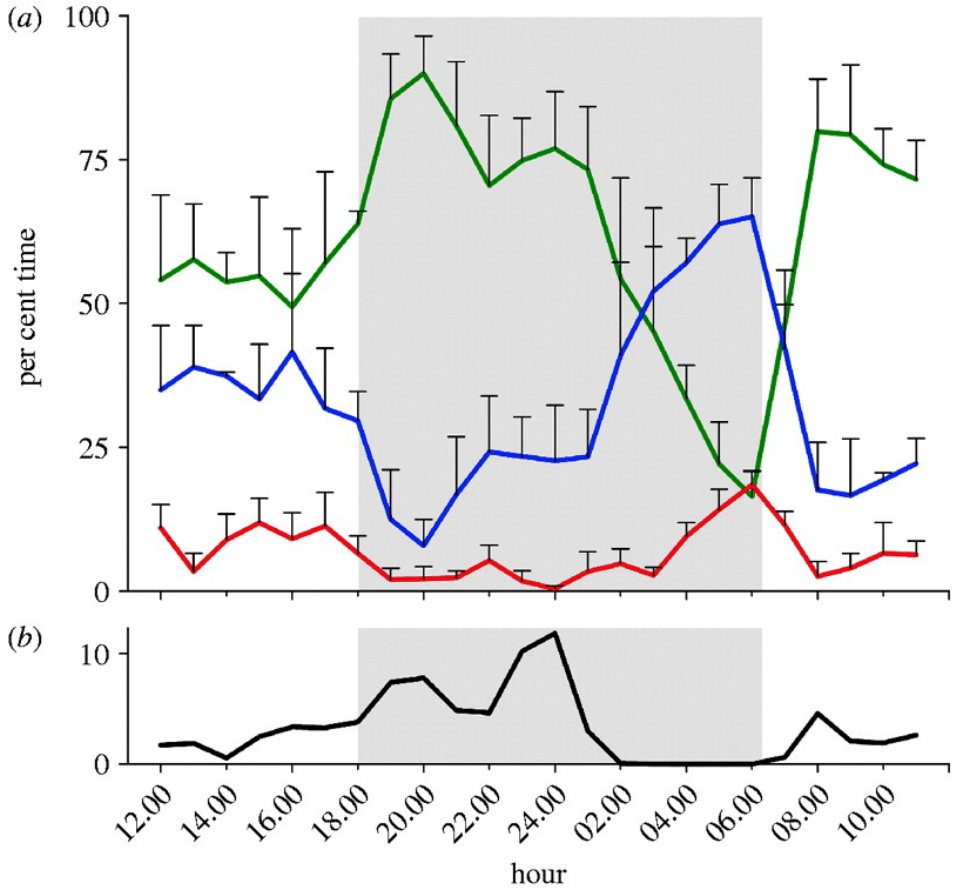


Figure 15 - (a) The percent time spent in wakefulness (green), non-REM sleep (blue) and REM sleep (red) for each hour of the day by wild sloth. (b) The time spent feeding (black) based on the occurrence of mastication artefacts in the EEG/EMG recordings. The area shaded in grey shows the time from sunset (18.00) to sunrise (06.20). *Reproduced from Rattenborg et al. (2008) with permissions (see appendix 4).*

A study by Scriba et al. (2013) demonstrated the successful use of both surface and subcutaneous electrodes for PSG recording in barn owls (*Tyto alba*). In their study, Scriba et al. (2013) examined adult American barn owls (*Tyto alba pratincola*) in captivity (surface electrodes) (Fig. 17) and nestlings from a free-ranging population of barn owls (*Tyto alba*) (subcutaneous electrodes). They demonstrate successful EEG signals from both groups and clearly define the owl's sleep states during the recording periods (Fig. 18). The study demonstrates that various techniques may be used to study animals in the wild depending on the suitability of the technology and regarding the scientific questions being asked. Scriba et al. (2013) also include that these devices are minimally invasive and that both techniques could improve the welfare of the species during the experiment compared to prior techniques.

Voirin et al. (2014) examined the ecology and sleep neurophysiology of two species of wild sloth in Panama; a mainland rainforest group living with predators (*Bradypus variegatus*) and an island mangrove group living without predators (*Bradypus pygmaeus*). They recorded both EEG and EMG and examined the effects of predation risk on sleep timing and duration. Voirin et al. (2014) demonstrate that predation pressure influences the timing of sleep but not the duration in these two sloth species. The nocturnal sleep preferences of the mainland sloth (compared with diurnal sleep preference in the island sloth) may be a strategy to reduce predation risk and avoid detection. In the case of the sloth, sleeping may be safer than being active as they lack anti-predator defenses (Montgomery and Sunquist, 1978). These findings are in agreement with a recent evolutionary strategic model that predicts sleep timing based on predator activity (Acerbi and Nunn, 2011). The model predicts that species that are safer whilst asleep should maintain sleep during their predators active periods and vice versa.

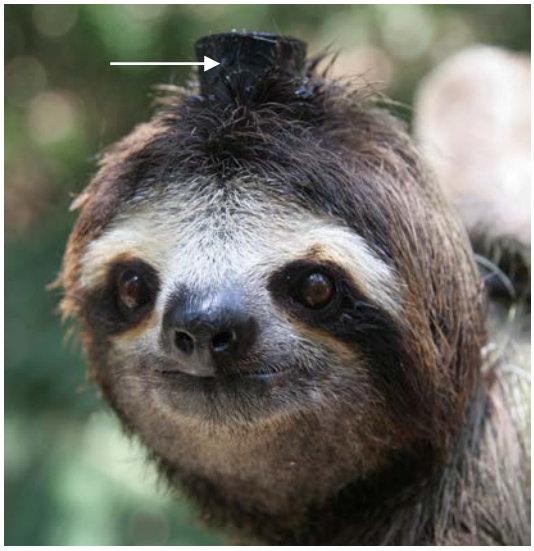


Figure 16 - Brown-throated three-toed sloth equipped with an EEG "hat" (arrow). *Reproduced from Voirin et al. (2014) with permissions (see appendix 4).*



Figure 17 - Adult barn owl with surface electrodes and a data logger attached (arrow). *Reproduced from Scriba et al. (2013) with permissions (see appendix 4).*

A study by Capellini et al. (2008b) examining the influence of laboratory procedures on sleep duration estimates showed that total sleep time observations based on EEG were greater than behavioural observations and thus make inferences, regarding the evolution of sleep, difficult as it could lead to erroneous conclusions. There are both obvious and subtle setbacks to most techniques used to examine sleep and there will most likely always be over- or underestimations of total sleep times. None the less, the advancement and refinement of technology will allow for improvement of data validity and consistency as well as flexibility in study design.

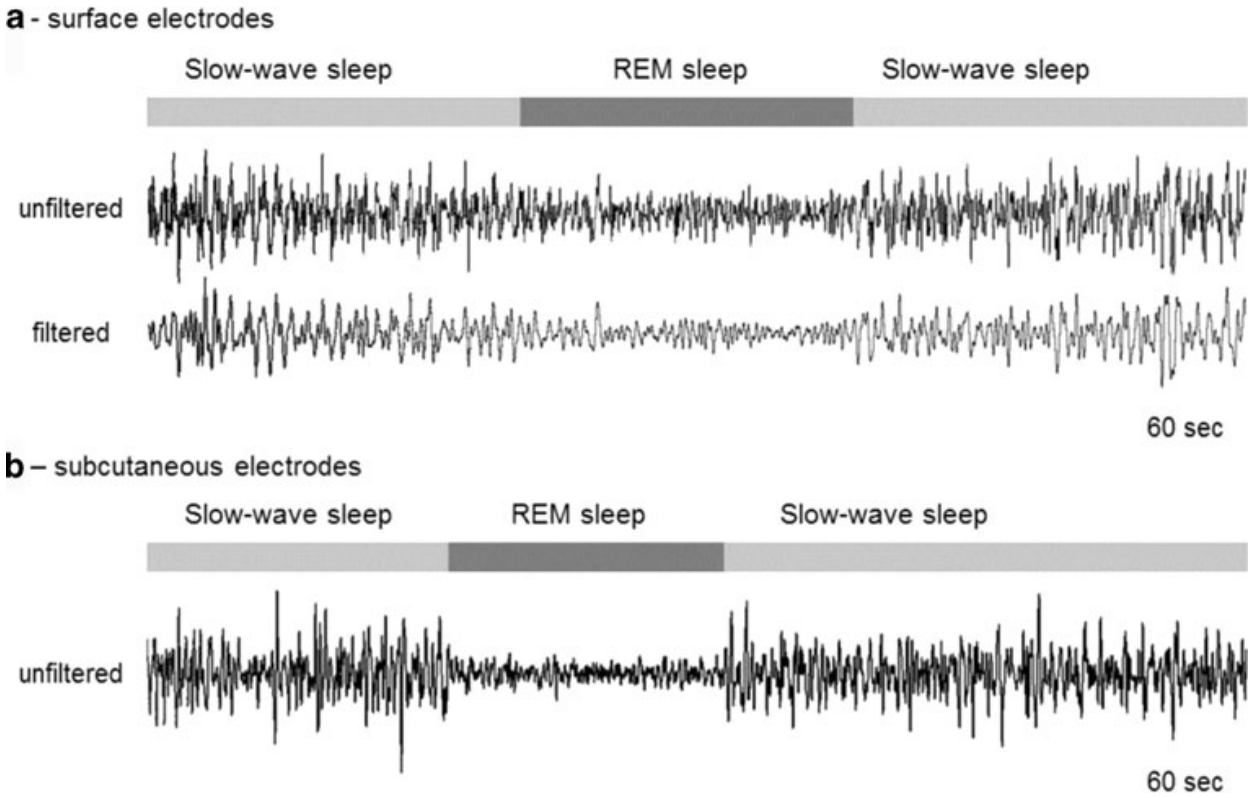


Figure 18 - Signal quality examples of SWS/REM sleep recorded from barn owls using (a) surface electrodes and (b) subcutaneous electrodes. *Reproduced from Scriba et al. (2013) with permissions (see appendix 4).*

2.5 Actigraphy: A Promising Tool for Sleep and Circadian Rhythm Research

Actigraphy (ACT, wrist-worn, activity-based, sleep-wake monitoring) has been shown to be an objective, indirect measurement of sleep and wakefulness that can be used as an alternative to PSG. Validation studies have shown the concordance of ACT and PSG in scoring sleep in humans' ranges from 83 to 90% (de Souza et al., 2003; Shambroom et al., 2011). Several studies have shown that periods of arm immobility correlate very strongly with sleep and can serve as a proxy for EEG or video identification of sleep (Hurelbrink et al., 2005). Additionally, this property has been used in the monitoring of insomnia, circadian sleep/wake disturbances and periodic limb disorders (Sadeh and Acebo, 2002; Ancoli-Israel et al., 2003).

Some advantages of using ACT over PSG are that it is more cost-effective, less intrusive, relatively easy to use in ambulatory settings, utilizes automatic scoring algorithms and most importantly it allows recording in a natural sleep environment while retaining full mobility (Ancoli-Israel et al., 2003; Sadeh, 2011; Rattenborg et al., 2017). Each actiwatch utilizes a piezoaccelerometer device connected to a microchip that sums and records the number of acceleration events for each minute (recordings up to 6 months are possible).

ACT is currently applied to subjects or populations where PSG recordings are difficult or impractical to attain such as with astronauts, oil rig workers, miners, Arctic and Antarctic based personnel and in animals under natural conditions (Monk et al., 1999; Ancoli-Israel et al., 2003, Hetem et al., 2012a, c; Arendt and Middleton, 2017; Rattenborg et al., 2017). ACT can be used not only for monitoring and understanding sleep and sleep disorders but also for circadian rhythms research (Frisbie and Sharma, 1992; Pollak et al., 2001; Ancoli-Israel et al., 2003). A shortcoming of ACT is that it is unable to discern between sleep and rest and particularly with the definition of any specific sleep state, namely NREM or REM sleep (Rattenborg et al., 2017). ACT-based studies usually report active and inactive data using manual or automatic-based algorithms. The inactive data reports with some level of over- or underestimation of total sleep time and requires further input through other means such as PSG, video, mobile heart monitor bands and sleep diaries or journals. The majority of ACT and accelerometry research has been conducted in humans and minimal literature exists for animal-based research (Miller et al., 2008; Mitani et al., 2010; Bäckman et al., 2017; Gravett et al., 2017; Rattenborg et al., 2017). Further validation between ACT and PSG is required to determine if it is a suitable alternative to PSG, especially for the study of sleep in animals in their natural environment (Lesku et al., 2012). As advances in technology improves the accuracy of such devices, both improved reliability and the ability to assess sleep states in a variety of settings, wild or laboratorial, may be possible.

2.6 Comparative Sleep and the Artiodactyls

Currently, there is a large void in the data that accurately describes sleep in Artiodactyls, both from a physiological and neuroanatomical point of view. The neuroanatomy of sleep appears to be highly predictable across species within the same mammalian order (Manger, 2005), whereas the physiology of sleep appears to be purely adaptive and unpredictable. Of the 240 species comprising the highly diverse Artiodactyl order, only a select few of these species have been studied with regard to their sleep phenomenology. For example, sleep has been recorded electrophysiologically in captivity for the okapi (Immelmann and Gebbing, 1962), cow (Ruckebusch et al., 1970; Ruckebusch, 1972; Hänninen et al., 2008), sheep (Ruckebusch, 1961; 1972; Bell and Itabisashi, 1973), pig (Ruckebusch and Morel, 1968; Ruckebusch, 1972; Campbell and Tobler, 1984; Robert and Dallaire, 1986), giraffe (Tobler and Schwierin, 1996), and goat (Klemm, 1966; Bell and Itabisashi, 1973) (See Table 4 for sleep quotas from Artiodactyls previously studied). As such, a large amount of work is required in order to cover sleep in such a large and highly ecologically relevant order.

Table 4 – Reported daily sleep quotas in Artiodactyl species

Species	Total sleep (rest) time/24 h (hr)	Reference
Sheep (*Ovis aries*)	3.8	Ruckebusch, 1972
Cow (*Bos Taurus*)	4.0	Ruckebusch et al., 1970
Pig (*Sus domesticus*)	7.8	Ruckebusch and Morel, 1968
Goat (*Capra aegagrus hircus*)	5.4	Bell and Itabisashi, 1973
Giraffe (*Giraffa Camelopardalis)*	4.6	Tobler and Schwierin, 1996
Okapi (*Okapia johnstoni)*	1.0	Immelmann and Gebbing, 1962

Most of the world's largest herbivorous mammals are Artiodactyls; they are indigenous to almost every continent and have been capable of adapting to extreme environments ranging from harsh desert habitats to subarctic forests. Artiodactyls provide for unique examination as the members of this order show great diversity in their body sizes, morphologies, phenotypes and lifestyles. Studies that have examined ungulate sleep have either been conducted under controlled laboratory conditions or in Zoological Gardens where methods have been purely observational in nature. The data available for total sleep times for the different species examined thus far have furthermore also been obtained using different methodologies, i.e. electrophysiological recordings vs. behavioural observations, different ages, sexes and sample size with no standardization with regards to length of recording (Campbell and Tobler, 1984).

Studies using immunohistochemistry that involve the identification and description of the sleep related nuclei in Artiodactyls have only been conducted in the sub-adult giraffe (*Giraffa Camelopardalis*) (Bux et al., 2010; Dell et al., 2012), sheep (*Ovis aries*) (Ferreira et al., 2001), cow (*Bos primigenius*) (Iqbal et al., 2001), and pig (*Sus scrofa domesticus)* (Ettrup et al., 2010; Mahady et al., 2017). No prior complete description of all systems neuroanatomy implicated in the control of sleep has been provided for any Artiodactyl species. For the purpose of this thesis, only the Artiodactyla sleep-related neuroanatomy will be discussed and not that of the Cetartiodactyla (see Table 5 for further references).

Table 5 - Literature describing neuroanatomical systems across Cetartiodactyla

Species	Systems Described	Reference
Sheep (*Ovis aries*)	Cholinergic, catecholaminergic, serotonergic	Tillet, 1987; Tillet and Thibault, 1989; Iqbal et al., 2001; Ferreira et al., 2001
Cow (*Bos primigenius*)	Catecholaminergic, Orexinergic	Kitahama et al., 1994; Tillet, 1994; Tillet & Kitahama, 1998; Leshin 1995a,b
Goettingen Mini Pig (*Sus scrofa domesticus)*	Cholinergic, GABAergic, Orexin	Ettrup et al., 2010; Mahady et al., 2017
Pig (*Sus scrofa domesticus)*	Catecholaminergic, Orexinergic	Østergaard et al., 1992; Kitahama et al., 1994; Tillet, 1994; Tillet & Kitahama, 1998; Leshin, 1995b, 1996
Giraffe (*Giraffa Camelopardalis*)	Cholinergic, Catecholaminergic, Serotonergic, Orexinergic	Bux et al., 2010; Dell et al., 2012
Harbour Porpoise (*Phocoena phocoena*)	Cholinergic, Noradrenergic, Serotonergic, Orexinergic, GABAergic	Dell et al., 2016a
Minke Whale (*Balaenoptera acutorostrata*)	Cholinergic, Noradrenergic, Serotonergic, Orexinergic, GABAergic	Dell et al., 2016b
Hippopotamus (*Hippopotamus amphibious*)	Cholinergic, Noradrenergic, Serotonergic, Orexinergic, GABAergic	Dell et al., 2016c

The cholinergic systems described in the sheep and giraffe brain follow that of the typical mammalian organization (Ferreira et al., 2001; Maseko et al., 2007; Gravett et al., 2009) however, some features exhibited variations in giraffe (Bux et al., 2010). Within the pontine region of the brain, the laterodorsal tegmental (LDT) cholinergic nuclei display an apparent larger size to that of the cholinergic nuclei of the pedunculopontine tegmental (PPT) region (Fig. 19) (Bux et al., 2010). In the Goettingen mini pig, the reverse was observed, with larger PPT neurons compared to the LDT neurons (Mahady et al., 2017) and thus the neuronal size variations appears to be a novel feature only observed within the Artiodactyl order. The LDT and PPT cholinergic nuclei are selectively active during wake and REM sleep, and are reportedly involved in the suppression of muscle tone during REM sleep (Gravett et al., 2009). The functional implications of the differing neuronal morphology between the LDT and PPT nuclei could possibly relate to functional aspects of the sleep-wake cycle specific to Artiodactyls and their environment but are presently unclear.

Another Artiodactyl specific feature observed in the giraffe brain relates to the orexinergic hypothalamic groups, a feature deemed to be novel. In all mammals previously studied, three clusters are normally found within the hypothalamus: (i) the main cluster, (ii) zona incerta

cluster and (iii) the optic tract cluster (Kruger et al., 2010b). The three hypothalamic clusters typically display a homogenous magnocellular neuronal morphology; however, in the giraffe brain an additional parvocellular cluster can be distinctly observed (Dell et al., 2012). The parvocellular cluster neurons display a small somal size and are found closest to the third ventricle while the larger magnocellular neurons of the main cluster are more laterally located (Fig. 20) (Dell et al., 2012). Whilst not specifically noted, the parvocellular cluster also appears to be present in the sheep (Iqbal et al., 2001) and Goettingen mini pig (Ettrup et al., 2010) suggesting that its occurrence may be found in all Artiodactyls. The orexinergic system within the hypothalamus has been associated with the generation of the sleep wake cycle, as well as with respiratory, feeding, satiety, neuroendocrine and locomotion function and damage to this region has also been linked to narcolepsy (Peyron et al., 1998; Mintz et al., 2001; Ferguson and Samson, 2003; Zeitzer et al., 2003; Kirouac et al., 2005; Takakusaki et al., 2005). The possible interactions that the parvocellular cluster has with the main cluster within the hypothalamus are so far unknown and require further study.

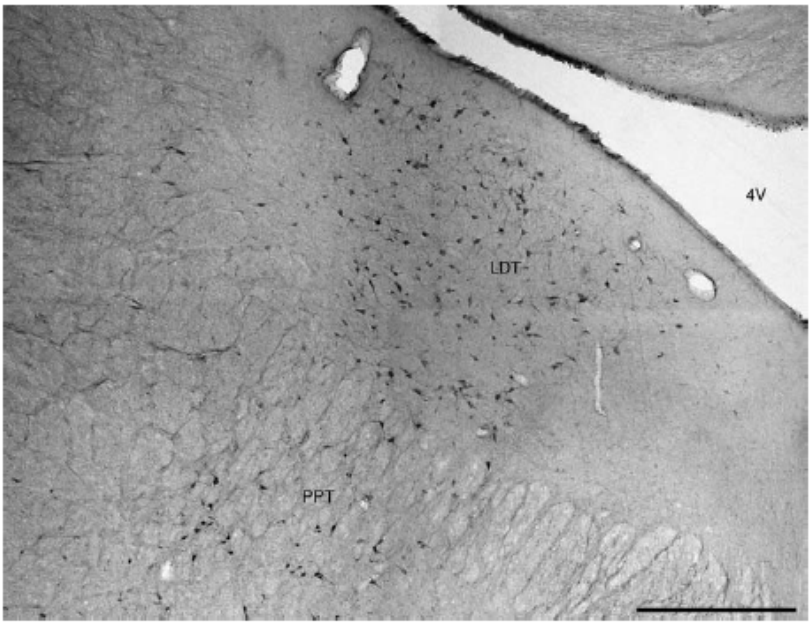


Figure 19 - Photomicrographic montage of a ChAT immunoreacted section through the dorsal pontine region of the giraffe brain showing the laterodorsal tegmental nucleus (LDT) in the periventricular grey matter and the pedunculopontine tegmental nucleus (PPT) in the adjacent pontine tegmentum. Scale bar = 1 mm. 4V – fourth ventricle. *Reproduced from Bux et al. (2010) with permissions (see appendix 4).*

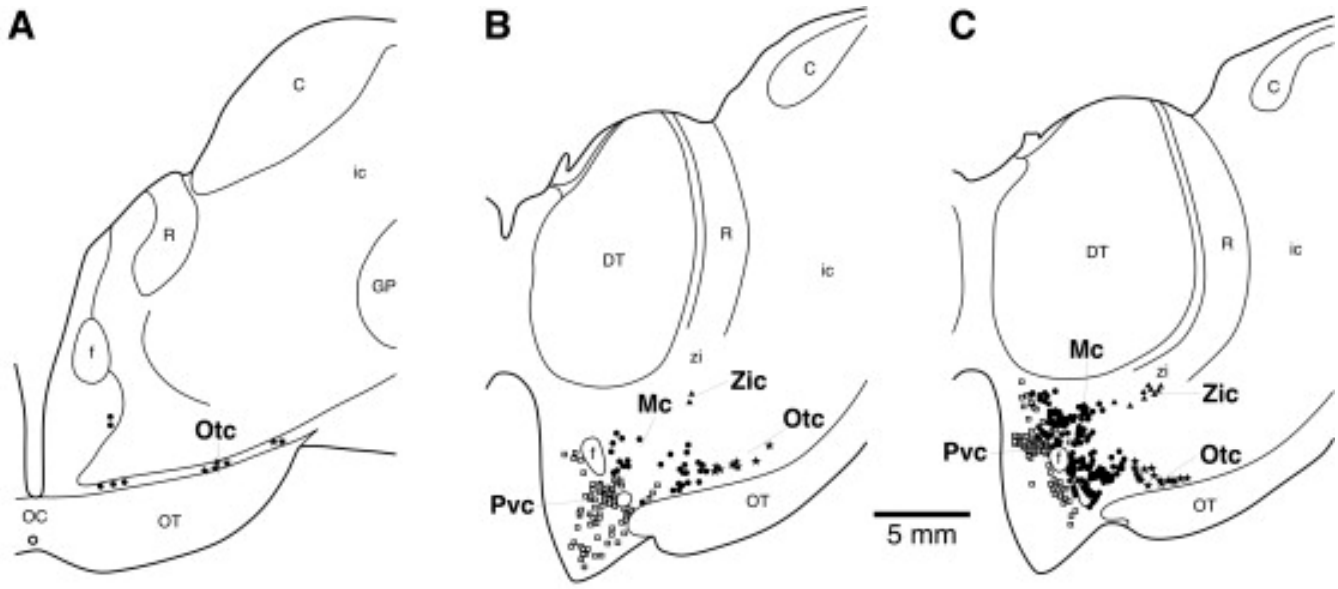


Figure 20 - Drawings of coronal sections through one half of the giraffe diencephalon illustrating orexin-A (Ox-A) immunoreactive neuron distribution. A single black dot indicates a single magnocellular orexinergic neuron while a single open square represents a single parvocellular orexinergic neuron. Drawing A represents the most rostral section, C the most caudal, and each drawing is approximately 500 mm apart. In all drawings, medial is to the left and dorsal to the top. Mc – main cluster, Zic – zona incerta cluster, Otc – optic tract cluster, Pvc – parvocellular cluster. *Reproduced from Dell et al. (2012) with permissions (see appendix 4).*

3. Circadian Rhythms

3.1 What are biological rhythms?

With the success of life on earth comes the necessary adaptation to certain rhythms dictated by the environment and driven by the earth's rotation around its axis. The four primary rhythms or geophysical cycles observed in nature are tidal, lunar, daily and seasonal (Aschoff, 1967, 1981). The most prevalent of these cycles with regards to biological life is that of daily/diel (24 h) and thereby nycthemeral rhythms. The circadian cycle is regulated by the timing of an endogenous clock synchronized by external cues yet also able to function in the absence of any environmental cycle (Refinetti, 2008). A further subdivision can be observed with ultradian (period of rhythm is shorter than ± 24 h), infradian (period of rhythm is longer than ± 24 h) and circannual (period of a year) rhythms (Halberg et al., 1965; Aschoff, 1981). Biological rhythms can be defined by four properties: 1) characteristics of the rhythm, like frequency and period; 2) the system in which the rhythm is detected; 3) the process that produces the rhythms; and 4) the function associated with the rhythm (Aschoff, 1981). Circadian rhythms are a cycle around a day involving biochemical, physiological and behavioural processes within a period of

approximately 24 h. The sleep-wake cycle and its regulation are an important feature of circadian rhythms (Franken et al., 2009; Van der Zee et al., 2009).

The control center of circadian rhythms is attributed to areas within the hypothalamus and are controlled via clock genes and oscillations of their protein expression (Johnson, 1939; Aschoff, 1960; Pittendrigh, 1960; Hamner et al., 1962; Richter, 1965; Pickard and Sollars, 2008; Sollars and Pickard, 2015). The autonomous oscillations within the control center cells involve two transcription/ translation feedback loops. The feedback system function to produce rhythms in gene expression which translate into a myriad of biochemical, physiological and behavioural outputs (Sollars and Pickard, 2015) (not discussed in depth for the purposes of this thesis).

The pervasive ability of animals to adapt behaviours to environmental rhythms is regulated primarily by the entrainment of endogenous circadian oscillators to the 24 h light-dark cycle (LD cycle) (Pittendrigh, 1960; Daan et al., 2011). The suprachiasmatic nucleus (SCN) is described as the primary circadian oscillator within the brain and is predominantly found within the hypothalamus in most species (Crosby and Woodburne, 1939; Sollars and Pickard, 2015). The SCN has been commonly recognized as the source of "Process C" from the two-process model (Ibuka and Kawamura, 1975; Ibuka et al., 1977; Mouret et al., 1978). The firing rate and gene expression patterns of the neurons within the SCN are responsible for most circadian rhythm outputs. The temporal signals of the SCN drive various rhythms such as activity patterns (including locomotion and rest), sleep-wake cycle, body temperature rhythms, feeding and foraging, to name but a few (Pickard and Turek, 1985; Lu et al., 2001; Saper et al., 2005; Todd et al., 2012; Sollars and Pickard, 2015; Vujovic et al., 2015). The SCN's involvement with the sleep-wake cycle and its temporal organization over a single 24 h period are not fully understood beyond the gating of "Process S" (Schwartz and Kilduff, 2015). It has been suggested that the sleep-wake state may provide modulatory input via serotonin in order to effect overall neuronal activity levels within the SCN in a reversed sense, especially during REM sleep (Fig. 21) (Decker et al., 2010).

In addition to the SCN, the eyes are considered an autonomous pacemaker site in most mammals and are a part of the peripheral oscillators (Tosini, 2000; Rensing and Ruoff, 2002). A projection from the retina terminating within the SCN, the retinohypothalamic tract (RHT), allows for direct synchronization, utilizing glutamatergic input to the SCN, with the LD cycle thus allowing for entrainment (Hendrickson et al., 1972; Moore and Lenn, 1972; Sollars and Pickard, 2015). Beyond the SCN master clock is a multi-oscillatory system involving circadian clocks distributed throughout all cells and tissue in the body, described as peripheral clocks (Sollars et al., 1995; Balsalobre et al., 1998; Welsh et al., 2004; Mohawk et al., 2012; Sollars and Pickard,

2015). The peripheral clocks are in turn entrained by the SCN through hormonal communication (e.g. pineal gland melatonin secretion), food availability and ingestion (Damiola et al., 2000; Stokkan et al., 2001), serum constituents (Balsalobre et al., 1998) and rhythmic shifts in Tb (Rensing and Ruoff, 2002).

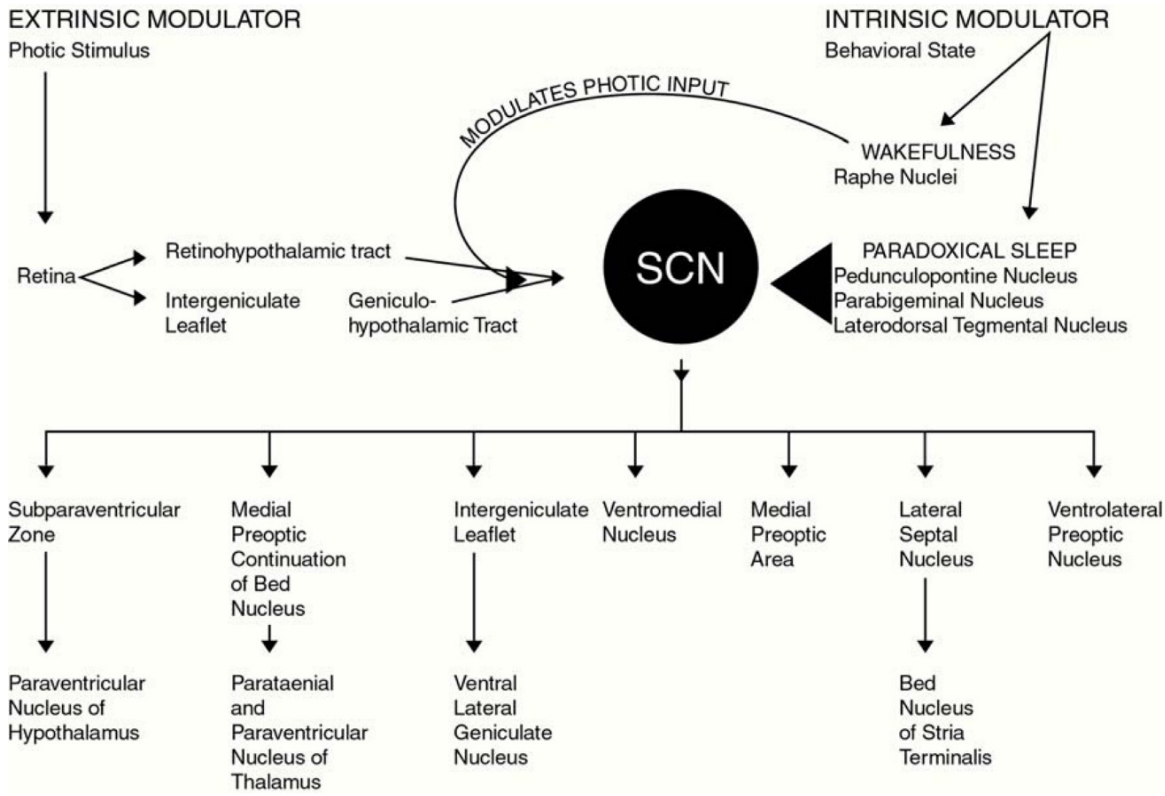


Figure 21 - Primary afferent and efferent pathways of the SCN. Illustrated graphically is the conceptual model through which intrinsic behavioral state-related stimuli could affect neuronal activity in the SCN. *Reproduced from Decker et al. (2010) with permissions (see appendix 4).*

The SCN master clock is made up of a multitude of autonomous cellular components that all interact in a complex neural network to control and regulate precise rhythms in relation to external and internal stimuli (Welsh et al., 2010; Sollars and Pickard, 2015). The most common neurotransmitters utilized within this nucleus are GABA and glutamate and subsequently a vast array of peptide neurotransmitters are utilized by individual neurons. The SCN has been described to be organized into a core and shell configuration (Moore, 2013) based on vasoactive intestinal peptide (VIP) and vasopressin (VP) distribution, although this pattern is highly dependent on various factors (Morin and Allen, 2006). The ventral VIP neurons receive photic input from the RHT and project to the VP neurons in the dorsal pacemaker region. From the SCN, there are three major output pathways that allow for the drive of circadian rhythms (Fig. 22, not further discussed for the purpose of this thesis). In addition there are sparse projections to the hypothalamic region responsible for orexin regulation (involved in wakefulness, mentioned earlier) (Aston-Jones et al., 2001) and to the VLPO related to sleep promotion (GABAergic,

mentioned earlier) (Saper et al., 2005). There is furthermore both hormonal (melatonin, cortisol) and humoral (suggested and not yet identified) output pathways in the circadian system not related to the neuroanatomical projections described (Pevet and Challet, 2011). It has been suggested that the SPZ is a vital region for SCN relay signals integration and may be the primary determinant in the temporal niche selection of an animal, such as diurnal or nocturnal activity (Todd et al., 2012). The entrainment or input of the SCN is dictated by various stimuli or time cues and this in turn allows for the output of biological rhythms.

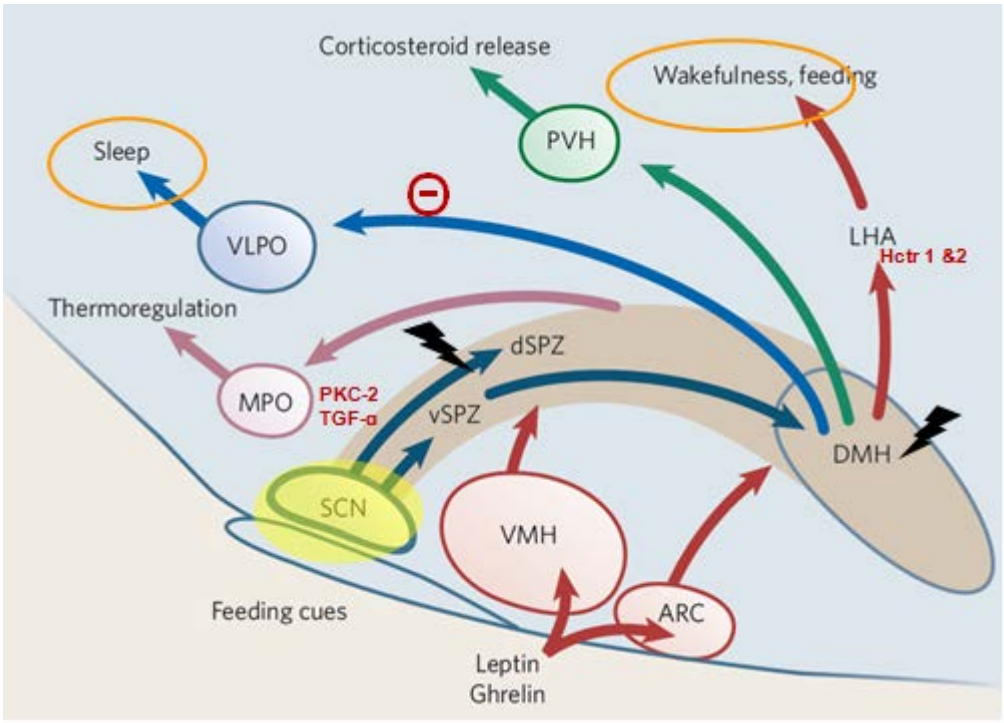


Figure 22 - Output pathways from the suprachiasmatic nucleus and various related functions. See abbreviations in the Preface section.
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Reproduced from Nami and Sadeghniaat (2011) with permissions (see appendix 4). Accessed on 15/06/2017.

3.2 Zeitgebers

Zeitgebers ("time-giver") are external stimuli that are powerful enough to entrain and cause phase shifting with intrinsic circadian rhythms (Van der Zee et al., 2009). There are multiple types of *Zeitgebers* that have been demonstrated to synchronize and alter circadian rhythms which can be divided into abiotic and biotic or photic and non-photic. Abiotic factors include light, Ta, sound and mechanical disruptions whilst biotic factors include intra-specific social interactions, exercise, food restrictions and food availability (Rensing and Ruoff, 2002; Refinetti, 2005, 2010, 2015; Vivanco et al., 2010; Gerkema et al., 2013). Photic variables are light based whilst non-photic variables include all previously mentioned abiotic and biotic

factors bar light. The entrainment of the SCN to the LD cycle is the synchronization of the period of endogenous SCN circadian oscillation to the relevant photoperiod (day length or amount of light an organism receives) (Dunlap et al., 2004; Sollars and Pickard, 2015). The entrainment of the SCN provides for a predicable phase relationship to photoperiod and the LD cycle (Pittendrigh and Daan, 1976; Sollars and Pickard, 2015). Entrainment itself allows for plasticity and adaptive potential for output rhythms (Pickard and Sollars, 2008; Sollars and Pickard, 2015). The two most dominant *Zeitgebers*, in terms of their ability to modulate circadian rhythms, are light and Ta (Fig. 23) with food availability placing third (Refinetti, 2005, 2015). The LD cycle and its relation to circadian rhythms has been extensively studied in the past (Daan and Aschoff, 2001; Refinetti, 2005, 2010) while the entraining capability of the non-photic variables has received far less attention (Wams et al., 2017). Of particular interest to the research included in this thesis are the effects of Ta on circadian rhythms.

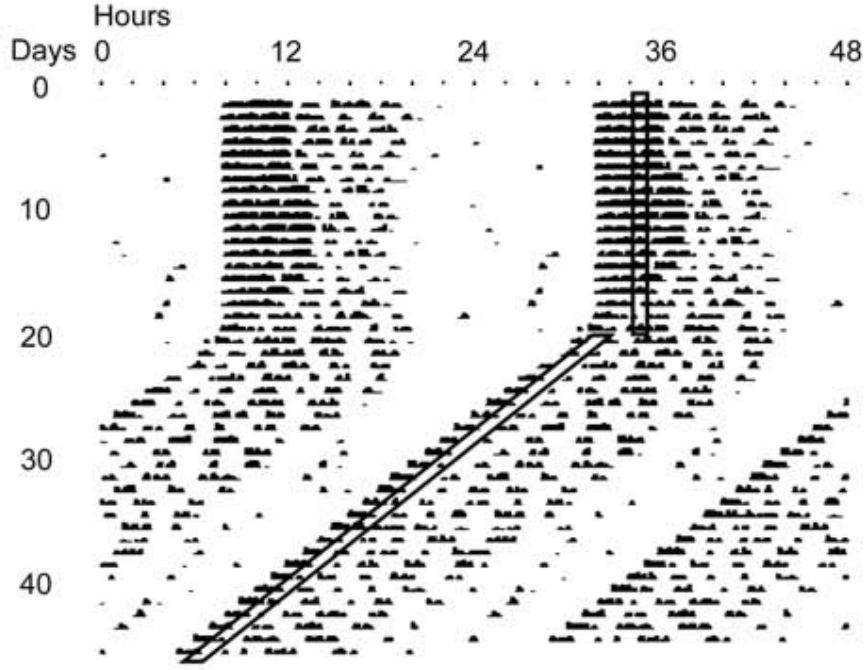


Figure 23 - Double-plotted actograms of running-wheel activity of a representative mouse subjected to ambient temperature T cycles with periods of 24 h (days 1 to 19) or 23 h (days 20 to 45). Time of day is indicated on the horizontal axis and number of days on the vertical axis. The narrow open rectangles denote the cold segment (12 C, 1 h) of the T cycle. *Reproduced from Refinetti (2010) with permissions (see appendix 4).*

Ta has been described to affect the circadian clock through direct and indirect pathways. Direct effects are from Ta either accelerating or retarding component cellular processes within the pacemaker biochemistry, an effect which can be considered global to the body and can affect any somatic cell and cellular process (Liu et al., 1998; Dunlap, 1999; Edery, 1999; Rensing and

Ruoff, 2002). The indirect effects of Ta may be through secondary messengers systems, such as hormones, or intracellular ion and metabolite concentration shifts within the clocks cellular environment (specific to the non-mammalian circadian system, effects remains unknown in mammals). These direct and indirect effects translate into variations of phase and frequency of circadian oscillation and are brought about through shifts of Ta (phasic) and through variation in the duration of temperature exposure (tonic) (Rensing et al., 1995; Rensing and Ruoff, 2002).

Ta as a form of *Zeitgeber* has been studied for almost half a century within ecto- and endothermic species such as reptiles and mammals (Hoffmann, 1969; Rensing and Ruoff, 2002; Refinetti, 2015). Over the years, the general conclusions have been that Ta strongly dictates the circadian rhythms of ectothermic species more so than endotherms, and that several variables determine the outcome and level of entrainment. These variables include timing differences between the endogenous rhythm and *Zeitgeber* period length, including its subsequent strength, and importantly on species-specific and intraspecific differences in sensitivity to Ta changes (Hoffmann, 1969; Rensing and Ruoff, 2002). Ta-affected locomotor patterns have been demonstrated in turn to act as a non-photic *Zeitgeber* and raise or lower Tb (Janik and Mrosovsky, 1993). Overall, the masking effect coupled with the entrainment signal of *Zeitgebers* ultimately leads to the adaptation of a species to its environment and its variations (Redlin, 2001; Rensing and Ruoff, 2002).

In contrast to changing Ta, different constant temperatures could alter the phasing of the clock and may lead to species exhibiting diurnality at one temperature and nocturnality at another (Rensing and Ruoff, 2002). Interestingly, among the ecto-endothermic gradient the heterothermic animals appear most sensitive to Ta fluctuations and subsequent entrainment (Lindberg and Hayden, 1974; Erkert and Rothmund, 1981; Pohl, 1998; El Allali, 2013; Bouâouda et al., 2014). In the case of the dromedary camel (*Camelus dromedarius*), an adaptive heterotherm living in extreme arid conditions, wide fluctuations of daily and seasonal Ta are a constant threat to survival (Schmidt-Nielsen, 1964; El Allali, 2013; Bouâouda et al., 2014). A study by El Allali et al. (2013) provided the first demonstration that daily Ta cycles can be used experimentally as *Zeitgebers* in mammals, and that the camel's Tb and melatonin rhythmicity, under certain conditions, is predominantly entrained by Ta shifts (Fig. 24). The effects of Ta on the camel appear to control the expression of daily Tb rhythms through either direct or indirect mechanisms as mentioned earlier, although specific mechanisms have not yet been described. El Allali et al. (2013) further suggested that through extended comparative study on wild species under similar arid conditions, a relationship between Ta and circadian rhythms may be more commonly observed.

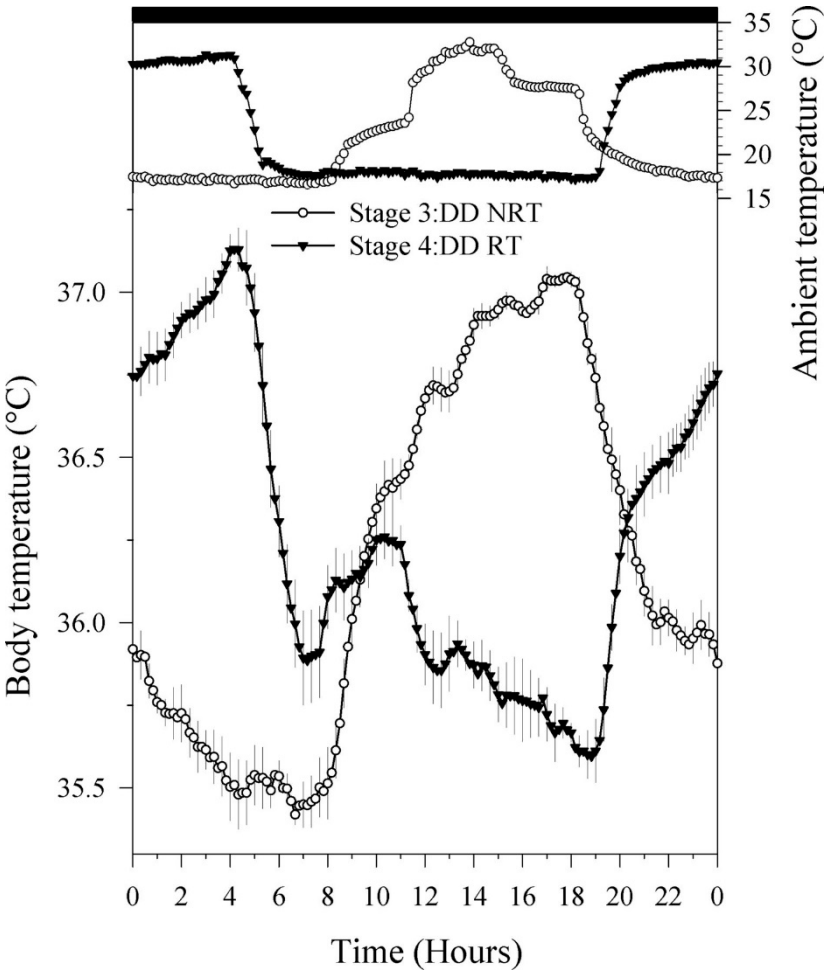


Figure 24 - Shift of the rhythm of Tb by a 12-h shift in the rhythm of Ta in DD (constant darkness) conditions (top black bar). Top: time course of Ta at each stage. Open circles, stage 3, Ta peak in the afternoon. Closed triangles, stage 4, Ta peak advanced by 12 h. Bottom (same symbols): corresponding Tb curves. RT – reversed Ta cycle, NRT – non-reversed Ta cycle. Means ± SE of 7 camels (pooled data) averaged for each animal on the last 5 days of the stage. *Reproduced from El Allali et al. (2013) with permissions (see appendix 4).*

3.3 Activity Patterns and Temporal niche switching

Varied activity patterns appear to have evolved in order to cope with the temporality and 24 h periodicity of the environment. Such activity varies highly amongst animals and this variation may hold important implications for ecology, physiology and evolution (Daan, 1981; Kronfeld-Schor and Dayan, 2003). The LD cycle and its effect on rest-activity rhythms and locomotion is a focus area of research in the field of circadian rhythms. Studied variables associated with this relationship include photoperiod (sunrise/sunset), Ta and food biomass availability as well as internal physiological processes including feeding patterns, hormone secretion, satiation/hunger and digestion/rumination (Aschoff, 1964, 1989; Gwinner, 1981; Nielsen, 1984; Krüll et al., 1985; Colman et al., 2001; Pépin et al., 2006). The rest-activity patterns and locomotion of mammals are primarily influenced by the physical aspects of light (lux and wavelength) and

photoperiod through entrainment of the internal circadian clock and additionally through masking (Redlin et al., 2005; Cohen et al., 2010). Masking occurs through the direct stimulatory (positive) or inhibitory (negative) effects of certain environmental factors on behaviours and only affects the expression of activity, not the condition of the circadian pacemaker (Refinetti, 2008; Chiesa et al., 2010).

Most species have been classified, based on behavioural and physiological rhythms, into three distinct categories or phenotypic preferences for certain temporal niches (time when an animal is active), namely diurnal, nocturnal or crepuscular (Aschoff, 1966; Refinetti, 2008; Otalora et al., 2010). Another temporal niche within this continuum is cathemerality (no specific preference for activity at any time of day, "lack" of rhythmicity) which is common amongst species of primates (Curtis and Rasmussen, 2006; Tattersall, 2006; Refinetti, 2008). These phenotypic variations appear to reflect evolutionary adaptations to specific temporal niches (Levy et al., 2012; Phillips et al., 2013b) The varying activity patterns of species has been proposed to be related to reducing interspecific resource and interference competition between potentially competing taxa and between predator and prey species (Johnston and Zucker, 1983; Kronfeld-Schor and Dayan, 2003). Schoener's (1974a) theoretical model suggests that temporal resource partitioning amongst competitors, by varying activity patterns, would only occur when a severe depletion of available resources came about. Daan (1981) suggested that closely related species, destined for competition would normally occupy similar temporal niches as diurnality and nocturnality require different evolutionary adaptations. An interesting case can be seen in golden spiny mice, where the majority of the species is diurnal throughout most of their habitat range in the wild, but become immediately nocturnal when introduced to lab conditions (Cohen et al., 2010). Across the species there are various adaptations that appear to support diurnality with dark skin pigmentations, and some supporting nocturnality with structural changes in the retina (Cohen et al., 2010). In this case of golden spiny mice, there appears to be evidence of an evolutionary shift between these adaptations to the LD cycle (Kronfeld-Schor and Dayan, 2008; Cohen et al., 2010). Shkolnik (1971) and Kronfeld-Schor et al. (2001c) report on spiny mouse in the rocky deserts of the Middle East in which common (*Acomys cahirinus*) (nocturnal) and Golden spiny (*Acomys russatus*) (diurnal) mice share foraging microhabitats. When the common spiny mice were removed from the area, the golden spiny mice became nocturnal indicating a forced temporal niche selection in lieu of resource competition (Shkolnik, 1971).

The general hypothesis states that species are evolutionarily constrained within their temporal niche (Daan, 1981; Kronfeld-Schor et al., 2001a, b, c; Roll and Dayan, 2002; Kronfeld-Schor and Dayan, 2003) and any form of plasticity is limited (Schoener, 1974b). This constraint is

possibly related to anatomical and physiological phenotypes and in the case of response to climate change, variables like body size and activity times play major roles in adaptation (McCain and King, 2014). Observed taxon-specific temporal niche preferences appear to support the general hypothesis (Daan, 1981; Roll and Dayan, 2002).

The majority of desert mammals studied, in terms of activity patterns, belong to the order Rodentia, which are primarily nocturnal and have relatively small body masses (Gerkema et al., 2013). The nocturnal behaviour of desert species such as the kangaroo rat (*Dipodomys merriami*) allows for reduction of predation risk and avoidance of excess heat load, but in turn adds costs to thermal energy regulation as nighttime temperatures are usually low, leading to a trade-off between losing heat energy and finding food (van der Vinne et al., 2014). Diurnal desert mammals are presented with different challenges in that they must both avoid excessive daytime heat loads and forage during daylight hours to avoid competition. Small sized diurnal mammals are further disadvantaged by having rapid potential thermal shifts compared with the slower thermal gradients found in larger mammals (Kenagy et al., 2004). They are, however, at an advantage in terms of access to a multitude of microclimates unavailable to larger mammals. A compromise trade-off between diurnal and nocturnal phenotypes is crepuscularity, a commonly employed 24 h phasing pattern in desert species (Aschoff, 1966; Leuthold, 1977). This broad variation in phenotypes amongst desert species allows for trade-offs between behavioural adaptations like avoidance of excess heat load, foraging strategies, reduction of predator risk and prevention of water loss (Kenagy et al., 2004; van der Vinne et al., 2014).

The majority of species examined throughout the literature present with a crepuscular phenotypic preference (activity with two peaks in a 24 h) more so than nocturnality or diurnality (single activity peak during the nighttime or daytime, respectfully) (Aschoff, 1966; Refinetti, 2008). Within this general preference; however, are several cases with strong disagreement and variation. Within the same species, some individuals may exhibit diurnality and others nocturnality (Johnston and Zucker, 1983; Iigo and Tabata, 1996; Oster et al., 2002; Oosthuizen et al., 2003; Sharma et al., 2004; Refinetti, 2006, 2008). Within the same individual of a species, both diurnal and nocturnal behaviours can be observed, for example degus (*Octodon degus*) (Refinetti, 1996, 2006, 2008; Labyak et al., 1997; Kas and Edgar, 1999, Kenagy et al., 2002). Lastly, an interspecies gradient can be observed for diurnality, like that demonstrated by Refinetti (2006, 2008) in several rodent species. The vast gradient of temporal niche preference and non-discrete classification, besides that of activity patterns, is compounded further depending on the variable being recorded and the current conditions. When measuring several variables such as locomotor activity, heart rate, blood pressure, Tb, glucose levels and

melatonin, there is no absolute classification possible for any animal into pure diurnal or nocturnal phenotypes (see Fig. 25 for examples specific to the horse) and thus the specificity of the scientific question is critical and any extrapolations should be limited to a single variable at a time (Moore-Ede and Sulzman, 1981; Piccione et al., 2005; Refinetti, 2008).

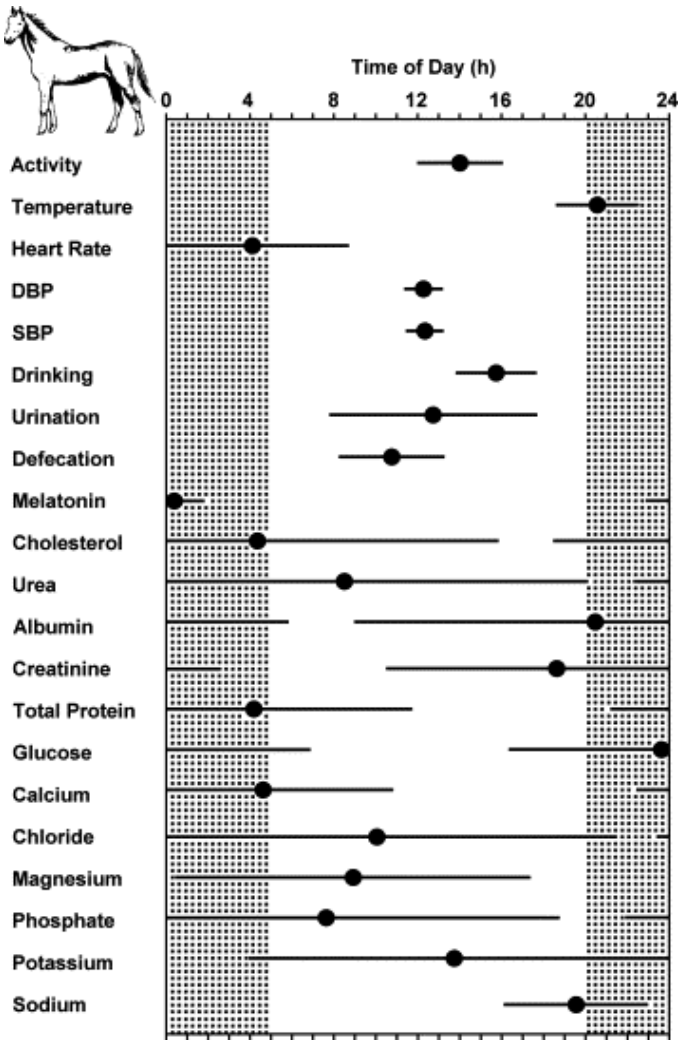


Figure 25 - Acrophases (peak of a cycle) of the rhythms of 21 variables in horse (*Equus caballus*). Circles indicate the means for five mares housed in individual indoor stalls under natural photoperiod with 15 hours of light per day. Horizontal lines indicate the 95% confidence intervals of the means. *Reproduced from Piccione et al. (2005) with permissions (see appendix 4).*

A particularly interesting and relevant variation in temporal niche preference is “temporal niche switching”, also described as “dual phasing” (Refinetti, 2008; Otalora et al., 2010). Some of the possible stimuli resulting in such plasticity includes: food availability and restrictions; temperature cycles and extremes; LD cycle variations; social pressure and interactions; developmental changes; and lastly, seasonal cues (Reebs, 2002; Mrosovsky, 2003; Mrosovsky and Hattar, 2005; Kempinger et al., 2009; Chiesa et al., 2010; Van der Vinne, 2014). There are several cases for temporal niche switching, both under natural and laboratorial conditions, with the latter comprising most of the literature. There are multiple cases of physiological or

molecular inductions of temporal niche switching related to sensory input from photoreceptors. Some of these include rats with hypothalamic lesions (Richter, 1978), mice lacking cryptochromes (retinal pigments) (Van Gelder et al., 2002), melanopsin knockout mice (Mrosovsky and Hattar, 2003), mice lacking melanopsin and functional rods (Hattar et al., 2003), vitamin-A depleted mice (Thompson et al., 2004) and mutant fruit flies (Rieger et al., 2003; Mrosovsky and Hattar, 2005).

In the degu (diurnal in the wild, Refinetti 1996; Kenagy et al., 2002), spontaneous switching from a clear nocturnal activity pattern to diurnal activity was demonstrated by wheel-running behaviour after a prolonged period (Refinetti, 2006). Additionally, it was shown that degus, Nile grass rats (*Arvicanthis niloticu*) and Mongolian gerbils (*Meriones unguiculatus*) can be diurnal in the lab without running wheels; however, when given access to running wheels, they exhibited nocturnal behaviours or vice versa (Kas and Edgar, 1999; Redlin and Mrosovsky, 2004; Weinert et al., 2007; Cohen et al., 2010). The same inversion of temporal niche preference was later demonstrated in degu using varying Ta cycles under lab conditions. An inversion of running wheel behaviour from a diurnal to a nocturnal preference was attributed to masking by alternating daily temperature cycles and entrainment to the LD cycle (Vivanco et al., 2010). Similar spontaneous shifts or switching between activity patterns was observed in cotton rats (*Sigmodon hispidus*) under lab conditions (Johnston and Zucker, 1983; Refinetti, 2008). Another remarkable laboratorial example is the Norway lobster (*Nephrops norvegicus*) which exhibits temporal niche switching under dramatic light intensity changes related to the depth of their deep-water habitat (Chiesa et al., 2010). Under dim light (deep depths) they are predominantly diurnal while under brighter light (shallow depths) they are predominantly nocturnal. Chiesa et al. (2010) attribute the switch to a circadian system that can generate both phenotypes under varied LD cycles and masking. There are currently three proposed mechanisms to account for the phenomenon of temporal niche switching (Fig. 26). Empirical evidence exists for each of the mechanisms and it appears they are not mutually exclusive to one another (Chiesa et al., 2010) (not discussed further for purposes of this thesis).

Temporal niche switching has been documented in several vertebrate and invertebrate species; however, the majority of the research was conducted under laboratorial conditions and rarely in relation to shifting environmental variables (Mrosovsky and Hattar, 2005; Kempinger et al., 2009; Chiesa et al., 2010; Otalora et al., 2010). Few studies have reported this phenomenon under natural conditions, especially in larger mammalian species. Wolves (*Canis lupus*) are usually nocturnal except when travelling for long periods and distances, during which time they exhibit predominantly daytime activity (Merrill and Mech, 2003). A study by Lourens and Nel

(1990) reported Bat-eared foxes (*Otocyon megalotis*) shifting between nocturnal activities during the hot South African summer to diurnal activity during the cold winter. They propose that the adaptive strategy is driven by prevailing temperatures and corresponding prey availability throughout the seasons.

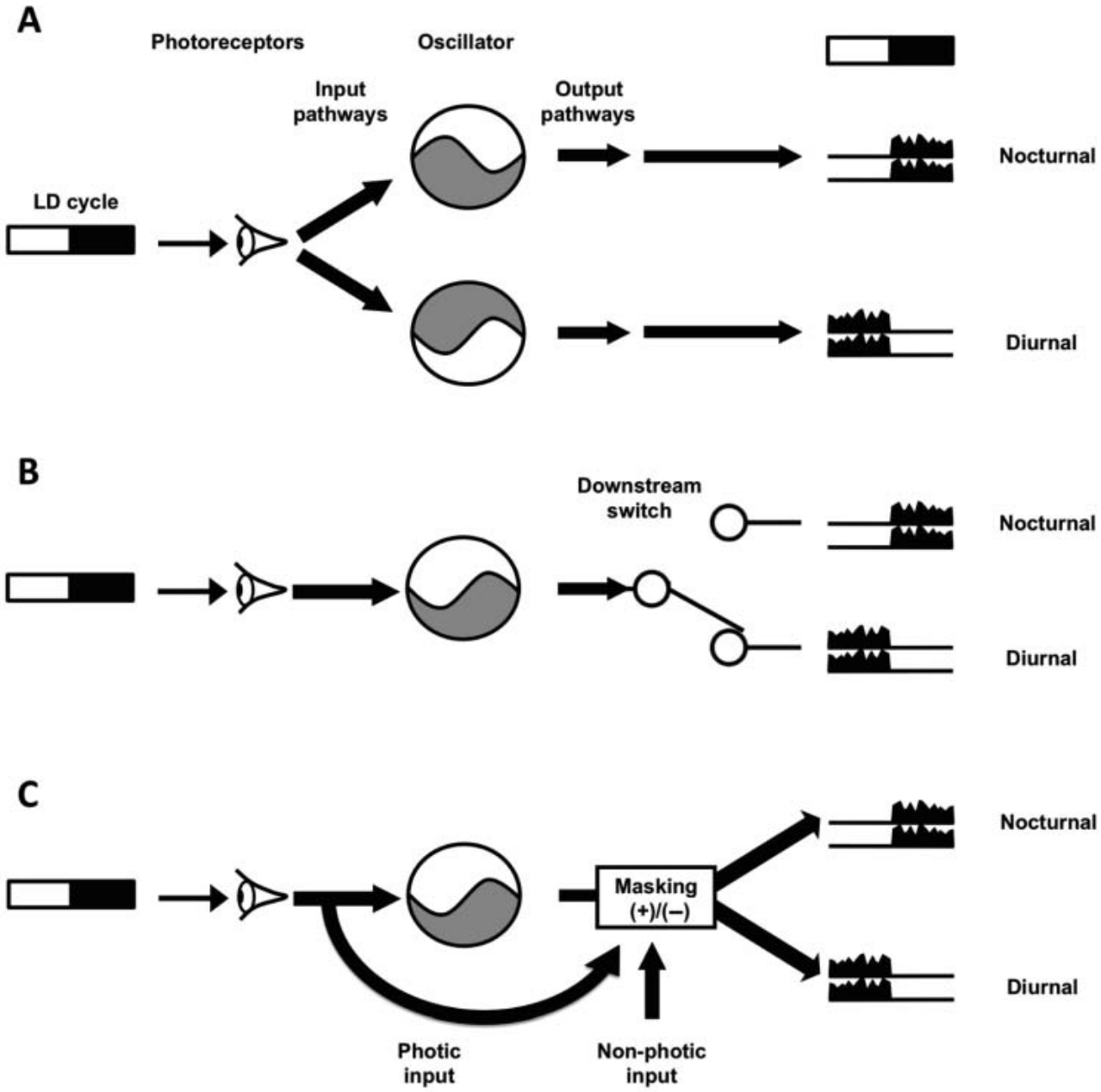


Figure 26 - Schematic representation of possible mechanisms by which a circadian system could switch between nocturnal or diurnal temporal patterns. (A) Circadian oscillator properties could change so that its phase relationship to the LD cycle is different by almost 180°, leading to oppositely phased rhythms of activity. (B) Oscillator properties could be unaltered, but a switch in the output pathways regulating locomotor activity could alternate between nocturnal and diurnal patterns of locomotor activity. (C) Oscillator properties could be unaltered, but positive and/or negative masking could determine the rhythmic pattern of activity regardless of the oscillator phase relationship to the LD cycle. *Reproduced from Chiesa et al. (2010) with permissions (see appendix 4).*

3.4 Circadian Rhythms and the Artiodactyls

Aspects of the behaviour and circadian rhythms (primarily activity patterns) of the Artiodactyla have been studied quite extensively. It has been demonstrated that the manner in which Artiodactyls (wild and domestic) manage their time for various activities and behaviours is strongly dependent on age, sex, reproductive status, social condition, ecological habitat and seasonality (Grubb and Jewell, 1966; Sadleir, 1969; Boy and Duncan, 1979; Turner, 1979; Clutton-Brock et al., 1982; Hudson and White, 1985; Alados, 1986; Shi et al., 2003; Pépin et al., 2006). The daily and annual organization of activity patterns results from an intricate balance and compromise between optimum foraging times, social activities and environmental limitations (Aschoff, 1963; Shi et al., 2003). Such organization is unique to each species and relates to an adaptation to their environment needed to maximize energy intake and allow for survival. These necessary adaptations suggest that time budgeting is a vital factor for balancing tradeoffs and life history strategies (Sadleir, 1969; Hudson and White, 1985; Shi et al., 2003; Pépin et al., 2006).

As described by Colman et al. (2001) most Artiodactyl species studied thus far, including varied high-Arctic reindeer species, domesticated sheep, Spanish ibex (*Capra pyrenaica*) (Alados, 1986), chamois (*Rupicapra rupicapra*) (Couturier, 1938), roe deer (*Capreolus capreolus*) (Bubenik, 1960; Turner, 1979; Cederlund, 1989), rocky mountain elk (*Cervus elaphus*) (Green and Bear, 1990), white tailed deer (*Odocoileus virginianus*) (Montgomery, 1963; Beier and McCullough, 1990) and most caribou (Maier and White, 1998), show the majority of their time spent active over the morning and evening periods in synchronization to light (sunrise and sunset) with no seasonal differences and a primary dominance to diurnal phasing. Conversely, in some species like wild high-Arctic reindeer (*Rangifer tarandus*) there is no synchronization of their daily activity peaks to sunrise or sunset during summer demonstrating that photoperiod may not be the dominant *zeitgeber* seasonally for this species (Colman et al., 2001). The majority of Artiodactyls employ crepuscular activity patterns in order to avoid predators (predators are typically diurnal or nocturnal and not active during twilight hours) (Leuthold, 1977). While high-Arctic reindeer do not naturally have predators, their activity peaks are not distinct around sunrise and sunset and vary seasonally in relation to other factors, such as peak insect biting times (Loe et al., 2007; Pan et al., 2011).

In most Artiodactyl species, a synchronicity exists between individuals of a herd or group to allow for group cohesion. Behavioural synchronicity usually applies to vigilance and reduction of predation risk but can also relate to the start and end time of active and resting bouts as well as the beginning and end of a feeding bout (Jarman, 1974; Arnold and Dudzinski, 1978; Pépin et

al., 2006). This behaviour is well documented in reindeer where the activity rhythms appear to be defined and highly synchronized within herd groups by internal physiological processes and intra-specific group interactions rather than by the LD cycle (Colman et al., 2004). When examining synchronicity between reindeer groups, there was almost a complete lack of overlap between foraging and activity rhythms and attributes this to intra-specific competition. The reindeer species exhibit a polycyclic activity rhythm during summer in order to maximize foraging efficiency and which appears to be independent to external *Zeitgebers* (Maier and White, 1998; Colman et al., 2001, 2004).

Ruminants are normally reported to exhibit a "… simple serial foraging-resting-foraging activity pattern" that is not distributed evenly over 24 h (Shi et al., 2003). As such, the patterns observed appear to conform to the "rumen fill" theory which states that ruminants are limited by the length of time required to digest food, not by the time taken to locate it (Westoby, 1974; Moen, 1978; Pépin et al., 2006). The bimodal crepuscularity mentioned earlier in conjunction with this serial activity rhythm appears typical across most temperate environment living species and some African Artiodactyls (Jarman and Jarman, 1973; Dunbar and Dunbar, 1974; Roberts and Dunbar, 1991; Shi et al., 2003). These patterns are, however, both adaptive and subject to an animal's energetic needs and the variability of the food supply in changing environments (Renecker and Hudson, 1989; Green and Bear, 1990; Defler, 1995; Moncorps et al., 1997; Shi et al., 2003). In addition, the age and sex of individuals plays a major role in the seasonal activity patterns and there appears to be some form of sexual dimorphism across activity budgets independent of metabolic rate and cost (Turner, 1979; Zhang, 2000; Shi et al., 2003).

The metabolic rates and rhythms observed amongst the artiodactyls living in desert environments appears to exhibit a summer time nadir (lowest value of an oscillatory function) (Williams et al., 2001; Ostrowski et al., 2006a, b) in association with low biomass quality and quantity (Piccione et al., 2002; Ahmed and El Kheir, 2004). This seasonal reduction in metabolic rate may be in response to reduced energy intake or to reduce the heat load associated with food metabolism (Piccione et al., 2002; Ahmed and El Kheir, 2004). Conversely, in northern region ruminants and non-ruminants, like the red deer and Przewalski horse (*Equus ferus przewalskii*), a winter nadir in body temperature and metabolism rate can be observed, termed "nocturnal hypometabolism" (Nilssen et al., 1984; Mesteig et al., 2000; Arnold et al., 2004, 2006).

Circadian rhythms allow for major anticipatory roles in physiology and behaviour in relation to environmental change. Animals have been reported to shift or adjust their status prior to environmental events taking place and do not become misled by minor disturbances, thus enhancing their overall fitness (Daan and Aschoff, 1982; Bennet, 1987; Gerkema, 1992;

Aronson et al., 1993; Horton, 2001; Kronfeld-Schor and Dayan, 2003). Through observational and behavioural studies of the Artiodactyls, our knowledge regarding their social organization, foraging ecologies, reproductive strategies, activity patterns, temporal niche preferences, and evolution has been vastly expanded. This noteworthy gain allows for assessing the effects of climate change and human disturbance promoting conservation and preservation (Geist, 1971; Geist and Walthers, 1974; Jarman, 1974; Schaller 1977; Stockwell et al., 1991; Fox et al., 1992; Beale and Monaghan, 2004; Pan et al., 2011; Fuller et al., 2016). In the case of many species, human interference can be analogous to predation and as such may initiate similar counter behaviours, like that of increased vigilance and abnormal social constructs (Lima and Dill, 1990; Roberts, 1996; Papouchis et al., 2001; Frid and Dill, 2002; Fernández-Juricic and Schroeder, 2003; Pan et al., 2011). In lieu of all the factors affecting the behaviour and activity patterns of wild Artiodactyls, a remarkable adaptive ability can be observed under natural and extreme conditions. The perpetuation of comparative wild-based studies on the sleep and circadian rhythms of animals is of vital significance for understanding the ecology and evolution of behavioural strategies, functions related to sleep, additional survival mechanisms and its succeeding plasticity in the face of climate change, human interference and nature.

4. Research Objectives

Within the literature of sleep and circadian rhythms, there is a continuous drive towards and a necessary demand for basic comparative research on species under natural conditions. The relatively extensive number of species examined still only includes a small percentage of total species and thus leaves a gap in our broad understanding. The noteworthy work of researchers within the previously mentioned literature has none the less paved the way towards understanding the evolution of sleep, behaviour and circadian rhythms and how species interact and adapt to life on Earth.

Considering the prior history of the Arabian oryx and its sustained threat of extinction, any further knowledge gained is a feat of its own and thus should merit any form of continued study. The comprehensive work on the Arabian oryx has identified this species as rather extraordinary in its plasticity and adaptive ability to survive under such extreme environments. The Arabian oryx may allow for a unique ethological and ecological perspective on the function and evolution of sleep in relation to extreme temperatures and for the importance of circadian rhythms in regards to their dictation of life.

The overarching aims of the current thesis were to study sleep in the Arabian oryx (*Oryx leucoryx*), explicitly the neuroanatomy, physiology and behavioural aspects of sleep specific to wild Arabian oryx living under natural conditions.

This was accomplished by the following objectives:

Article I:-

1. To provide the first complete description of the sleep systems in the brain of an Artiodactyl using immunohistochemistry and stereology, namely the Arabian oryx.
2. To determine whether the extreme desert environment of Saudi Arabia has resulted in the evolution of any specific neural novelties related to the nuclei described in objective 1.

Article II:-

3. To examine the daily inactivity patterns and body temperature at a seasonal level using Actigraphy in Arabian oryx under extreme natural conditions.
4. To determine whether daily inactivity patterns examined in objective 3 shift between summer and winter or remain similar with respect to ambient temperature and photoperiod under natural conditions.

Article III:-

5. To investigate the 24 h temporal activity budgets and phasing profiles of wild Arabian oryx continuously over one year in two separate varied locations within the Arabian Desert.
6. To further determine if differences in habitat size and climate between the two locations in objective 5 relate to any variations in activity budgets and phasing.
7. To determine the relationship between seasonal environmental variables and continuous year-round activity patterns and time budgets described in objective 5.

Article IV:-

8. To provide the first complete polysomnographic-based examination of sleep in a wild, unrestricted Cetartiodactyl species, namely the Arabian oryx.

9. To determine whether the extreme desert environment of Saudi Arabia has resulted in novelties related to the sleep architecture described in objective 8 during summer and winter.
10. To verify whether previously reported seasonal temporal niche switching developed in objectives 3 and 4 reflects similarly in terms of sleep physiology.

The above stated comprehensive approach focusing on the aspects of sleep in wild Arabian oryx is addressed by the succeeding article publications in Part II.

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PART II

Published Works

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Neurochemical organization and morphology of the sleep related nuclei in the brain of the Arabian oryx, *Oryx leucoryx*.

Joshua G. Davimes, Abdulaziz N. Alagaili, Nigel C. Bennet, Osama B. Mohammed, Adhil Bhagwandin, Paul R. Manger, Nadine Gravett.

Journal of Chemical Neuroanatomy. 2017 81:53-70

Journal of Chemical Neuroanatomy 81 (2017) 53–70

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu

Neurochemical organization and morphology of the sleep related nuclei in the brain of the Arabian oryx, *Oryx leucoryx*

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ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form 30 January 2017
Accepted 1 February 2017
Available online 3 February 2017

Keywords:
Artiodactyla
Arabian oryx
Mammalia
Sleep
Evolution
Neuroanatomy

ABSTRACT

The Arabian oryx, *Oryx leucoryx*, is a member of the superorder Cetartiodactyla and is native to the Arabian Desert. The desert environment can be considered extreme in which to sleep, as the ranges of temperatures experienced are beyond what most mammals encounter. The current study describes the nuclear organization and neuronal morphology of the systems that have been implicated in sleep control in other mammals for the Arabian oryx. The nuclei delineated include those revealed immunohistochemically as belonging to the cholinergic, catecholaminergic, serotonergic and orexinergic systems within the basal forebrain, hypothalamus, midbrain and pons. In addition, we examined the GABAergic neurons and their terminal networks surrounding or within these nuclei. The majority of the neuronal systems examined followed the typical mammalian organizational plan, but some differences were observed: (1) the neuronal morphology of the cholinergic laterodorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei, as well as the parvocellular subdivision of the orexinergic main cluster, exhibited Cetartiodactyl-specific features; (2) the dorsal division of the catecholaminergic anterior hypothalamic group (A15d), which has not been reported in any member of the Artiodactyla studied to date, was present in the brain of the Arabian oryx; and (3) the catecholaminergic tuberal cell group (A12) was notably more expansive than previously seen in any other mammal. The A12 nucleus has been associated functionally to osmoregulation in other mammals, and thus its expansion could potentially be a species specific feature of the Arabian oryx given their native desert environment and the need for extreme water conservation.

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Abbreviations: III, oculomotor nucleus; Vmot, motor nucleus of trigeminal nerve; Vsens, sensory nucleus of trigeminal nerve; 3V, third ventricle; 4V, fourth ventricle; 5n, trigeminal nerve; A6d, diffuse portion of locus coeruleus; A7d, nucleus subcoeruleus, diffuse portion; A7sc, nucleus subcoeruleus, compact portion; A8, retrorubral nucleus; A9l, substantia nigra, lateral; A9m, substantia nigra, medial; A9pc, substantia nigra, pars compacta; A9v, substantia nigra, ventral, pars reticulata; A10, ventral tegmental area; A10c, ventral tegmental area, central; A10d, ventral tegmental area, dorsal; A10dc, ventral tegmental area, dorsal caudal; A11, caudal diencephalic group; A12, tuberal cell group; A13, zona incerta cell group; A14, rostral periventricular nucleus; A15d, anterior hypothalamic group, dorsal division; A15v, anterior hypothalamic group, ventral division; Arc, hypothalamic arcuate nucleus; B9, supralemniscal serotonergic nucleus; C, caudate nucleus; ca, cerebral aqueduct; CLi, caudal linear nucleus; Diag.B, diagonal band of Broca; DRc, dorsal raphe, caudal division; DRd, dorsal raphe, dorsal division; DRif, dorsal raphe, interfascicular division; DRl, dorsal raphe, lateral division; DRp, dorsal raphe, peripheral division; DRv, dorsal raphe, ventral division; DT, dorsal thalamus; f, fornix; fr, fasciculus retroflexus; GC, central gray matter; GiCRt, gigantocellular reticular nucleus; GP, globus pallidus; Hyp.d, dorsal hypothalamic cholinergic nucleus; Hyp.l, lateral hypothalamic cholinergic nucleus; Hyp.v, ventral hypothalamic cholinergic nucleus; IC, inferior colliculus; ic, internal capsule; IP, interpeduncular nucleus; Is.Call/TOL, islands of Calleja/olfactory tubercle; LDT, laterodorsal tegmental nucleus; Ll, lateral lemniscus; LOT, lateral olfactory tract; LV, lateral ventricle; Mc, main cluster of orexinergic neurons; mcp, middle cerebellar peduncle; mlf, medial longitudinal fasciculus; MnR, median raphe nucleus; N.Acc, nucleus accumbens; N.Bas, nucleus basalis; OT, optic tract; OTc, optic tract cluster of orexinergic neurons; P, putamen nucleus; PBg, parabigeminal nucleus; PC, cerebral peduncle; PCRt, parvicellular reticular nucleus; PIR, piriform cortex; PPT, pedunculopontine tegmental nucleus; Pvc, parvocellular orexinergic cluster; R, thalamic reticular nucleus; Rmg, raphe magnus nucleus; RtTg, reticulotegmental nucleus of the pons; SC, superior colliculus; scp, superior cerebellar peduncle; Sep.L, lateral septal nucleus; Sep.M, medial septal nucleus; SON, supraoptic nucleus; STN, subthalamic nucleus; VPO, ventral pontine nucleus; xscp, decussation of the superior cerebellar peduncle; zi, zona incerta; Zic, zona incerta cluster of orexinergic neurons.

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http://dx.doi.org/10.1016/j.jchemneu.2017.02.002
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1. Introduction

The Arabian oryx, *Oryx leucoryx*, is the smallest member of the genus *Oryx*, and is grouped within the order Cetartiodactyla, which comprises cetaceans (whales and dolphins) and artiodactyls (even-toed ungulates). Arabian oryx are adaptive heterotherms, allowing them to survive in the harsh climatic conditions of the deserts and steppes of the Arabian Peninsula (Ostrowski et al., 2003). Given the environment occupied by the Arabian oryx under natural conditions, the question naturally arises as to whether there are any special neurological adaptations that have evolved to enable the Arabian oryx to regulate sleep.

The examination of sleep in natural conditions, especially under extreme circumstances has received very little attention in the literature. Understanding the ecology of a species, its sleep physiology and the underlying neuronal architecture regulating such patterns could provide an improved understanding of the evolution of sleep and its functions. For species that reside in desert environments a range of conditions may act as stress factors that affect survival, and may lead to specific adaptations and behaviours. Factors such as food quantity and quality, availability of water and extreme ambient temperatures are aspects of daily life in the desert that may alter adaptive patterns and timing of sleep (Degen et al., 1997; Schmidt-Nielsen, 1997).

The Arabian oryx has been previously examined in terms of its ecology and morphophysiological adaptations to the aforementioned environmental extremes. Adaptations such as lower total evaporative water loss rates, adaptive heterothermy and selective brain cooling are among several physiological responses to high temperatures (Williams et al., 2001; Ostrowski et al., 2003; Hetem et al., 2010, 2012a). Additionally the Arabian oryx has been shown to exhibit seasonal shifts in the timing of daily inactivity patterns, switching between winter diurnality and summer crepuscularity/nocturnality (Seddon and Ismail, 2002; Hetem et al., 2012b; Davimes et al., 2016). Despite these studies, no examination has been conducted on the underlying neuronal architecture that has been implicated in sleep control in other mammals for the Arabian oryx.

The sleep related neuronal systems are comprised of nuclei that extend from the level of the basal forebrain to the pontine region and are comprised of nuclei that are cholinergic, catecholaminergic, serotonergic and orexinergic as well as GABAergic interneurons. The cholinergic nuclei located within the basal forebrain and pons are primarily involved in cortical activation and are mostly active during wakefulness and REM sleep (for reviews, see Harris, 2005; Jones, 2005; Siegel, 2006). The catecholaminergic nuclei are predominantly involved in sleep state homeostasis, wakefulness and REM sleep. The norepinephrine containing neurons of the locus coeruleus (LC) complex discharge most during wakefulness, have reduced discharge rates during non-REM sleep and cease to discharge during REM sleep (Jones, 2005). The midbrain serotonergic nuclei are most active during reduced states of arousal and regulate behaviours such as grooming and muscle tone. This system shows reduced activity during non-REM sleep and the neurons cease to discharge during REM sleep (Jones, 2005). The orexinergic neurons, located within the hypothalamus, play an integral role in the maintenance of arousal through its wide spread projections to the histaminergic, norepinephrine and serotonergic systems (for review, see Siegel, 2004). The GABAergic neurons and terminal networks found throughout and projecting to the aforementioned nuclei are thought to promote non-REM sleep by inhibiting those neurons that are involved in arousal. Certain groups of GABAergic neurons have a maximal discharge rate at the onset of sleep, continue to discharge during non-REM sleep and in some instances also continue to discharge during REM sleep (Siegel, 2004).

The order Artiodactyla is the fifth most speciose mammalian order, with over 220 species (Grzimek, 1990, 2003; Nowak, 1999), but despite this diversity, only a few studies have described certain aspects of the systems that have been implicated in sleep control within this order. The nuclei of the cholinergic, catecholaminergic, serotonergic and orexinergic systems have been described in the giraffe (Bux et al., 2010; Dell et al., 2012), the cholinergic system has been described in the Goettingen miniature pig (Mahady et al., 2016), a partial description of the cholinergic, and full descriptions of the catecholaminergic and serotonergic systems have been provided for sheep (Ferreira et al., 2001; Iqbal et al., 2001), and aspects of the catecholaminergic and orexinergic systems have been described in the cow and pig (Tillet and Thibault, 1989; Østergaard et al., 1992; Kitahama et al., 1994; Tillet, 1994; Leshin et al., 1995a,b, 1996; for review, see Tillet and Kitahama, 1998; Ettrup et al., 2010). While, for the most part, the nuclear organization of these systems can be thought of as typically mammalian, certain features have been identified that could be described as specific features of the Artiodactyl brain. In the giraffe it has been noted that the neurons of the cholinergic laterodorsal tegmental (LDT) nucleus are larger than those of the pedunculopontine tegmental (PPT) nucleus (Bux et al., 2010), whilst the opposite was reported for the Goettingen mini pig (Mahady et al., 2016). The orexinergic neurons of the hypothalamus, while having the three clusters generally observed in mammals, form an additional medially located parvocellular cluster not observed in most other mammals, but is seen in the Cetartiodactyls studied to date, including the harbour porpoise, minke whale and hippopotamus, and also in the Afrotherian African elephant (Dell et al., 2012, 2016a, 2016b, 2016c; Maseko et al., 2013).

While the organization of these nuclei appears to be predictable across species within the same mammalian order (for review, see Manger, 2005), the pressure of surviving within the harsh Arabian desert may have led to the evolution of specific specializations within the brain of the Arabian oryx. Thus, the aim of the current study is twofold: (1) to provide the first complete description of the systems that have been implicated in sleep control in the brain of an Artiodactyl; and (2) to determine whether the harsh desert environment has led to the evolution of any specific neural novelties related to these nuclei in the Arabian oryx.

2. Materials and methods

2.1. Brain acquisition

Two adult female Arabian oryx (average body mass of 65 kg, average brain mass of 166 g, not pregnant, not lactating), from the National Wildlife Research Centre near Taif in the Kingdom of Saudi Arabia, that were part of a planned management cull, were overdosed on the same day using sodium pentobarbital (200 mg/kg i.v.) under permission from the Saudi Wildlife Authority. All animals were handled according to the guidelines of the University of the Witwatersrand Animal Ethics Committee (clearance number 2008/36/1), which parallel those of the National Institute of Health (NIH) for the care and use of animals in scientific experimentation. The brains of these animals were perfusion fixed *via* the internal carotid arteries with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4, 4 °C) (Manger et al., 2009). The brains were then removed from the skulls (Fig. 1) and post-fixed for 48 h in 4% paraformaldehyde in 0.1 M PB at 4 °C. The brains were then transferred to a 30% sucrose in 0.1 M PB solution at 4 °C until equilibrated (approximately 7 days), and then placed in an antifreeze solution for the same length of time. The brains were stored in the antifreeze solution at −20 °C until immunohistochemical processing.

2.2. Immunohistochemistry

Blocks of tissue extending from the basal forebrain to the pons were dissected from the remainder of the brain, allowed to equilibrate in a 30% sucrose in 0.1 M PB solution at 4 °C, and then frozen in crushed dry ice. The blocks were attached to an aluminium stage, and sectioned at 50 μm in the coronal plane using a sliding microtome. A one in twenty series of serial sections was taken, and the first eight of each series was stained for Nissl, myelin, choline acetyltransferase (ChAT), tyrosine hydroxylase (TH), serotonin (5HT), orexin (OxA), calbindin (CB) and calretinin (CR). The remaining sections were placed in antifreeze and stored at −20 °C for later use. Sections used for the Nissl series were mounted on 1% gelatine coated glass slides and once dried, cleared overnight in a solution of 1:1 chloroform and absolute alcohol and stained with 1% cresyl violet. Myelin sections were first stored in 5% formalin for two weeks at 4 °C then mounted on 1.5% gelatine-coated slides and stained with a silver solution to reveal myelin sheaths (Gallyas, 1979).

For the immunohistochemical staining each section was treated with endogenous peroxidase inhibitor (49.2% methanol: 49.2% 0.1 M PB: 1.6% of 30% H_2O_2) for 30 min and subsequently subjected to three 10 min 0.1 M PB rinses. Sections were then pre-incubated for 2 h, at room temperature, in blocking buffer (containing 3% normal goat serum for the TH, 5-HT, OxA, CB and CR sections or 3% normal rabbit serum for the ChAT sections, plus 2% bovine serum albumin and 0.25% Triton-X in 0.1 M PB). This was followed by three 10 min rinses in 0.1 M PB. The sections were then placed in the primary antibody solution that contained the appropriately diluted primary antibody in blocking buffer for 48 h at 4 °C under gentle agitation. Anti-choline acetyltransferase (AB144P, Millipore, raised in goat) at a dilution of 1:3000 was used to reveal cholinergic neurons. Anti-tyrosine hydroxylase (AB151, Millipore, raised in rabbit) at a dilution of 1:7500 revealed the putative catecholaminergic neurons. Serotonergic neurons were revealed using anti-serotonin (AB938, Millipore, raised in rabbit) at a dilution of 1:7500. Orexinergic neurons were revealed using anti-Orexin A (AB3704, Millipore, raised in rabbit) at a dilution of 1:3000. Neurons and terminal networks containing calbindin and calretinin were revealed using anti-calbindin (CB38, SWant, raised in rabbit, 1:10 000) and anti-calretinin (7699/3H, SWant, raised in rabbit, 1:10 000). This incubation was followed by three 10 min rinses in 0.1 M PB and the sections were then incubated in a secondary antibody solution (1:1000 dilution of biotinylated anti-rabbit IgG, BA-1000, Vector Labs, for TH, 5-HT, OxA, CB and CR sections, or a 1:1000 dilution of biotinylated anti-goat IgG, BA-5000, Vector Labs, for ChAT sections, in a blocking buffer containing 3% NGS/NRS and 2% BSA in 0.1 M PB) for 2 h at room temperature. This was followed by three 10 min rinses in 0.1 M PB, after which the sections were incubated for 1 h in avidin-biotin solution (at a dilution of 1:125, Vector Labs), followed by three 10 min rinses in 0.1 M PB. Sections were then placed in a solution of 0.05% diaminobenzidine (DAB) in 0.1 M PB for 5 min, followed by the addition of 3 μl of 3% hydrogen peroxide to each 1 ml of solution in which each section was immersed. Chromatic precipitation was visually monitored and verified under a low power stereomicroscope. Staining was continued until such time as the background stain was at a level that would assist architectural reconstruction without obscuring the immunopositive structures. Development was arrested by placing sections in 0.1 M PB, followed by two more rinses in this solution. Sections were then mounted on 0.5% gelatine coated glass slides, dried overnight, dehydrated in a graded series of alcohols, cleared in xylene and coverslipped with Depex. To test for non-specific staining of the immunohistochemical protocol, in selected sections the primary antibody was omitted and replaced with antibody diluent followed by secondary antibody incubation and detection agents which resulted in no staining of the tissue. Primary antibody pre-incubation absorption controls were also conducted with each specific antigen in selected sections with no staining results.

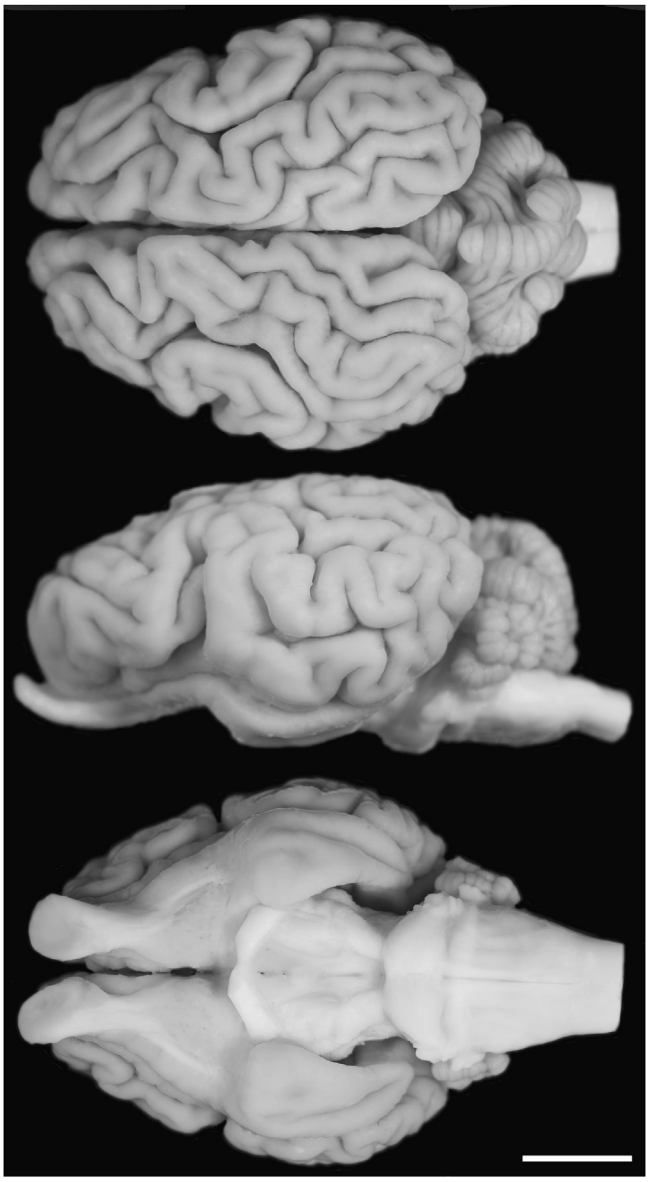


Fig. 1. Photographs of the dorsal (top), lateral (middle) and ventral (bottom) aspects of the Arabian oryx brain. Scale bar = 2 cm.

2.3. Analysis of results

Sections were examined with a low power stereomicroscope and the architectonic borders were traced according to the Nissl and myelin stained sections using a camera lucida (Leica MZ75). Corresponding immunostained sections were matched to the drawings and the immuno-positive neurons and terminal networks densities were marked or noted. All drawings were then scanned and redrawn using the Canvas 8 drawing program (Figs. 2–4). The nomenclature used for the cholinergic nuclei was adopted from Woolf (1991, review), Limacher et al. (2008), Bhagwandin et al. (2008), Gravett et al. (2009), Pieters et al. (2010), and Calvey et al. (2013), the catecholaminergic nuclei from Hökfelt et al. (1984), Smeets and González (2000), Limacher et al. (2008), Bhagwandin et al. (2008), Gravett et al. (2009), Pieters et al. (2010)

and Calvey et al. (2013), the serotonergic nuclei from Törk (1990), Limacher et al. (2008), Bhagwandin et al. (2008), Gravett et al. (2009), Pieters et al. (2010) and Calvey et al. (2013), and the orexinergic nuclei from Kruger et al. (2010), Bhagwandin et al. (2011), Gravett et al. (2011), Dell et al. (2012), Dell et al. (2016a), Dell et al. (2016b), Dell et al. (2016c) and Calvey et al. (2013).

2.4. Stereological analysis

Using stereological analysis, as described in Dell et al. (2016a, 2016b, 2016c), the number of orexinergic immunopositive neurons (OxA) in the hypothalamus, number of cholinergic immunopositive neurons (ChAT) in the laterodorsal tegmental nucleus (LDT) and pedunculopontine tegmental nucleus (PPT) as well as the number of noradrenergic immunopositive neurons (TH) in the locus coeruleus were determined. A Zeiss Z2 vario axioimager equipped with a three-plane motorized stage, video camera, and integrated Stereo-Investigator software (MicroBrightField, Colchester, VT, version 11.08.1) was used for the stereological counts. Independent pilot studies for the LDT, PPT, locus coeruleus, and hypothalamus were conducted on individual brain slices to optimize sampling parameters for cell counting (Table 1). Counting frames and grid sizes were optimized to achieve a mean coefficient of error of 10% or less (Gundersen and Jensen, 1987), and a guard zone of 2 μm was set so as to not include errors due to sectioning artifacts (West et al., 1991). Section thickness was measured at every fifth sampling site, and the number of immunopositive neurons was counted in accordance with the principles of the optical fractionator method (West et al., 1991). In the PPT, LDT, hypothalamus, and locus coeruleus, a standardized stereological approach using simple random sampling was implemented with counting frames of 400 × 400 μm for the PPT and LDT and 350 × 350 μm for the locus coeruleus and hypothalamus. Corresponding grid sizes of 400 × 400 μm were used for the PPT and LDT and 450 × 450 μm for the locus coeruleus, and hypothalamus (Table 1). The optical fractionator method was used to computationally determine the number of ChAT+ neurons in the LDT and PPT as well as the number of TH+ neurons in the locus coeruleus and the OxA+ neurons in the hypothalamus using the following formula: N = Q/(SSF x ASF x TSF) where N is the total estimated neuronal number, Q is the number of neurons counted, SSF is the section sampling fraction, ASF is the area subfraction (ratio of the size of the counting frame to the size of the sampling grid), and TSF is the thickness subfraction (ratio of the dissector height relative to cut section thickness) (Table 1). The nucleator probe was used to estimate the mean volume and cross-sectional area of the immunopositive neurons, in conjunction with fractionator sampling (Gundersen, 1988). To measure the size of the PPT, LDT and orexin neurons, we used a modified unbiased stereological approach by performing exhaustive total counts using a counting frame and grid size of 400 × 400 μm.

3. Results

Nuclei from the following sleep related neuronal systems were immunohistochemically identified in the brain of the Arabian oryx: the cholinergic, catecholaminergic, serotonergic, orexinergic as well as the GABAergic neurons and terminal networks revealed using antibodies to the calcium binding proteins calbindin and calretinin. The results revealed that the majority of these nuclei

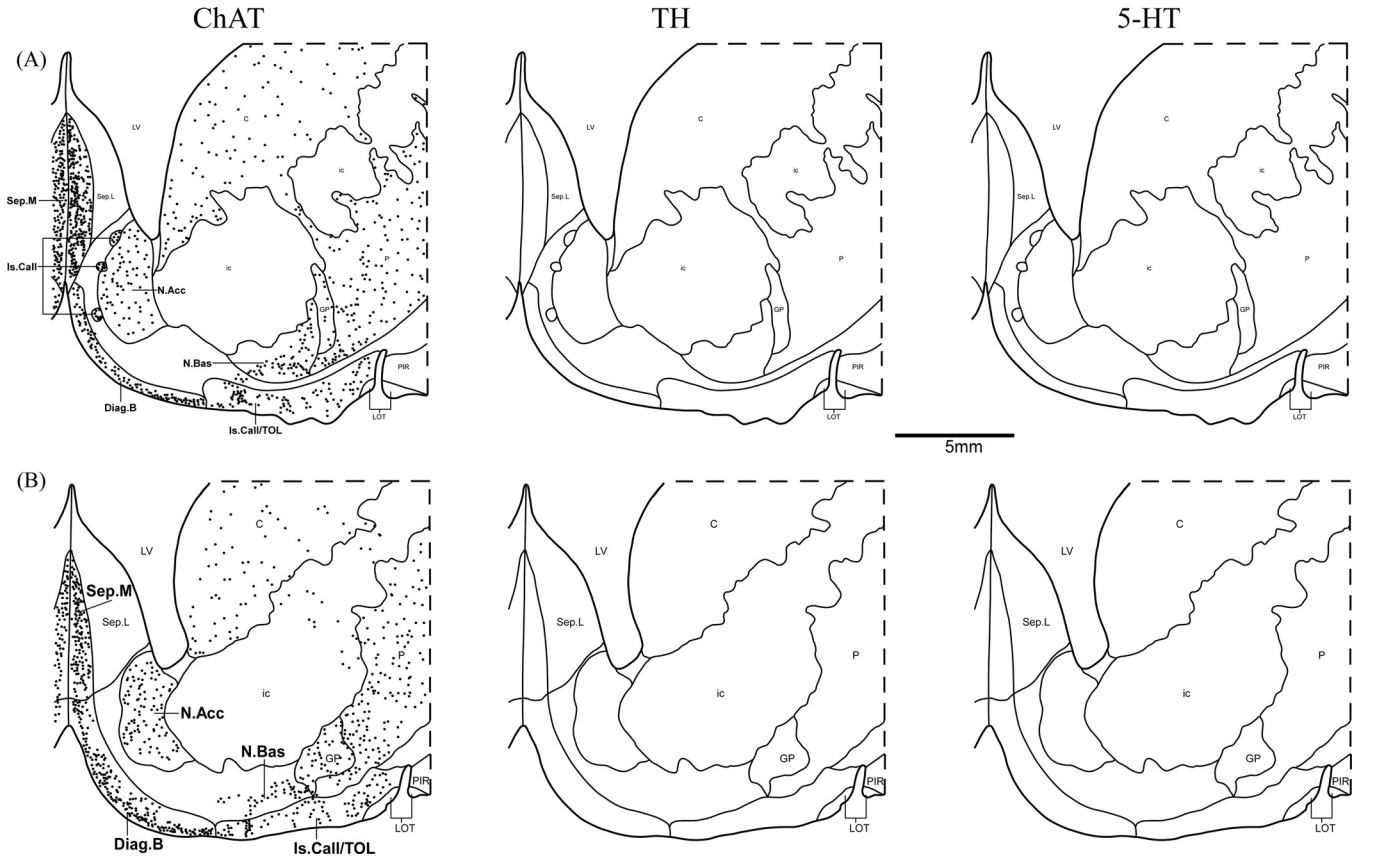


Fig. 2. Diagrammatic reconstructions of two coronal sections through the basal forebrain of the Arabian oryx brain illustrating the location of neurons forming nuclei immunopositive for choline acetyltransferase (ChAT, closed circles), tyrosine hydroxylase (TH, closed triangles) and serotonin (5-HT, closed squares). A is the more rostral section, B the more caudal. The drawings are approximately 1000 μm apart. Each symbol represents a single cell body. Reconstructions represent a single Arabian oryx brain (no architectural or organizational differences were observed between both brains examined). See list for abbreviations.

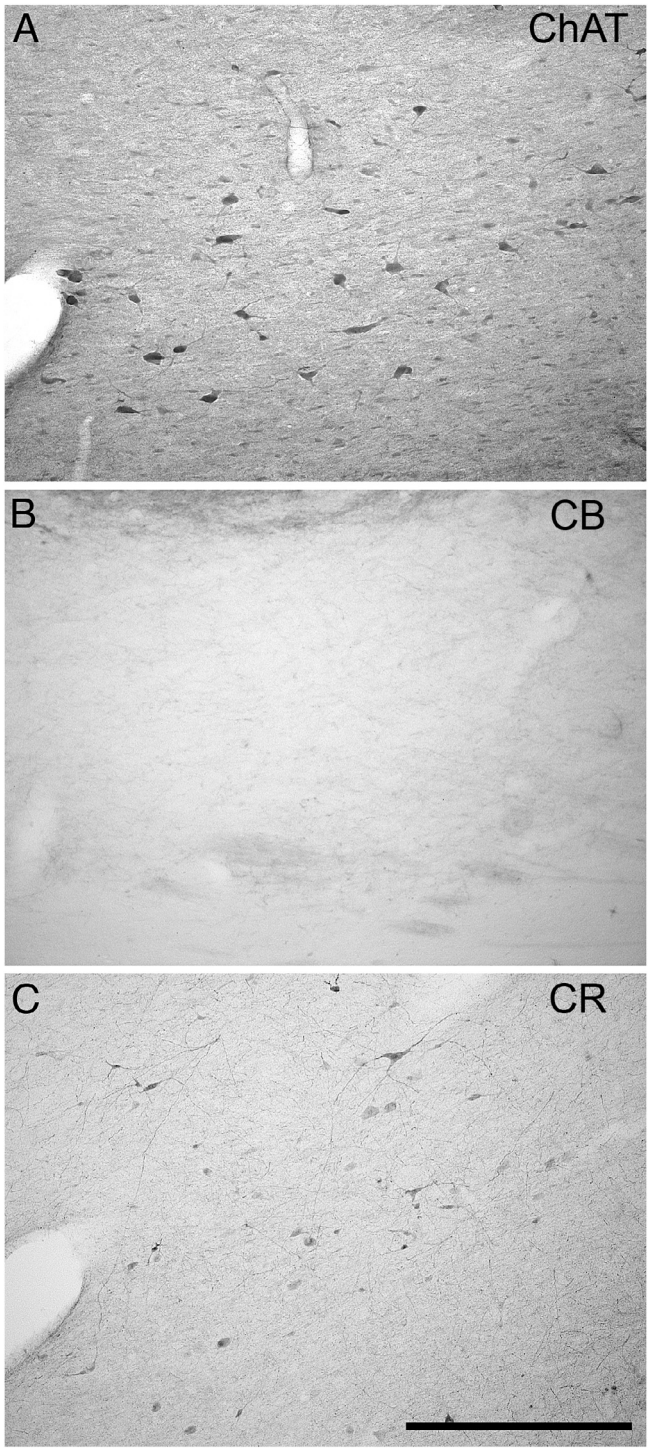


Fig. 3. Photomicrographs showing (A) the cholinergic (ChAT) immunopositive neurons of the nucleus basalis, (B) the virtual lack of calbindin (CB) immunoreactivity, and (**C**) the presence of calretinin (CR) immunopositive neurons and terminal networks. In each photomicrograph dorsal is to the top and medial to the left. Scale bar in C = 500 μm and applies to all.

followed the typical mammalian organizational plan of these systems (e.g. Bhagwandin et al., 2013); however, some features unique to this species, as well as some order specific features (Bux et al., 2010; Dell et al., 2012, 2016a, 2016b, 2016c; Mahady et al., 2016), were also identified in the Arabian oryx. The differences when compared to most mammals were: (1) the cholinergic neurons of the pedunculopontine tegmental nucleus (PPT) were larger than those of the laterodorsal tegmental nucleus (LDT); (2) the presence of a medially located parvocellular subdivision of the hypothalamic orexinergic neurons, seen only in Cetartiodactyls (Dell et al., 2012, 2016b, 2016c) and the African elephant (Maseko et al., 2013); (3) the presence of the dorsal division of the catecholaminergic anterior hypothalamic group (A15d), an Arabian oryx specific feature; and (4) an expansion of the catecholaminergic tuberal cell group (A12), an Arabian oryx specific feature.

3.1. Cholinergic nuclei of the basal forebrain and pons

The following cholinergic sleep-related nuclei located within the basal forebrain and pons of the Arabian oryx were identified: the medial septal nucleus, diagonal band of Broca, islands of Calleja and olfactory tubercle, nucleus basalis, laterodorsal tegmental nucleus (LDT) and the pedunculopontine tegmental nucleus (PPT). The medial septal nucleus was located within the septal nuclear complex in the rostral half of the medial wall of the cerebral hemisphere (Fig. 2). The region contained a high density of choline acetyltransferase immunopositive (ChAT+) bipolar and multipolar neurons. The islands of Calleja and olfactory tubercle were located in the ventral most portion of the cerebral hemisphere at a level ventrolateral to the nucleus accumbens (Fig. 2). These nuclei extend from the level of the anterior horn of the lateral ventricle to the level of the anterior commissure. The islands of Calleja were identified as clusters of moderately dense bipolar and multipolar ChAT+ neurons. Also noted were three small, low density clusters of bipolar ChAT+ neurons along the medial border of the nucleus accumbens forming the islands of Calleja distinct to the olfactory tubercle. The areas surrounding the ventrally located islands of Calleja were assigned to the olfactory tubercle and within this region a moderate density of ChAT+ neurons showing similar neuronal morphologies to the islands of Calleja was observed. The diagonal band of Broca was identified as a cluster of moderately dense ChAT+ neurons located rostral to the hypothalamus in the ventromedial corner of the cerebral hemisphere (Fig. 2). This nucleus consisted of a mixture of bipolar and multipolar neurons. A low to moderately dense cluster of ChAT+ neurons located slightly ventromedial to the globus pallidus at the level of the anterior commissure was identified as the nucleus basalis (Fig. 2). This nucleus consisted of a mixture of both bipolar and multipolar neurons (multipolar predominating) (Fig. 3A).

The PPT nucleus was located within the dorsal aspect of the pontine tegmentum in a position both superior (rostrally) and inferior (caudally) to the superior cerebellar peduncle (Fig. 4). This nucleus was characterised by a moderate to high density of bipolar and multipolar ChAT+ neurons. Within the ventrolateral region of the pontine periventricular grey matter, a moderately dense cluster of ChAT+ neurons surrounded by a moderate neuropil density was identified as the LDT nucleus (Figs. 4 and 5A). A mixture of bipolar and multipolar, ChAT+, neurons was observed within this nucleus. It was noted that the PPT ChAT+ neurons had larger somata than those of the LDT. The LDT cells had a mean somal volumes ranging from 1 000 to 3 500 μm^3 (average volume 2 823.82 μm^3, SD ± 1 863.38) and average surface areas ranging from 100 to 300 μm^2 (average area 222.42 μm^2, SD ± 97.74) (Table 2). The PPT cells had a mean somal volumes ranging from 2 500 to 6 000 μm^3 (average volume 3 899.76 μm^3, SD ± 1 871.58), and average surface areas ranging from 150 to 400 μm^2 (average area 279.31 μm^2, SD ± 90.45) (Table 2). The LDT neurons were significantly smaller than the size of the PPT neurons (Mann-Whitney, $p < 0.001$). Stereological analysis revealed that there were approximately 10 251 cholinergic neurons in the PPT, and approximately 10 874 cholinergic neurons in the LDT.

3.2. Catecholaminergic nuclei of the hypothalamus and pons

Within the hypothalamus and pons of the Arabian oryx, tyrosine hydroxylase immunostaining revealed a number of nuclei in both regions of the brain that are similar to that observed in many mammalian species (Dahlström and Fuxe, 1964; Dell et al., 2010; Calvey et al., 2013). Within the brain of the Arabian oryx two additional features of note were observed: (1) the presence of the dorsal division of the anterior hypothalamic group (A15d), which is usually absent in Artiodactyls; and (2) an expansive tuberal cell group (A12). For the purpose of this study only the aforementioned two nuclei, as well as those directly related to the systems that have been implicated in sleep control in other mammals, the nuclei of the locus coeruleus complex, will be described.

A low to moderate density of tyrosine hydroxylase immunopositive (TH+) bipolar and multipolar neurons, forming the dorsal division of the anterior hypothalamic nucleus (A15d), was located in the dorsomedial aspect of the hypothalamus (Fig. 6). The TH+ neurons forming the tuberal cell group (A12) were located medially within the ventromedial hypothalamus, medial and ventral to the arcuate nucleus (Fig. 6). The A12 nucleus was characterised by a moderate to high density of bipolar TH+ neurons that extended as far dorsally as the A14 nucleus, and for almost the entire rostrocaudal extent of the hypothalamus.

The locus coeruleus complex was identified as a large group of TH+ neurons within the pons that could be readily subdivided into three distinct nuclear groups: the subcoeruleus compact portion (A7sc), subcoeruleus diffuse portion (A7d) and the locus coeruleus diffuse portion (A6d) (Figs. 4 and 7A). Within the dorsal portion of the pontine tegmentum, between the ventrolateral aspect of the periventricular grey and medial to the superior cerebellar peduncle a high density of TH+ neurons were identified as the A7sc nucleus

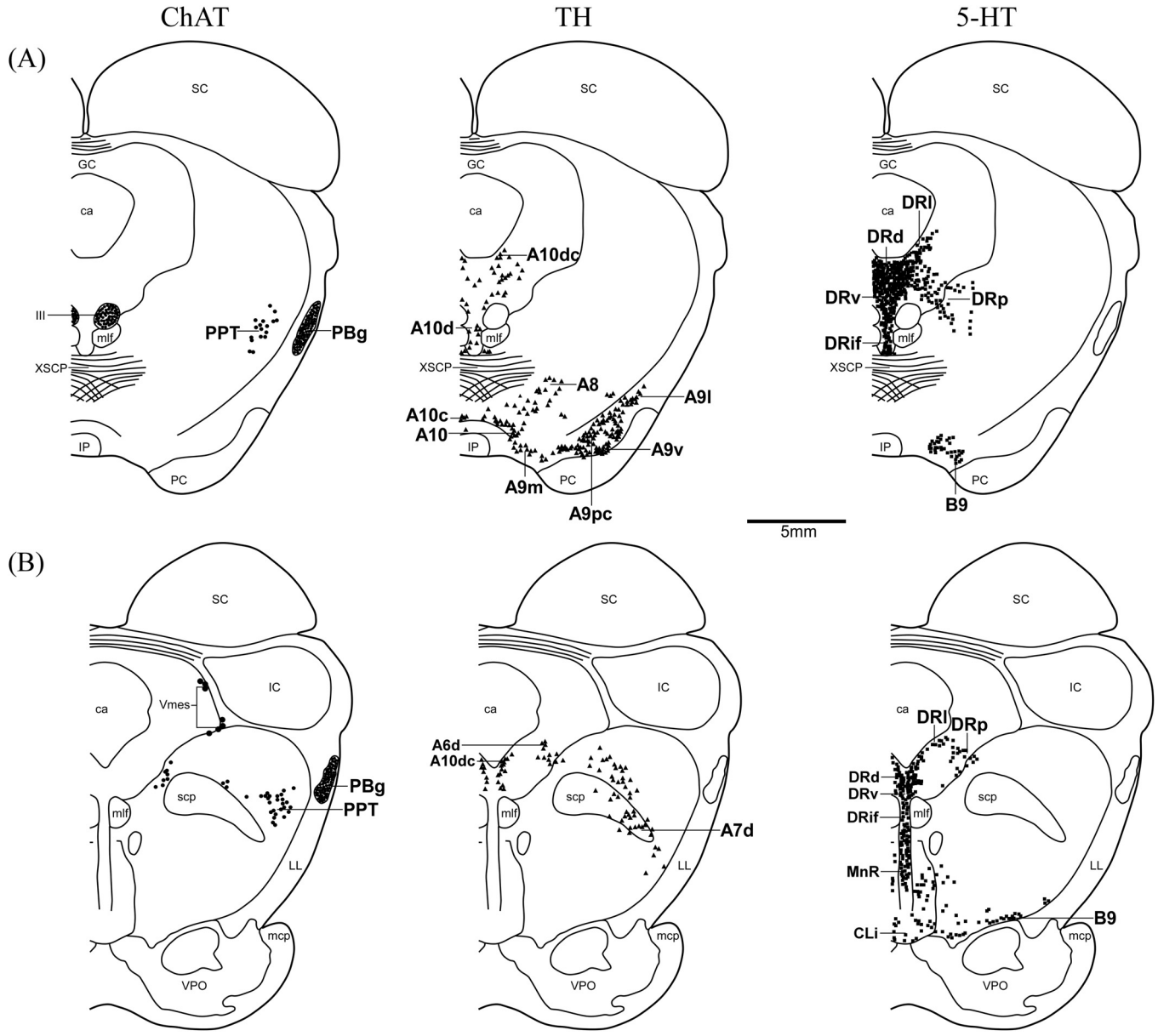


Fig. 4. Diagrammatic reconstructions of a series of coronal sections through the midbrain and pons of the Arabian oryx illustrating the location of neurons immunohistochemically reactive for choline acetyltransferase (ChAT, closed circles), tyrosine hydroxylase (TH, closed triangles) and serotonin (5-HT, closed squares). Drawing A represents the most rostral section, E the most caudal. The drawings are approximately 1000 μm apart. Each symbol represents a single cell body. Reconstructions represent a single Arabian oryx brain (no architectural or organizational differences were observed between both brains examined). See list for abbreviations.

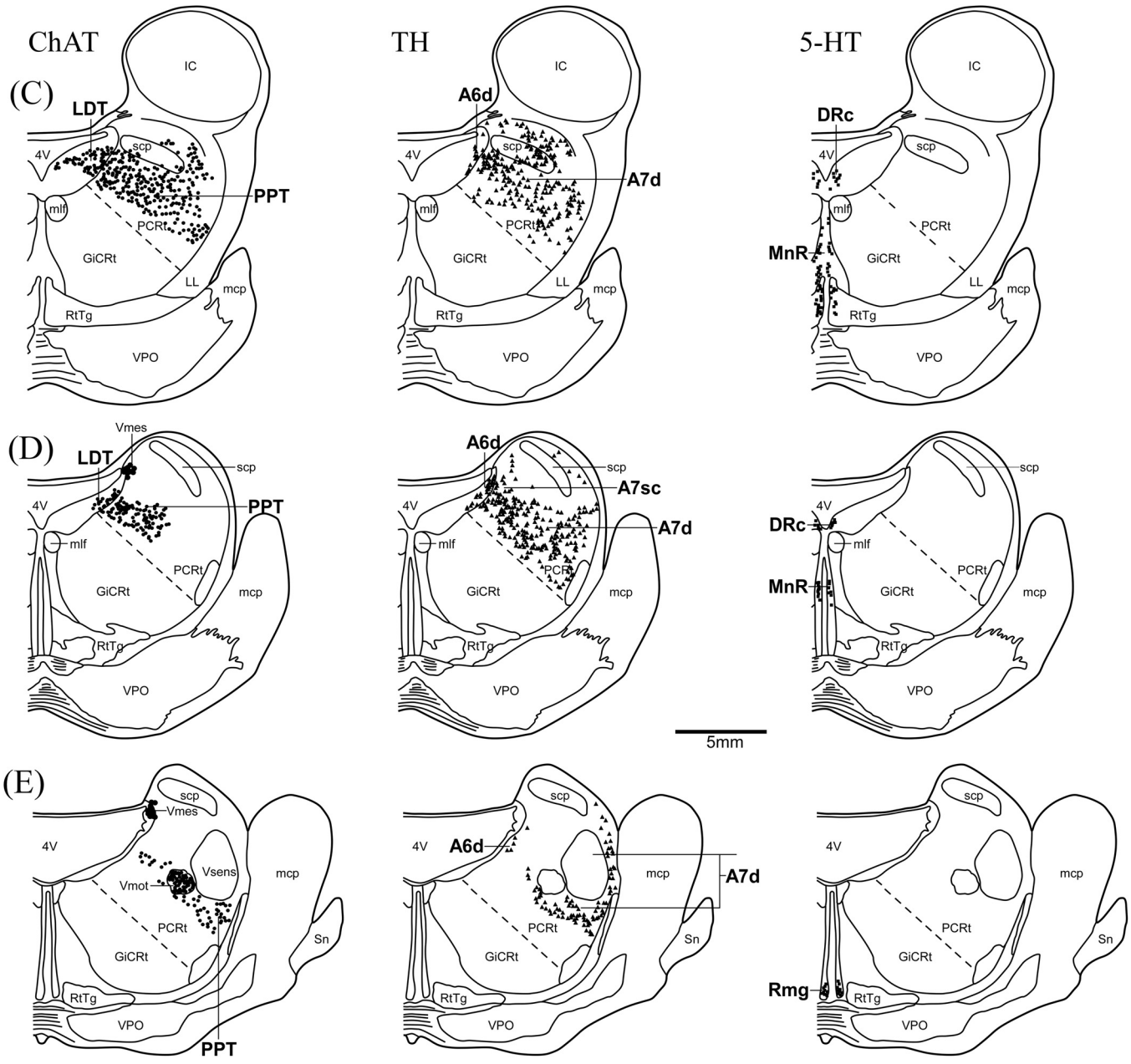


Fig. 4. (Continued)

Table 1
Stereological parameters used for estimating cell numbers in the Arabian oryx brain.

Nucleus examined	Counting frame size (μm)	Sampling grid size (μm)	Dissector height (μm)	Cut thickness (μm)	Average mounted thickness (μm)	Vertical guard zones (top and bottom, μm)	Section interval	No. of sections	No. of sampling sites	Average CE (Gundersen m = 1)
LDT (ChAT+)	400 × 400	400 × 400	16	50	21.8	2	18	3	164	0.07
PPT (ChAT+)	400 × 400	400 × 400	16	50	21.8	2	18	4	759	0.08
LC (TH+)	350 × 350	450 × 450	15	50	20.3	2	18	9	2437	0.05
Orexin (OxA+)	350 × 350	450 × 450	14	50	18.7	2	18	6	954	0.06

Abbreviations: LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine nucleus; LC, locus coeruleus complex; ChAT+, neurons immunopositive for choline acetyltransferase; TH+, neurons immunopositive for tyrosine hydroxylase; OxA+, neurons immunopositive for orexin-A.

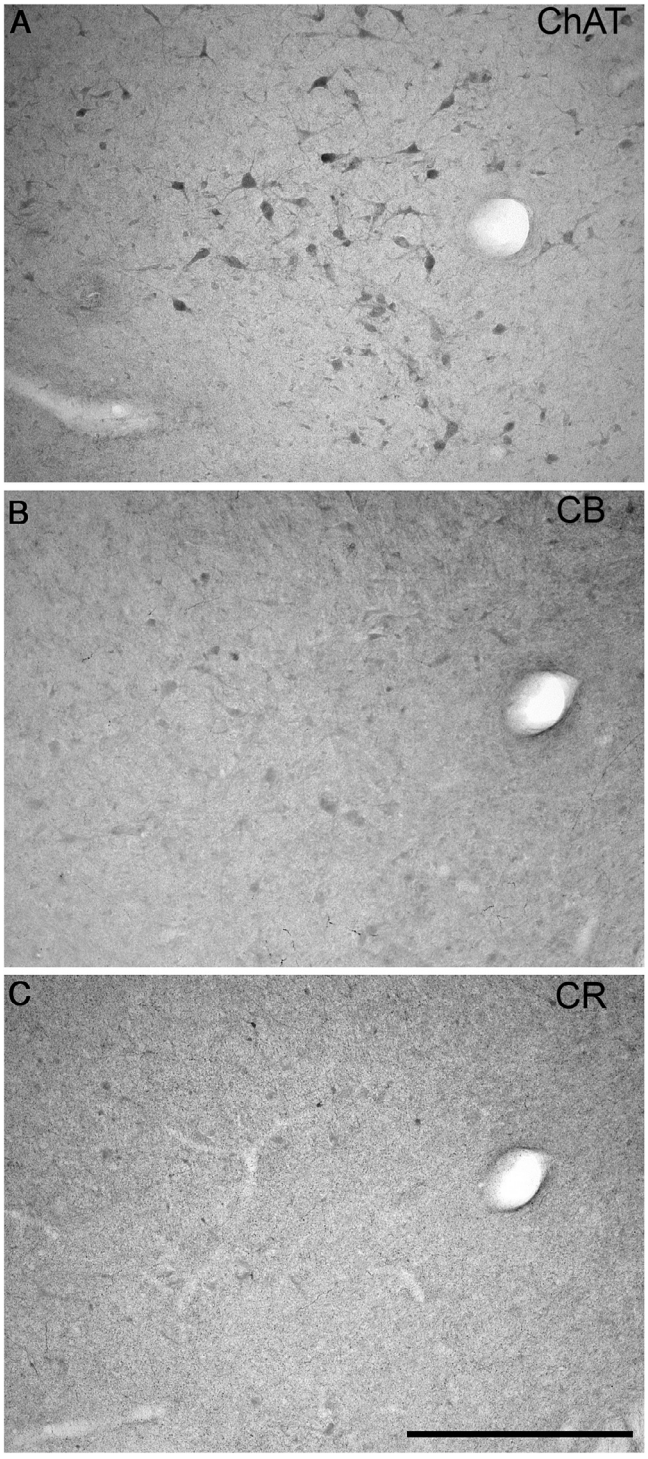


Fig. 5. Photomicrographs showing (A) the cholinergic (ChAT) immunopositive neurons of the laterodorsal tegmental nucleus (LDT), (B) the moderate density of calbindin (CB) immunopositive neurons and the high density calbindin immunopositive terminal network, and (C) the low density of calretinin (CR) immunopositive neurons, and the moderate density calretinin immunopositive terminal network. In each photomicrograph dorsal is to the top and medial to the left. Scale bar in **C** = 500 μm and applies to all.

(Fig. 4D). This group consisted predominantly of bipolar neurons interspersed with occasional multipolar neurons. A moderate to low density of TH+ neurons surrounding the superior cerebellar peduncle on both the dorsolateral and ventromedial borders was identified as A7d (Fig. 4B–E). Caudally this nucleus extended from the dorsolateral border of the superior cerebellar peduncle ventrally between the lateral border of the sensory division of the trigeminal nerve nucleus and the medial border of the lateral lemniscus. Both bipolar and multipolar neuronal morphologies were observed in this nucleus. The A6d nucleus was identified as a low to moderate density of bipolar and multipolar TH+ neurons located within the ventrolateral region of periventricular grey matter contiguous to the A7sc nucleus (Figs. 4B–E and 7A). Stereological analysis revealed that the locus coeruleus complex as a whole contained approximately 32 967 neurons. The cells had a mean somal volume ranging from 1 000 to 8 500 μm^3 (average volume 5 361.49 μm^3, SD ± 3 676.54) and average surface areas ranging from 150 to 550 μm^2 (average area 336.84 μm^2, SD ± 152.54) (Table 2).

3.3. Serotonergic nuclei

The sleep related nuclei containing serotonergic immunopositive (5HT+) neurons were located within the midbrain and pons and formed part of the rostral serotonergic nuclear cluster (for review, see Törk, 1990; Bjarkam et al., 1997). These nuclear groups followed the typical Eutherian mammal organizational plan (Maseko et al., 2007; Dell et al., 2010) and extended from the level of the decussation of the superior cerebellar peduncle through to the level of the trigeminal motor nucleus (Fig. 4). While other serotonergic nuclei were present in this region of the brain, those associated with sleep regulation and control described in the current study belongs to the dorsal raphe nuclear complex. Within the periaqueductal and periventricular grey matter six divisions of the dorsal raphe (DR) complex were identified: the interfascicular (DRif), ventral (DRv), dorsal (DRd), peripheral (DRp), lateral (DRl) and caudal (DRc) divisions. The DRif division consisted of a moderate to high density of bipolar 5HT+ neurons flanked on either side by the medial longitudinal fasciculi. A high density of scattered, bipolar 5HT+ neurons was observed immediately dorsal to the DRif division and caudal to the oculomotor nerve nuclei and was identified as the DRv division. The DRd division consisted of a high density of both bipolar and multipolar 5HT+ neurons located directly dorsal to the DRv division and ventral to the inferior border of the cerebral aqueduct (Fig. 8A). The DRp division was identified as a cluster of low to moderately dense 5HT+ neurons located lateral to the DRd and DRv divisions, in the ventrolateral portion of the periaqueductal grey matter. A low density of morphologically similar neurons considered part of the DRp division was also observed outside the periaqueductal grey matter in the adjacent midbrain tegmentum. The DRl division was located dorsolateral to the DRd division and adjacent to the ventrolateral borders of the cerebral aqueduct (Fig. 8A). The DRl consisted of a moderate density of both multipolar neurons that had distinctly larger soma than the 5HT+ neurons in the other divisions. The DRc division was located further caudally within the periventricular grey matter. A low to moderate density of 5HT+ neurons were observed along the ventrolateral border of the fourth ventricle in the rostral portion of this division, whereas further caudally the neurons became sparser and were located along the ventromedial border of the fourth ventricle. This nuclear division displayed an identical neuronal morphology to that of DRl, including that of the larger somal size (Fig. 4C and D).

3.4. Orexinergic nuclei

Orexin-A immunopositive neurons (OxA+) were identified throughout the hypothalamus of the Arabian oryx and could be divided into two cell types based on somal size, parvocellular and magnocellular. The magnocellular neurons were readily divided into three distinct clusters, while the parvocellular neurons formed a single cluster (Fig. 6). The parvocellular cluster was located

Table 2
Stereological results for bilateral cell numbers, volume and area in the Arabian oryx brain.

Nucleus examined	Total estimated population using mean section thickness	Average estimated cell volume (μm^3)	Average estimated cell area (μm^2)
LDT (ChAT+)	10,874	2823.82	222.42
PPT (ChAT+)	10,251	3899.76	279.31
LC (TH+)	32,967	5361.49	336.84
Orexin (OxA+) all cells	24,549	4786.87	306.32
Orexin (OxA+) magnocellular	–	7118.51	406.47
Orexin (OxA+) parvocellular	–	2408.44	206.58

Abbreviations: LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine nucleus; LC, locus coeruleus complex; ChAT+, neurons immunopositive for choline acetyltransferase; TH+, neurons immunopositive for tyrosine hydroxylase; OxA+, neurons immunopositive for orexin-A.

within the medial zone of the hypothalamus between the lateral edge of the third ventricle and the fornix. The parvocellular cluster was characterised by a moderate to high density of OxA+ neurons that were predominantly bipolar in nature with smaller soma sizes than the remaining orexinergic neurons.

The magnocellular neurons could be divided into three distinct clusters: a main cluster, a zona incerta cluster and an optic tract cluster (Fig. 6). The OxA+ neurons forming the main cluster were located within the perifornical region of the hypothalamus, extending into the lateral hypothalamic zone. Within this region a moderate to high density of mostly multipolar OxA+ neurons were observed (Fig. 9A). A low to moderate density of OxA+ neurons extended from the dorsolateral margin of the main cluster into the hypothalamic region adjacent to the zona incerta. These neurons were assigned to the zona incerta cluster and displayed a mixture of both bipolar and multipolar neurons. The optic tract cluster was characterised by a low density of OxA+ neurons. The neurons of this cluster were mostly bipolar in nature and extended from the ventrolateral margins of the main cluster towards the ventrolateral hypothalamus adjacent to the optic tract (Fig. 6). Stereological analysis revealed that the orexinergic hypothalamic cluster as a whole (all neurons) contained approximately 24 549

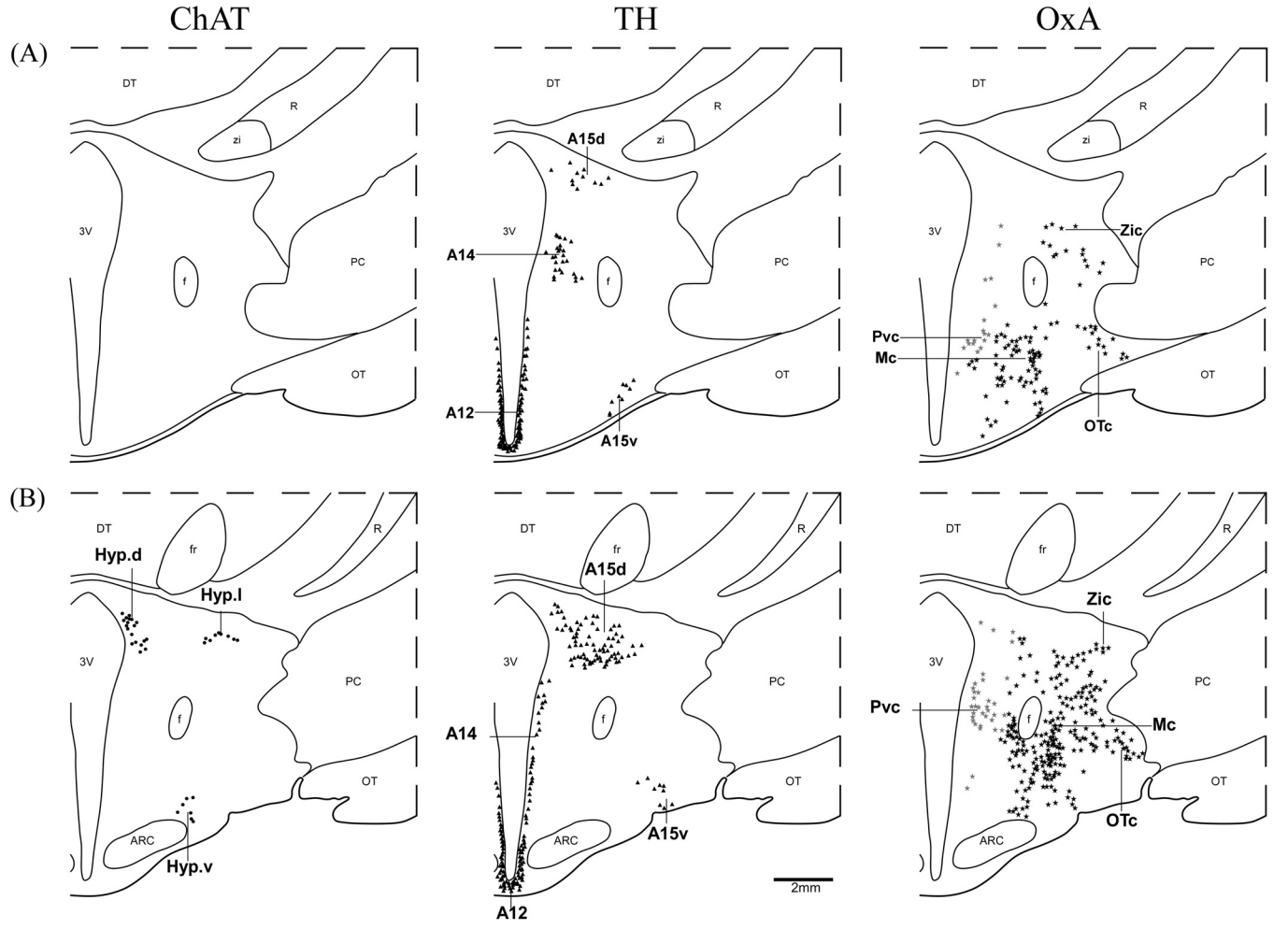


Fig. 6. Diagrammatic reconstructions of a series of coronal sections through the hypothalamus of the Arabian oryx brain illustrating the location of neurons forming nuclei immunopositive for choline acetyltransferase (ChAT, closed circles), tyrosine hydroxylase (TH, closed triangles) and orexin-A (OxA, parvocellular neurons – lighter grey stars, magnocellular neurons – closed black stars). Drawing A represents the most rostral section, D the most caudal. The drawings are approximately 1000 μm apart. Each symbol represents a single cell body. Reconstructions represent a single Arabian oryx brain (no architectural or organizational differences were observed between both brains examined). See list for abbreviations.

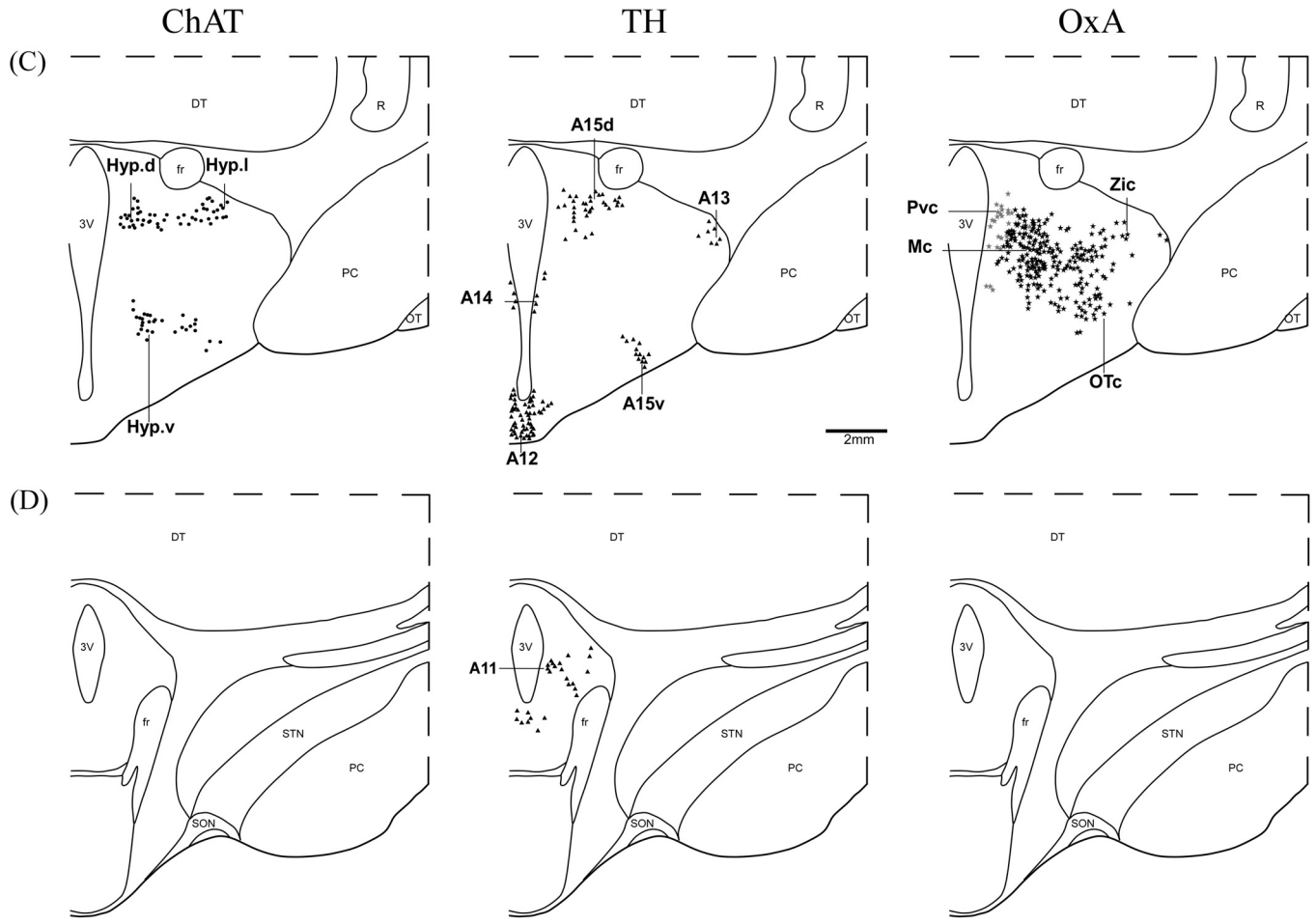


Fig. 6. (Continued)

neurons, with a mean somal volume of 4 786.87 μm^3 (SD ± 4 537.32) and an average surface area of 306.32 μm^2 (SD ± 159.55) (Table 2). The parvocellular orexinergic cells had mean somal volumes ranging from 1000 to 4000 μm^3 (average volume 2 408.44 μm^3, SD ± 797.00) and average surface areas ranging from 100 to 300 μm^2 (average area 206.58, SD ± 49.46). The magnocellular orexinergic cells had a mean somal volumes ranging from 5 000 to 10 000 μm^3 (average volume 7 118.51 μm^3, SD ± 1 410.21) and average surface areas ranging from 300 to 600 μm^2 (average area 406.47 μm^2, SD ± 79.10), being significantly larger than the parvocellular neurons (Mann-Whitney, $p < 0.001$).

3.5. Neurons and terminal networks containing calcium binding proteins

Potential GABAergic neurons and terminal networks were labelled immunohistochemically with antibodies targeted against the calcium binding proteins calbindin (CB) and calretinin (CR). Homogeneous immunostaining was observed for both oryx brains examined with regards to CB and CR immunopositive neurons and surrounding terminal networks. These proteins are generally found in specific subsets of GABAergic neurons that are involved sleep-wake state transitions (Siegel, 2004; Jones, 2007); however, within the basal forebrain and brainstem this general rule does not always hold true as other neurons can contain these calcium binding proteins (Gritti et al., 2003). The immunolabelling allowed us to provide descriptions of the CB immunopositive (CB+) and CR immunopositive (CR+) neuron and terminal network densities associated with the immunohistochemically identified cholinergic, catecholaminergic, serotonergic and orexinergic systems implicated in sleep control (summarized in Table 3). Refer to Fig. 10 for examples of low, moderate and high neuron and terminal network densities as described throughout the reported neuronal systems.

3.5.1. Neurons and terminal networks containing calcium binding proteins in the basal forebrain and pontine cholinergic nuclei

Within the medial septal nucleus a low density of CB+ neurons and terminal networks were identified. In contrast, a high density of both CR+ neurons and terminal networks were observed in this nucleus. A low density of CB+ neurons and terminal networks were observed in the diagonal band of Broca, while a moderate density of CR+ neurons and terminal networks were found in this nucleus. Within the olfactory tubercle and islands of Calleja, a moderate density of CB+ and CR+ neurons was observed, associated with a low density of CB+ terminal networks and a moderately dense CR+ terminal network. No CB+ neurons were observed in the nucleus basalis (Fig. 3B), but a low density CB+ terminal network was present. A moderate density of CR+ neurons and CR+ terminal networks were observed in the nucleus basalis (Fig. 3C).

Moderate densities of CB+ neurons were observed in the regions of the PPT and LDT nuclei, but the CB+ terminal network densities

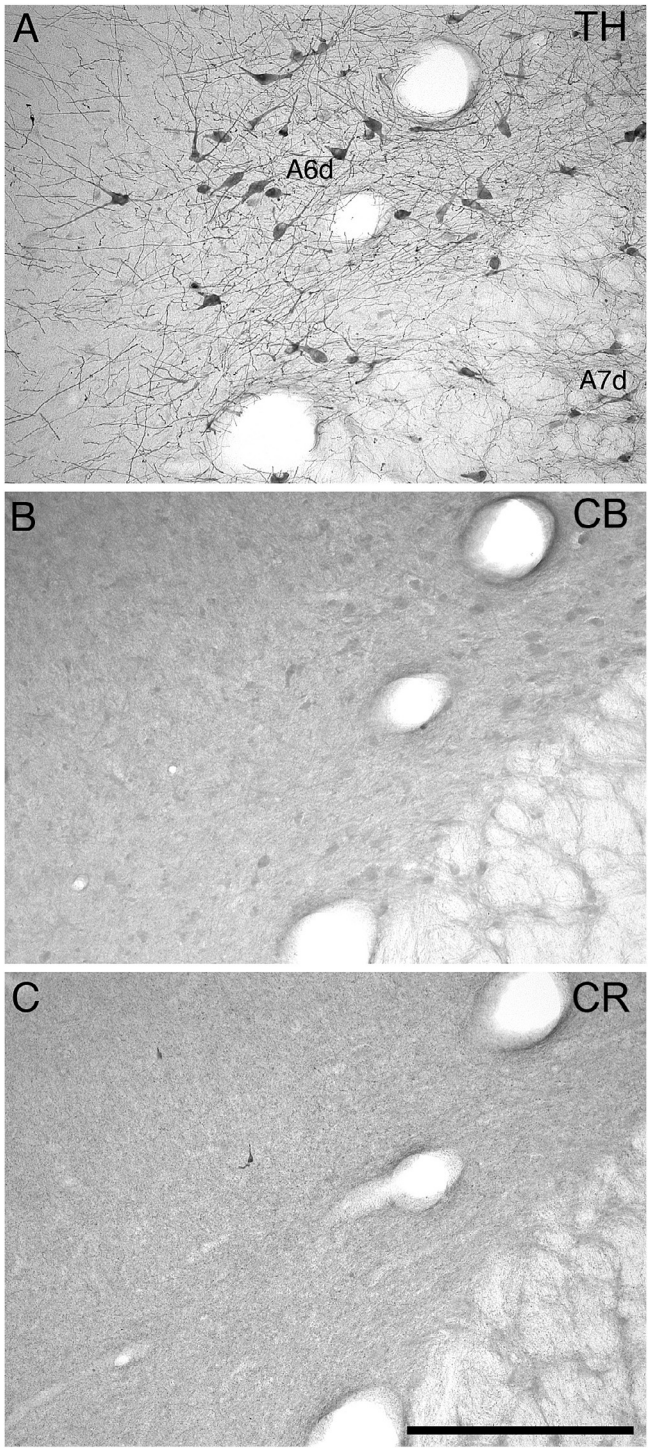


Fig. 7. Photomicrographs showing (A) tyrosine hydroxylase (TH) immunopositive neurons of the diffuse portion of the locus coeruleus (A6d) and a partial view of the diffuse portion of the subcoeruleus (A7d) nuclei, (B) the virtual lack of calbindin (CB) immunopositive neurons and the low density calbindin immunopositive terminal network, and (C) the occasional calretinin (CR) immunoreactive neuron, with a moderately dense calretinin immunopositive terminal network. In each photomicrograph dorsal is to the top and medial to the left. Scale bar in C = 500 μm and applies to all.

were moderate in the PPT nucleus and high for LDT nucleus (Fig. 5B). CR+ neuron densities were low in the region of the LDT nucleus and moderate in the PPT nucleus, whereas the CR+ terminal network densities were moderate in both the LDT and PPT nuclei (Fig. 5C).

3.5.2. Neurons and terminal networks containing calcium binding proteins in the locus coeruleus complex

A low density of CB+ neurons was observed in all three subdivisions of the locus coeruleus complex (A6d, A7sc and A7d) (Fig. 7B). In contrast the density of CB+ terminal networks ranged from low in A7d, to moderate in A7sc, through to high in A6d. In all three subdivisions a low density of CR+ neurons and a moderate density of CR+ terminal networks were observed (Fig. 7C).

3.5.3. Neurons and terminal networks containing calcium binding proteins in the dorsal raphe complex

Within the dorsal raphe nuclear complex, two different patterns of CB+ and CR+ staining could be observed. This pattern of staining separated the midline nuclei (DRif, DRv and DRd) from the more lateralized nuclei (DRl, DRp and DRc) which exhibited higher densities of both CB+ and CR+ structures. In the DRif, DRv and DRd no CB+ neurons could be observed (Fig. 8B), but a moderately dense CB+ terminal network was observed in all three subdivisions. CR+ neurons were absent in the DRif, and only in low density in the DRv and DRd (Fig. 8C), while all three subdivisions exhibited a moderately dense CR+ terminal network.

A moderate density of CB+ neurons were observed in the DRp, while both the DRl and DRc exhibited low densities of CB+ neurons (Fig. 8B). The DRc exhibited a high density CB+ terminal network, while both the DRl and DRp exhibited a moderately dense CB+ terminal network. The DRc had a low density of CR+ cells, while only the DRl and DRp exhibited a moderate density of CR+ neurons (Fig. 8C). All three of these subdivisions exhibited a high density CR + terminal network.

3.5.4. Neurons and terminal networks containing calcium binding proteins in the hypothalamic orexinergic complex

As with the serotonergic system, the clusters of orexinergic neurons could be separated into two distinct groups based on the densities of CB and CR immunolabelled neural structures, with the parvocellular and main clusters having substantially more immunolabelled structures than the zona incerta and optic tract clusters. Within the parvocellular and main clusters a moderate density of CB+ neurons, as well as a moderately dense CB+ terminal network was observed (Fig. 9B). While no CR+ neurons were observed in either cluster (Fig. 9C), a moderately dense CR+ terminal network was observed in both clusters. No CB+ neurons were evident in the region of the optic tract cluster, while only a low density of CB+ neurons were in the region of the zona incerta cluster. In both clusters a low density CB+ terminal network was observed. No CR+ neurons were observed in the zona incerta cluster, while a low density of CR+ neurons were revealed in the optic tract cluster. Both clusters evinced a moderately dense CR+ terminal network.

3.5.5. Neurons and terminal networks containing calcium binding proteins in the thalamic reticular nucleus

The thalamic reticular nucleus observed in the Arabian oryx brain occupies a similar position relative to the dorsal thalamic mass and has a similar appearance to that reported for other mammals (Jones, 2007). No CB+ neurons or terminal networks were noted for this nucleus, but a low density of CR+ neurons and a moderately dense CR+ terminal network was observed in this nucleus.

4. Discussion

The global aim of the current study was to provide a complete description of the nuclear organization of the sleep related neuronal systems in the brain of an Artiodactyl, the Arabian oryx. Furthermore, the current study also sought to reveal whether any

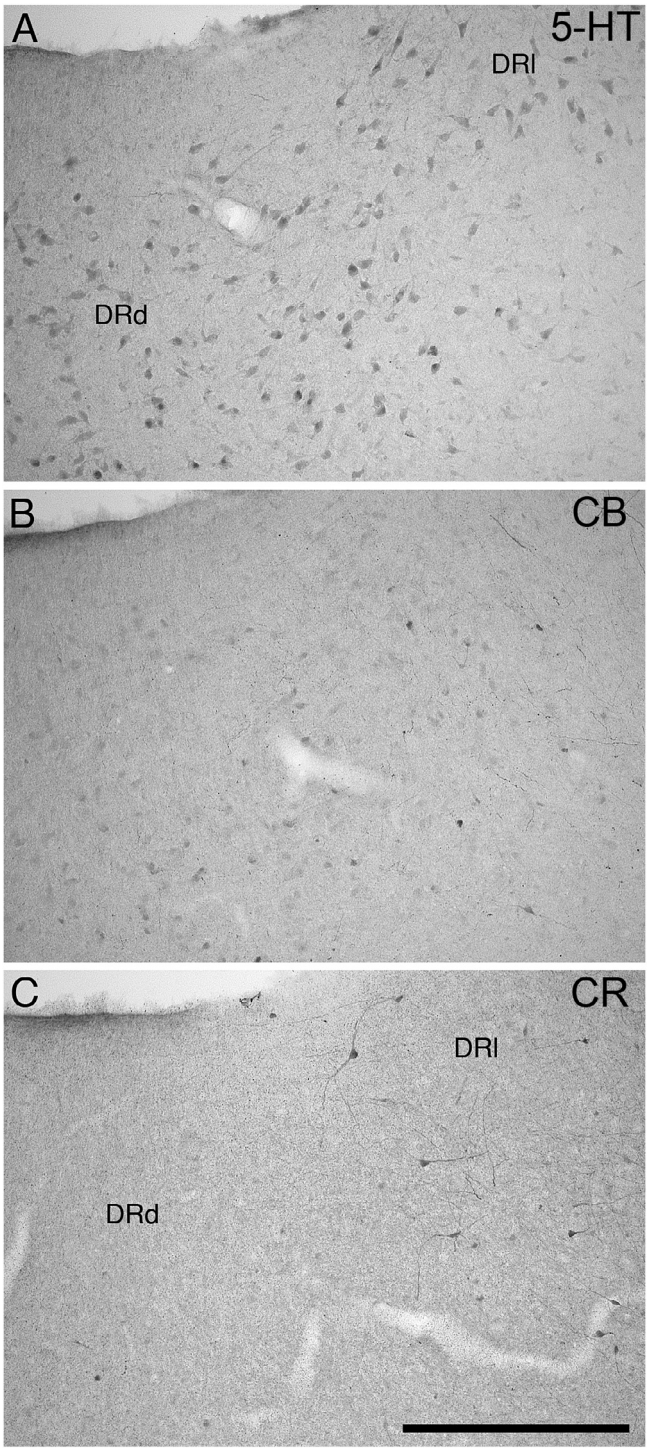


Fig. 8. Photomicrographs showing (A) serotonergic (5-HT) immunopositive neurons within the dorsal (DRd) and lateral (DRl) subdivisions of the dorsal raphe nuclear complex, (B) the low density of calbindin (CB) immunopositive neurons, and the moderate density of the calbindin immunopositive terminal network associated with these two divisions, and (C) the presence of a moderate to high density of calretinin (CR) immunopositive neurons and terminal networks. In each photomicrograph dorsal is to the top and medial to the left. Scale bar in C = 500 μm and applies to all.

specific neural novelties related to the sleep-wake cycle, or other physiological processes, may have evolved within the Arabian oryx brain in relation to the harsh desert environment that it inhabits. The results revealed that the nuclear organization of these systems, for the most part, followed that normally seen in other mammals (Maseko et al., 2007; Bhagwandin et al., 2013; Dell et al., 2010; Calvey et al., 2013), and in many respects were similar to previous observations made for other Cetartiodactyla studied to date (Bux et al., 2010; Dell et al., 2012, 2016a, 2016b, 2016c; Mahady et al., 2016). Despite this global similarity, some features were noted to be potentially either order-specific and or species-specific traits within the cholinergic, catecholaminergic and orexinergic systems that could possibly be related to the specific phylogenetic history or life history of the Arabian oryx (see Table 4). The expression of the GABAergic neuronal subtypes revealed with calbindin and calretinin immunohistochemistry and their association to the various sleep related nuclei exhibit both variation and similarity in terms of both neuron and terminal network density to that previously reported for rodents and Cetartiodactyls (Bhagwandin et al., 2013; Dell et al., 2016a, 2016b, 2016c).

4.1. Similarities in the nuclear organization of the sleep related neuronal systems across mammals and specifically artiodactyls

The cholinergic nuclei of the basal forebrain and pons, the locus coeruleus complex, the dorsal raphe complex, and the hypothalamic orexinergic clusters in the Arabian oryx were similar, in terms of their nuclear complement, the appearance of these nuclei in terms of location and neuronal distribution, and the neuronal morphology within these nuclei, to that previously reported in other artiodactyls (Tillet, 1987; Bux et al., 2010; Dell et al., 2012) and mammals in general (Steinbusch, 1981; Maseko et al., 2007; Gravett et al., 2009; Dell et al., 2010; Calvey et al., 2013). In a previous report on the giraffe and hippopotamus (Bux et al., 2010; Dell et al., 2016c), it was noted that the somal area of the cholinergic neurons of the LDT were larger than those of the PPT (LDT magnocellular neurons in the case of the hippopotamus). In contrast, a recent report on the Goettingen mini pig showed that the somal area of the cholinergic neurons of the PPT were larger than those of the LDT (Mahady et al., 2016). A similar observation to the Goettingen mini pig can be made for the Arabian oryx, potentially indicating that this size difference in the neurons of the pontine cholinergic nuclei is a variable Artiodactyl specific feature and may be related to brain size. A second specific feature reported in other Artiodactyls (Dell et al., 2012), and the African elephant (Maseko et al., 2013) and cetaceans (Dell et al., 2012, 2016b), is the medially located parvocellular orexinergic cluster in the hypothalamus. This feature was also readily observable in the Arabian oryx. Thus, it can be concluded that the nuclei potentially implicated in sleep control within the brain of the Arabian oryx conform to that seen generally across mammals, and very specifically to that observed in other Cetartiodactyls studied to date. This allows for speculation that the physiological manifestations of sleep in the Arabian oryx could be very similar to that seen in other mammals, but that there may be Artiodactyl specific features of sleep physiology in this species. In order to further relate the above mentioned neuronal adaptions to sleep physiology, examinations of sleep patterns from wild Arabian oryx under natural conditions in future studies are necessary.

4.2. Similarities and differences of the GABAergic systems across mammals

Certain groups of GABAergic neurons have been shown to promote non-REM sleep through inhibition of the arousal systems (Lin et al., 1989). The discharge patterns of the neurons containing calbindin or calretinin (which represent specific subsets of the GABAergic neurons) differ substantially, in that calbindin-containing neurons discharge tonically, whereas calretinin-containing neurons discharge in rapid bursts with an irregular pattern. The tonic discharge pattern of calbindin-containing neurons correlates

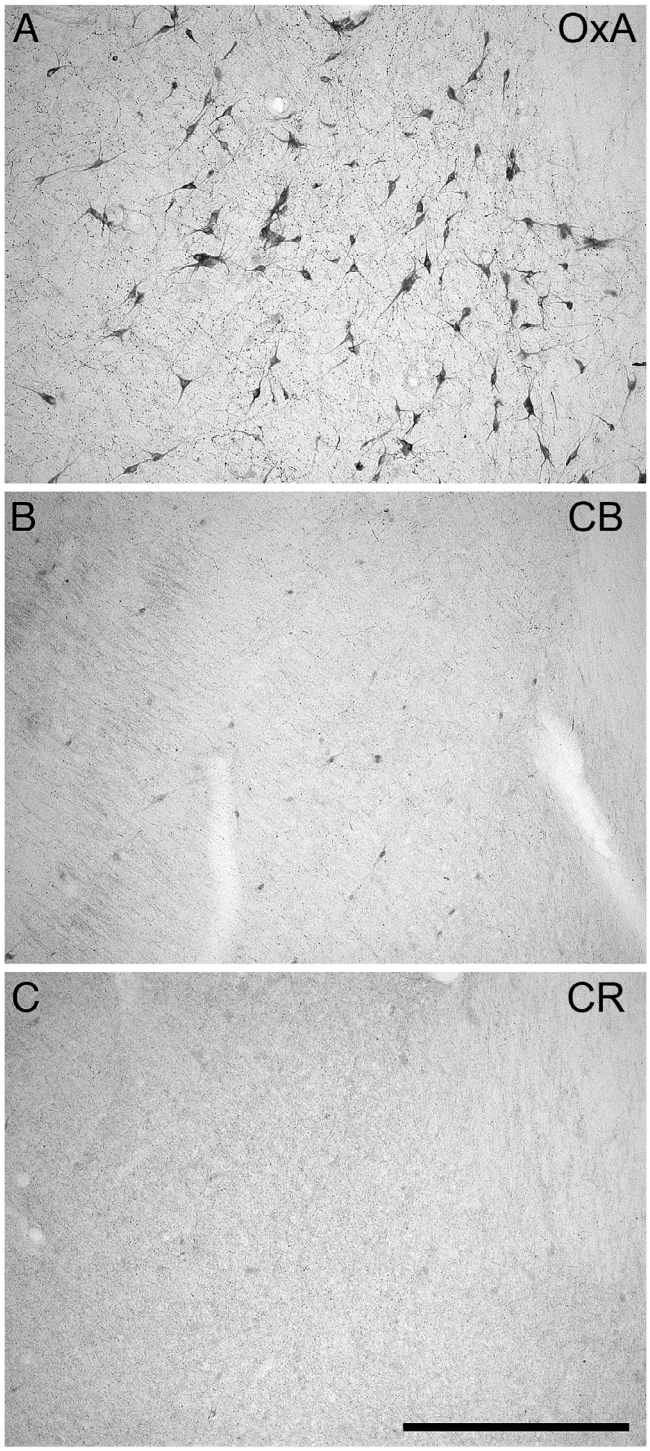


Fig. 9. Photomicrographs showing (A) the high density of orexin-A (OxA) immunopositive neurons and terminal networks of the main cluster, (B) the moderate density of calbindin (CB) immunopositive neurons and terminal networks in this hypothalamic region, and (C) the lack of calretinin (CR) immunopositive neurons, but the presence of a moderately dense calretinin immunopositive terminal network. In each photomicrograph dorsal is to the top and medial to the left. Scale bar in C = 500 μm and applies to all.

with sleep state maintenance, whereas the irregular discharge pattern of calretinin-containing neurons correlates with changes in sleep states (Cauli et al., 1997; Ascoli et al., 2010).

In general, the Arabian oryx exhibited a similar organization and density of neurons and terminal networks immunopositive for calbindin and calretinin within the sleep associated nuclei (see Table 3) as seen in other Cetartiodactyls and mammals studied to date (Bhagwandin et al., 2013; Dell et al., 2016a, 2016b, 2016c); however, various differences have been noted. Overall, the densities of calbindin neurons and terminal networks in the sleep-associated nuclei appear lower than that observed in rodents, minke whale, harbour porpoise and the hippopotamus (Bhagwandin et al., 2013; Dell et al., 2016a, 2016b, 2016c). Within the cholinergic complexes, the densities of calbindin neurons and terminal networks are lower than that reported for the hippopotamus and minke whale, while being similar to that of the harbour porpoise. No calbindin neurons were reported for the Arabian oryx in the nucleus basalis, whilst moderate to high densities were observed for three Cetartiodactyls (Dell et al., 2016a, 2016b, 2016c). Within the locus coeruleus complex the densities of calbindin neurons and terminal networks are higher than that reported for rodents, lower than that reported for the harbour porpoise and similar to that of the hippopotamus and minke whale (Dell et al., 2016a, 2016b, 2016c). Calbindin neuron densities in the serotonergic complexes were substantially lower (no neurons reported for the DRif, DRv and DRd complexes) in the Arabian oryx compared with all three Cetartiodactyls studied previously (Dell et al., 2016a, 2016b, 2016c), and the same densities of calretinin neuron as observed in rodents, minke whale and harbour porpoise, but not the hippopotamus. Calbindin neuron densities and terminal networks were similar in the orexinergic complexes with the exception of the optic tract cluster (no neurons in the Arabian oryx) compared to other Cetartiodactyls.

Calretinin neuron and terminal network densities were higher in the Arabian oryx than that reported for rodents, harbour porpoise and hippopotamus (neuron densities only) within the cholinergic complexes. Calretinin neuron densities in the hypothalamic orexinergic neuronal clusters were substantially lower in the Arabian oryx (no neuron densities reported except for the optic tract cluster) compared to the rodents and Cetartiodactyls and was especially varied in both neuron and terminal network densities compared to the harbour porpoise. Calbindin and calretinin neuron and terminal network densities were reported for rodents, minke whale and the harbour porpoise within the thalamic reticular nucleus whereas only calretinin was present in the Arabian oryx and hippopotamus.

These qualitative comparisons indicate that variation in the GABAergic innervation of the sleep-associated nuclei occurs across mammals, both within and between different orders. This variation in the GABAergic innervation may underlie differences in the expression of certain physiological features of sleep, such as the time spent in a particular sleep state, the rapidity in changing from one sleep state to another, and the periodicity of sleep in the different species. Marrying the results of anatomical studies with polysomnographic studies of sleep across a variety of mammals, may provide clues as to how the variation in certain sleep parameters across mammals are brought about by the systems involved in their production and regulation.

4.3. Artiodactyl species features and those unique to the Arabian oryx – are they indicative of changes in the expression of sleep in these species?

Two key sleep-wake cycle nuclei specific to Artiodactyls have been noted previously (Dell et al., 2012; Mahady et al., 2016), and observed in the current study of the Arabian oryx. First, the somal size of the cholinergic PPT neurons appears to be larger than those of the LDT neurons, as seen in the Gottingen mini pig (Mahady et al., 2016). The LDT and PPT nuclei have been shown to be selectively active during wake and REM sleep and appear to serve as powerful modulators of REM sleep initiation but not REM sleep maintenance (Shouse and Siegel, 1992; for review, see Siegel, 2004;

Table 3
Neuronal and terminal network densities of the calcium binding proteins calbindin and calretinin in relation to the sleep associated nuclei in the brain of the Arabian oryx. (–) absence, (+) low density, (++) moderate density, (+++) high density.

Sleep related nuclei	Calbindin		Calretinin	
	Neurons	Terminal Networks	Neurons	Terminal Networks
Cholinergic				
Medial septal nucleus	+	+	+++	+++
Diagonal band of Broca	+	+	++	++
Islands of Calleja and olfactory tubercle	++	+	++	++
Nucleus basalis	–	+	++	++
Pedunculopontine tegmental nucleus	++	++	++	++
Laterodorsal tegmental nucleus	++	+++	+	++
Catecholaminergic				
Compact subcoeruleus (A7sc)	+	++	+	++
Diffuse subcoeruleus (A7d)	+	+	+	++
Diffuse locus coeruleus (A6d)	–	+	+	++
Serotonergic				
Dorsal raphe, interfascicular (DRif)	–	++	–	++
Dorsal raphe, ventral (DRv)	–	++	+	++
Dorsal raphe, dorsal (DRd)	–	++	+	++
Dorsal raphe, lateral (DRl)	+	++	++	+++
Dorsal raphe, peripheral (DRp)	++	++	++	+++
Dorsal raphe, caudal (DRc)	+	+++	+	+++
Orexinergic				
Parvocellular cluster	++	++	–	++
Main cluster	++	++	–	++
Optic tract cluster	–	+	+	++
Zona incerta cluster	+	+	–	++
Thalamic reticular nucleus	–	–	+	++

van Dort et al., 2015). The varying difference in the size of the cholinergic LDT and PPT neurons in the Artiodactyls may indicate that REM sleep is manifested somewhat differently in the Arabian oryx and other Artiodactyls compared to other mammals. This speculation can be resolved by undertaking polysomnographic studies in the Arabian oryx and other Artiodactyls to determine whether a clear difference in REM sleep compared to other mammals is apparent.

The second feature of the sleep associated nuclei that is unusual compared to other mammals is the presence of a medially located parvocellular orexinergic cluster in the hypothalamus of the Arabian oryx, giraffe, harbour porpoise, African elephant, minke whale and hippopotamus (Dell et al., 2012, 2016a, 2016b, 2016c; Maseko et al., 2013). While this parvocellular orexinergic cluster has not been specifically noted in other species, the photomicrograph supplied for the sheep (Iqbal et al., 2001) and Gottingen mini-pig (Ettrup et al., 2010) indicate its broad presence across Artiodactyls. The orexinergic system has been associated with the generation of the sleep-wake cycle, as well as with respiratory, feeding, satiety, neuroendocrine and locomotor functions, while neuronal loss has also been linked to narcolepsy (Thannickal et al., 2000; Mintz et al., 2001; for review, see Ferguson and Samson, 2003; Zeitzer et al., 2003; Kirouac et al., 2005; Takakusaki et al., 2005). With regards to sleep and arousal, the orexinergic system neurons are both located and project to various sleep related neurons throughout the brain (Peyron et al., 1998). In the case of the Arabian oryx, the projections appear typical to those previously reported and include the locus coeruleus, dorsal raphe, paraventricular nucleus, intralaminar nucleus, epithalamus and habenular complex (Chen et al., 1999; Cutler et al., 1999; Hagan et al., 1999; Wagner et al., 2000; McGranaghan and Piggins, 2001; Zhang et al., 2002, 2004; Espana et al., 2005; Kruger et al., 2010; Bhagwandin et al., 2011; Gravett et al., 2011). The connectivity of the orexinergic system throughout the sleep systems may play important roles in how sleep physiologically manifests itself and has been shown to be strongly related to circadian rhythms (Taheri et al., 2000; Fujiki et al., 2001; Yoshida et al., 2001; Kukkonen et al., 2002). The means in which the nuclei described in the Arabian oryx relate to the unique and adaptive patterns observed are currently unclear. The sleep systems described are implicated in control and generation of sleep and are not necessarily involved in the timing of the sleep patterns. It may be possible that the suprachiasmatic nucleus and its circadian clock are more heavily involved in the case of the oryx, however further examination is necessary and no further speculation can be made here.

The medial hypothalamus, in which the parvocellular neurons are located, is additionally known to play an important role in the initiation of various types of behaviour such as copulation, aggression and appetite. It has been speculated that the control of appetitive behaviours and the need to be awake to ingest food, may have been the selection pressure leading to the evolution of this medially located orexinergic cluster in the Artiodactyls and African elephant (Dell et al., 2012; Maseko et al., 2013). The Arabian oryx feeds on medium quality vegetation, which becomes increasingly depleted of nutrients in the summer months. In order to cope with this seasonal deficit in nutrient content, the Arabian oryx appears to be able to decrease its metabolic rate and increase its digestive efficiency (Ostrowski et al., 2006; Williams et al., 2001). During the climatically extreme summer months, a specialized orexinergic nucleus, involved with increasing appetite and promoting wakefulness, may play a vital role as it searches large areas of harsh landscape to find fodder (Nowak, 1999).

In addition to these two Artiodactyl specific features, there appears to be two species specific features of the hypothalamic catecholaminergic system in the Arabian oryx – the presence of the dorsal division of the anterior hypothalamic group (A15d) and the increase in cell numbers of the tuberal cell group (A12). The A15d nucleus, while present in many mammals including the minke whale, harbour porpoise, hippopotamus, Cape hare, most Afrotheria and Primates (for review, see Smeets and González, 2000; Maseko et al., 2007; Dell et al., 2010; Calvey et al., 2013, 2015), is absent in the sheep, pig and possibly giraffe (Leshin et al., 1996;

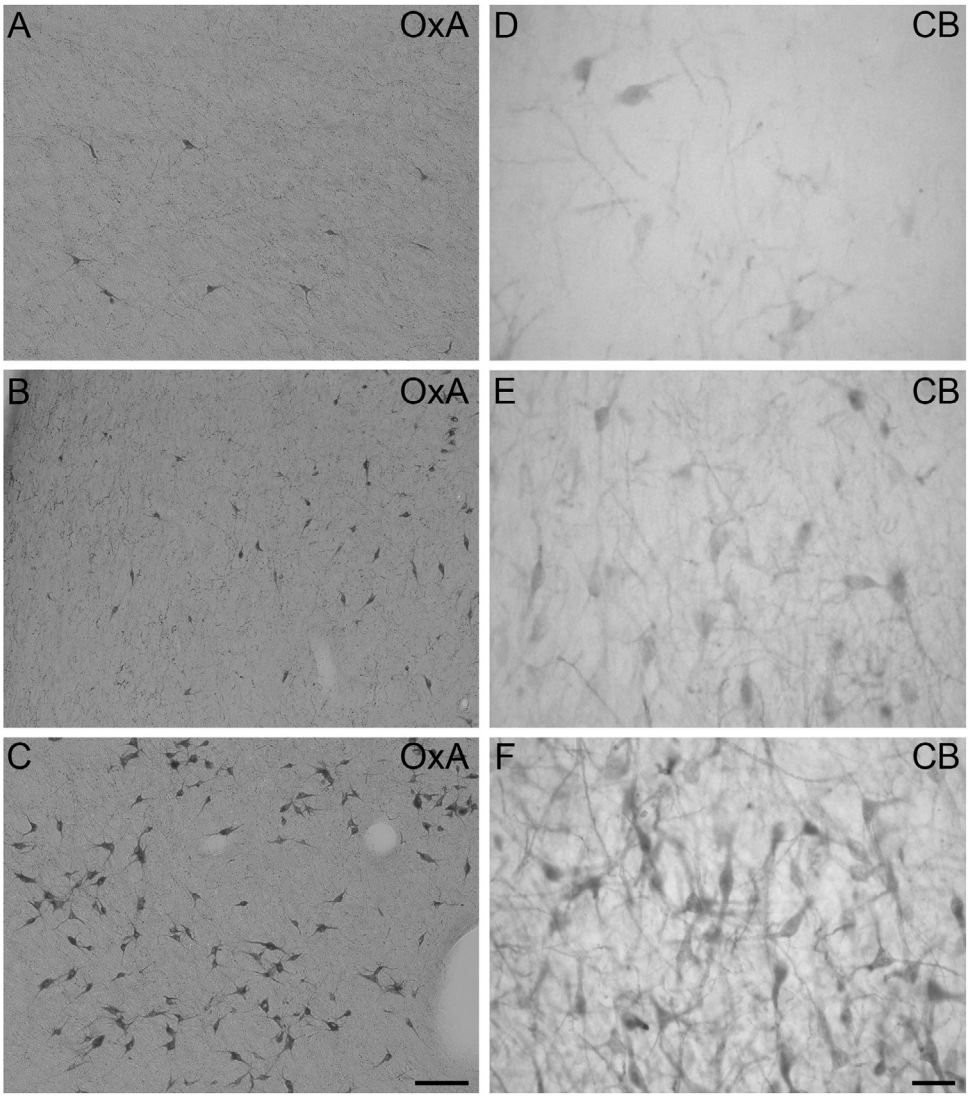


Fig. 10. Reference photomicrographs showing examples of (A) a low density of neurons (OxA + neurons in the optic tract cluster), (B) a moderate density of neurons (parvocellular OxA + neurons in the main cluster), (C) a high density of neurons (OxA + neurons in the main cluster), (D) a low density of terminal networks (CB + neurons and terminal networks in the medial septal nucleus), (E) a moderate density of terminal networks (CB + neurons and terminal networks in the compact subcoeruleus nucleus), (F) a high density of terminal networks (CB + neurons and terminal networks in the laterodorsal tegmental nucleus). Scale bar in C = 100 μm and applies to A, B, C. Scale bar in F = 20 μm and applies to D, E, F. Figures D, E, F are at a higher magnification so as to clearly show the terminal networks.

Table 4
Comparison of the highlighted sleep-related features across Cetartiodactyls.

	Species Specific Sleep-Related Features	General Mammalian Features	General Cetartiodactyl Features
Arabian Oryx Goettingen Mini Pig (Mahady et al., 2016) Giraffe (Bux et al., 2010) Hippopotamus (Dell et al., 2016c) Minke Whale (Dell et al., 2016b) Harbour Porpoise (Dell et al., 2016a)	– PPT neurons larger than LDT neurons – PPT neurons larger than LDT neurons – LDT neurons larger than PPT neurons – Supernumerary hypothalamic cholinergic neurons – LDT neuron divisions (parvo- and magnocellular) – Intralaminar cholinergic neurons – VTA cholinergic neurons – Large number of LDT, PPT and orexinergic neurons – Expanded peripheral division of the dorsal raphe complex – Expanded peripheral division of the dorsal raphe complex	– Three clusters of Orexinergic neurons in the hypothalamus – Similar LDT and PPT neuron morphologies – Typical neuronal architecture of the dorsal raphe complex	– Parvocellular and magnocellular division of Orexinergic main cluster in hypothalamus – Various morphology differences between LDT and PPT neurons – Expanded peripheral division of the dorsal raphe complex (Cetaceans only)

Iqbal et al., 2001; Bux et al., 2010), indicating that the presence of this nucleus presents with variable occurrence. The A15 nuclei have been reported to provide dopaminergic innervation to the supraoptic nuclei (SON), an area responsible for vasopressin release and osmoregulation (van Vulpen et al., 1999), plus the A15 neurons have been shown to be involved in the seasonal control of reproduction in sheep (Smeets and González, 2000), and likely play a similar role in the Arabian oryx.

Through comparative qualitative comparisons, the A12 nucleus described herein for the Arabian oryx brain appears more expansive and appears to have more neurons than previously reported for other Artiodactyls including pigs, sheep, giraffe and the hippopotamus, and other mammals (Tillet and Thibault, 1989; Tillet, 1994; Leshin et al., 1995a, 1995b, 1996; Tillet and Kitahama, 1998; Bux et al., 2010; Dell et al., 2016c) although quantitative comparisons are required. The A12 nuclear group belongs to the tuberoinfundibular pathway that projects to the median eminence (for review, see Moore, 1987; Ben-Jonathan et al., 1989; De Maria et al., 1998). The release of dopamine from the A12 neurons regulates prolactin secretion, which plays an important role in milk production in females, metabolism and regulation of the immune system (Zoli et al., 1993; Tillet, 1994). Differential levels of activity in the A12 neurons has been shown to either inhibit or stimulate prolactin secretion and has been linked to states of stress (Demarest et al., 1987; Tucker et al., 1991). The presence of the A15d nucleus and the expansion of the A12 nucleus in the Arabian oryx are currently not understood, but one could speculate that they may serve a specialized role in water regulation and conservation in an extreme environment such as the Arabian Desert.

Acknowledgments

This work was mainly supported by funding from the South African National Research Foundation (N.G. and P.R.M.), by a Masters fellowship within the South African National Research Foundation and German Academic Exchange Service, DAAD (J.G. D.), the Vice Deanship of Research Chairs at the King Saud University (A.N.A., O.B.M.), and the South Africa Research Chair for Mammal Behavioural Ecology (NCB). Opinions expressed and conclusions arrived at are those of the authors and are not necessarily attributed to the DAAD-NRF.

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Journal of Biological Rhythms. 2016 31:365-374

Arabian Oryx (*Oryx leucoryx*) Respond to Increased Ambient Temperatures with a Seasonal Shift in the Timing of Their Daily Inactivity Patterns

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Abstract The Arabian oryx inhabits an environment where summer ambient temperatures can exceed 40 °C for extended periods of time. While the oryx uses a suite of adaptations that aid survival, the effects of this extreme environment on inactivity are unknown. To determine how the oryx manages inactivity seasonally, we measured the daily rhythm of body temperature and used fine-grain actigraphy, in 10 animals, to reveal when the animals were inactive in relation to ambient temperature and photoperiod. We demonstrate that during the cooler winter months, the oryx was inactive during the cooler parts of the 24-h day (predawn hours), showing a nighttime (nocturnal) inactivity pattern. In contrast, in the warmer summer months, the oryx displayed a bimodal inactivity pattern, with major inactivity bouts (those greater than 1 h) occurring equally during both the coolest part of the night (predawn hours) and the warmest part of the day (afternoon hours). Of note, the timing of the daily rhythm of body temperature did not vary seasonally, although the amplitude did change, leading to a seasonal alteration in the phase relationship between inactivity and the body temperature rhythm. Because during periods of inactivity the oryx were presumably asleep for much of the time, we speculate that the daytime shift in inactivity may allow the oryx to take advantage of the thermoregulatory physiology of sleep, which likely occurs when the animal is inactive for more than 1 h, to mitigate environmentally induced increases in body temperature.

Keywords Arabian oryx, circadian rhythms, masking, temperature, sleep, seasonality

The Arabian oryx is the smallest member of the genus *Oryx* and is native to the gravel and hard sands of the deserts and steppes of the Arabian Peninsula. In the summer months, peak ambient temperatures in the Arabian Desert regularly surpass 40 °C for prolonged periods of time. To cope with this hot and dry

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JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 31 No. 4, August 2016 365–374
DOI: 10.1177/0748730416645729
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environment, the Arabian oryx uses a range of behavioral and morphophysiological responses to high temperatures. During the summer, Arabian oryx have been observed to increase the time spent inactive in the shade, where they appear to dig shallow depressions in which to rest (Stanley Price, 1989; Seddon and Ismail, 2002), creating a cooler microclimate (Hetem et al., 2012b). In addition to these behavioral strategies, the Arabian oryx has morphophysiological capacities that assist its survival, such as reduced fasting metabolic rates, lower total evaporative water loss rates, lower water influx/turnover rates compared with other similar-sized artiodactyls (Williams et al., 2001), adaptive heterothermy (Ostrowski et al., 2003; Hetem et al., 2010), and selective brain cooling (Hetem et al., 2012a). This suite of behavioral and morphophysiological features undoubtedly assists the Arabian oryx to survive in the desert of the Arabian Peninsula, although these features appear to be common to all species of oryx living in arid environments and are not specific adaptations of the Arabian oryx (Taylor, 1966, 1968, 1969, 1970a,b; Taylor and Lynn, 1972; King et al., 1975; King, 1979; Stanley Price, 1985).

Seddon and Ismail (2002) and Hetem et al. (2012b) indicated the possibility of an altered daily activity pattern between the cooler and hotter months in the Arabian oryx, although this was not explored in detail in either of those studies. Among many mammals, the dominant, or primary, zeitgeber for entraining circadian rhythms, including daily activity patterns, is ambient light (e.g., Daan and Aschoff, 1975; LeGates et al., 2014); however, under natural conditions, activity patterns may not be driven exclusively by the endogenous circadian clock but can be influenced by environmental conditions that bypass or act downstream of the clock ("masking"). Intricately linked with the entrained daily activity pattern is the timing of sleep and wakefulness, as sleep mostly occurs during the phase of general and prolonged inactivity (Monk, 1991).

The Arabian oryx can undergo substantial seasonal changes in the amplitude of its daily body temperature rhythm, although the timing of the rhythm does not appear to change seasonally (Hetem et al., 2010). To mitigate the effects of seasonal changes in ambient temperature, the Arabian oryx might alter its daily activity pattern, but it is unclear whether the timing of inactivity, and potentially of sleep, also changes in relation to the seasons (Seddon and Ismail, 2002; Hetem et al., 2012b). To determine whether daily inactivity patterns change seasonally or remain the same in the Arabian oryx, we used actigraphy (Ancoli-Israel et al., 2003) to analyze the timing of daily inactivity patterns during both summer and winter months.

MATERIALS AND METHODS

Animals

In the current study, a total of 10 adult Arabian oryx were used. These animals formed 2 groups: Group 1 consisted of 9 adult males (mean body mass 79.8 ± 11.0 kg) being used for fine-grain actigraphy and body temperature recording in the Mahazat as-Sayd Protected Area, northeast of Taif, Saudi Arabia (22° 12′12″N, 41° 49′36″E) in February and July 2014, and group 2 consisted of 1 adult male (85 kg body mass) being used for fine-grain actigraphy near Richmond in the Karoo region of South Africa (31° 32′29″S, 23° 41′34″E) during February 2015. Ethical clearance for the current study was obtained from the University of the Witwatersrand Animal Ethics Committee (clearance certificate number 2014/53/D), and all animals were treated according to the guidelines of this committee, which parallel those of the National Institutes of Health (NIH) for the care and use of animals in scientific experimentation.

Anesthesia of the Arabian Oryx

All animals were remotely injected using a Dan-Inject dart gun (Daninject, Børkop, Denmark) with etorphine hydrochloride (Captivon 98, Wildlife Pharmaceuticals Ltd., White River, South Africa; 19 µg/kg; a semisynthetic opioid that has an analgesic effect and is an opioid receptor antagonist); ketamine (Ketaminol Vet., MDS Animal Health, Intervet International B. V., Boxmeer, The Netherlands; 0.3 mg/kg; a dissociative anesthetic agent that is an NMDA receptor antagonist); midazolam (Wildlife Pharmaceuticals Ltd., White River, South Africa; 0.13 mg/kg; a benzodiazepine class anesthetic agent that acts by enhancing the effect of GABA on $GABA_A$ receptors); and medetomidine (Zalopine 10 mg/ml, Orion Pharma, Espoo, Finland; 5 µg/kg; a sedative analgesic that is an α_2-adrenergic agonist). All animals received oxytetracycline (Terramycin LA, Pfizer, Brazil; 20 mg/kg IM; a broad-spectrum antibiotic) and ketoprofen (Ketovet, Vemedim Animal Health, Can Tho City, Vietnam; 2 mg/kg IM; a propionic acid class of nonsteroidal anti-inflammatory that acts to inhibit the production of prostaglandin). After the procedure, anesthesia was reversed using naltrexone hydrochloride (Naltrexone, APL, Kungens Kurva, Sweden; 40 mg IM; an opiate antagonist) and atipamezole hydrochloride (Antisedan, Orion Pharma; 2 mg IM; a synthetic α_2-adrenergic receptor antagonist). After the recording period, the animals were again immobilized using a similar drug combination, and the implants were removed. Again, oxytetracycline

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and ketoprofen were administered, and the animals were allowed to recover in a shaded pen prior to release to their normal environment.

Surgery for Fine-Grain Actigraphy and Body Temperature Recording

Following recumbency, the animals were moved to a shaded area for surgery. With aseptic techniques, a subcutaneous pocket was created on the left lateral aspect of both the neck and thigh, and an Actiwatch embedded in an inert wax (SasolWax 1276, Sasol, Johannesburg, South Africa) was placed into each pocket. The Actiwatch ambulatory device is commonly used for measuring sleep in humans. Within each Actiwatch is a piezo accelerometer device connected to a microchip that sums and records the number of acceleration events for each minute. The Actiwatch Spectrum (Philips Respironics, Philips) was used in the current study, which has a weight of 25 g and approximate dimensions of 35 × 35 × 12 mm. Each Actiwatch, factory calibrated, was programmed (data acquisition rate set at 1-min intervals) with Philips Respironics Actiware 5 software (Actiwatch communication and sleep analysis software) prior to implantation. The wristbands from the Actiwatches were removed, and the watches were insulated with standard electrical insulation tape and covered with 2 coats of biologically inert wax. The implantation sites of the Actiwatches (i.e., the side of the neck and the hindleg) were shaved, washed with chlorhexidine, and disinfected with alcohol. Sterile drapes were taped in place over the respective surgical sites to isolate the disinfected area. A small incision (less than 50 mm in length) was made at each of the respective implantation sites, and a subcutaneous pocket extending approximately 100 mm ventrally from the incision site was created. The Actiwatch, sterilized within a container with formalin pellets for 48 h prior to implantation, was inserted into the respective pockets, and the incisions were closed in an everting pattern with interrupted sutures, sterilized once again, and liberally sprayed with Necrospray (Bayer Animal Health). A data logger that measured temperature was inserted intra-abdominally (AIC001: Abdominal implant, Africa Wildlife Tracking, South Africa). For recording body temperature, the device was calibrated against an accurate thermometer in an insulated water bath, providing an accuracy of better than 0.06 °C. A small incision (less than 80 mm in length) was made through the skin at the implantation site, and the muscle layers of the abdominal wall were separated using blunt dissection. The implant, coated in inert wax and sterilized within a container with formalin pellets for 48 h prior to implantation, was inserted untethered into the abdomen; the incisions were closed and liberally sprayed with Necrospray.

Following reversal of the anesthesia, the animals were released into a 2 × 1-km fenced area of natural oryx habitat and were not provided with supplementary food or water for the entire recording period. For the single animal studied in South Africa, the body temperature logger was not implanted, but a radio transmitter collar was attached to the neck of the animal and the animal was released back to the wider game reserve (which measured 25 × 25 km) and not provided with supplementary food or water. After the recording period, the implanted animals were relocated and reanesthetized, and the Actiwatches and body temperature loggers were retrieved.

Data Analysis for Fine-Grain Actigraphy

Phillips Respironics Actiware 5 was used to retrieve the recorded data from each of the implanted Actiwatches. The raw data were exported to Microsoft Excel, where they were manually scored and analyzed. Data obtained from the neck and leg actigraphs were scored concurrently in 1-min 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. From the 1-min scored data, the modal state for 5 min was calculated and used to determine total active-inactive time, number of active-inactive episodes, and the average duration of active-inactive episodes. The data presented in the Results section is the grouped data for all individuals, with the standard deviation provided in parentheses following averages.

Data Analysis for Body Temperature

The temperature data obtained from the abdominal implants were downloaded into Microsoft Excel files for processing. The animals from each season were analyzed individually and the data were then pooled. Body temperature from each 10-min recording period was averaged across the recording period, and the data were then pooled to create an overall average body temperature.

Recording of Weather Conditions

A permanent weather station within the 1 × 2-km pen at the Mahazat as-Sayd Protected Area recorded weather parameters throughout the recording periods in Saudi Arabia. This weather station provided a reading of

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Table 1. Weather parameters measured during the recording periods in February and July 2014 in Saudi Arabia, plus the recording made in South Africa in February 2015

Location	Month/Year	Highest Temperature Recorded, °C	Average High Temperature, °C	Average Temperature, °C	Average Low Temperature, °C	Lowest Temperature Recorded, °C	Rainfall, mm	Sunrise, h	Sunset, h	% of 24-h Daylight
Mahazat	February 2014	32.0	26.1	19.3	11.6	5.7	0	0649	1815	47.7
Mahazat	July 2014	41.6	39.7	33.1	25.7	21.1	0	0545	1903	55.4
Richmond	February 2015	31.4	25.4	21.6	17.8	14.4	12.6	0539	1846	54.6

ground temperature, air pressure, air temperature, humidity, wind speed, wind direction, rainfall, and solar radiation every 15 min throughout both recording periods. The date and time stamps on the data were used to match the different recordings. In Richmond, South Africa, a portable solar-powered weather station (Environdata, www.environdata.com.au/easidata-mark4), which recorded, every 10 min, air temperature, black globe temperature, relative humidity, wind speed, wind direction, rainfall, solar radiation, and illuminance (lux), was placed within the game reserve during the recording period. Sunrise and sunset times were obtained from various freely accessible databases.

RESULTS

The current report outlines our findings regarding seasonal effects on daily inactivity patterns in Arabian oryx studied in 2 different environments. Initially, we used fine-grain actigraphy (1-min resolution, 2 devices per animal, subcutaneous neck and hindleg) for 28 days in both a cold month (February) and a hot month (July) in the Mahazat as-Sayd Protected Area, northeast of Taif, Saudi Arabia, to reveal that there was a seasonal shift in the pattern of daily inactivity from essentially nocturnal in the cold month to bimodal (with the additional appearance of inactivity during the daytime) in the hot month. In addition, we used fine-grain actigraphy to examine an oryx from the Karoo region of South Africa (28 days) in February (i.e., exposed to a photoperiod comparable to the Saudi Arabian summer but ambient high temperatures resembling the Saudi Arabian winter); this individual expressed a daily inactivity pattern similar to the animals recorded in the Saudi Arabian winter.

Seasonal Variation in the Daily Inactivity Pattern

Nine male oryx (mean body mass 79.8 ± 11.0 kg), 4 animals in February 2014 (winter) and 5 animals in July 2014 (summer), were used for this portion of the study (see Table 1 for environmental data for each month). During the winter recording period, the average daily inactivity measured was 5.01 h (±1.71 h). The animals displayed polyphasic inactivity with an average of 18 (±4) bouts of inactivity per day. The average daily onset of the main inactive bout (that being the longest period of inactivity) occurred between 0330 and 0400 h, with this bout having an average length of 127 min, just over 2 h (±63.5 min). Thus, during the winter recording period, the oryx displayed a nocturnal inactivity pattern, with the vast majority (94% ± 7.25%) of inactivity occurring during the night (Figs. 1 and 2).

During the summer recording period, the average daily inactivity measured was 6.62 h (±1.93 h). The animals displayed polyphasic inactivity with an average of 27 (±6) bouts of inactivity per day. In this summer recording session, the main inactive bouts were found to have onsets between 0100 and 0130 h or between 1330 and 1400 h. Thus, prolonged periods of inactivity were observed at 2 different times during the 24-h period (Fig. 2). These main inactivity bouts had an average length of 137 min, just over 2 h (±73.4 h). Thus, when compared with winter, during the summer the daily inactivity pattern of the oryx changed dramatically, showing a bimodal pattern and having an extra 1 h and 37 min of inactivity (Mann-Whitney, nonparametric test, $P = 1.25 \times 10^{-12}$). The additional inactivity occurred mostly during the afternoon but was also present in the predawn hours (Figs. 1 and 2). During the summer, 68% (±24.6%) of inactivity occurred during the daytime compared with 6% in the winter (statistically significant difference, Mann-Whitney, nonparametric test, $P = 2.49 \times 10^{-30}$), while only 32% (±24.6%) of inactivity occurred during the nighttime in summer compared with 94% in the winter (statistically significant difference, Mann-Whitney, nonparametric test, $P = 4.21 \times 10^{-8}$). Despite this seasonal variation in the inactivity pattern, we could find no seasonal change in the timing of the daily body temperature rhythm, although the amplitude did change (Fig. 1), confirming the results of an earlier study (Hetem et al., 2010).

Is Photoperiod or Ambient Temperature the Dominant Factor Changing the Daily Inactivity Pattern?

To begin to distinguish whether photoperiod or ambient temperature is the factor causing the seasonally switching daily inactivity patterns in the Arabian

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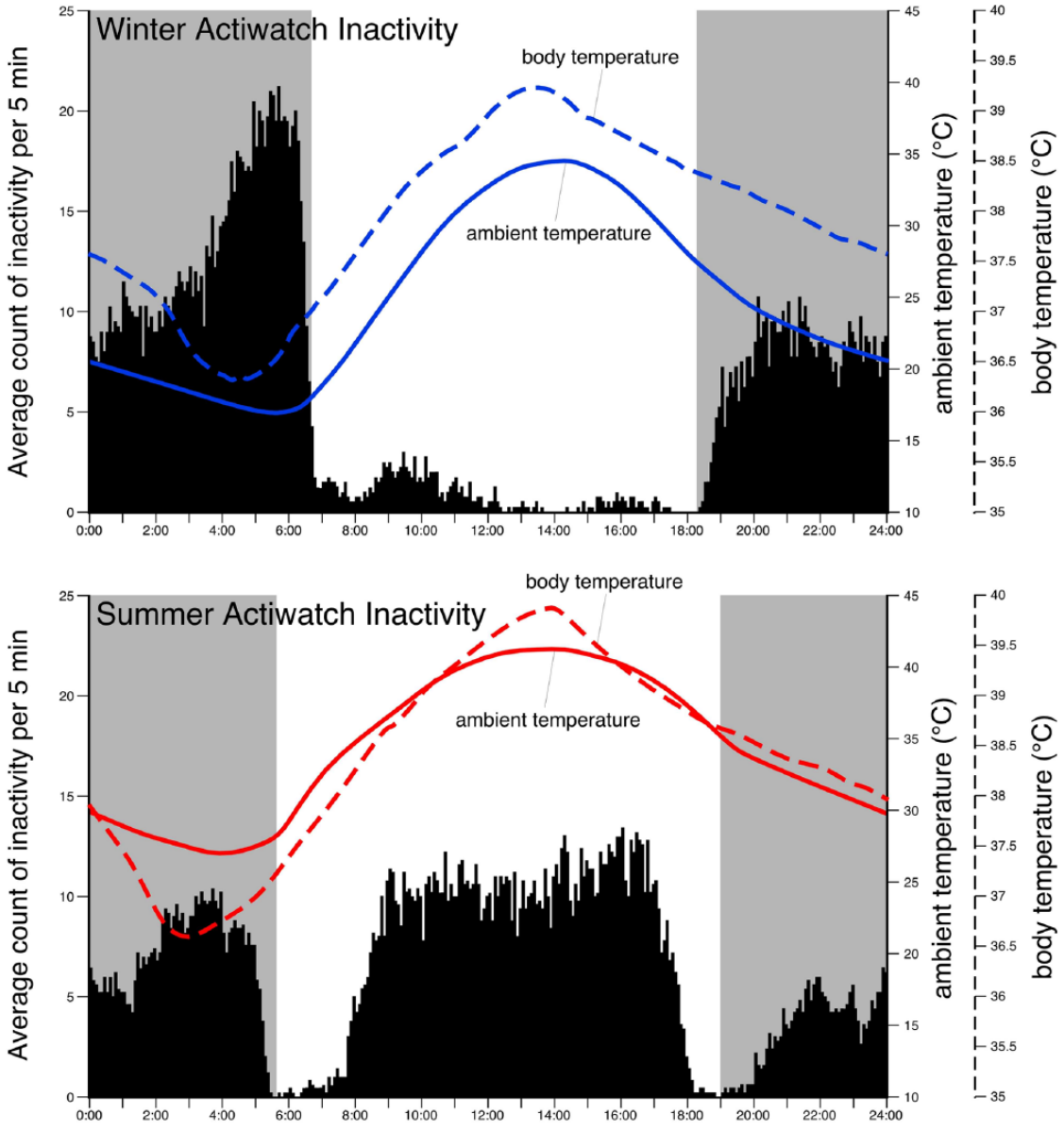


Figure 1. Graphs illustrating the average count of inactivity for any given 5-min period scored over 28 days in the winter month (upper graph, February 2014, Saudi Arabia, pooled from the 4 animals recorded) and the summer month (lower graph, July 2014, Saudi Arabia, pooled from the 5 animals recorded). Note the nocturnal pattern of inactivity during the winter month, with little inactivity occurring during the daytime. In contrast, in the summer month we observed a bimodal inactivity pattern, with the additional appearance of inactivity during the daytime. The average ambient (solid lines) and body (dashed lines) temperatures, recorded every 10 min, across the month of recording are overlain on the activity graphs (online, blue for winter, red for summer). Note the substantially higher average ambient temperatures in the summer month. Note that the timing of the daily rhythm of body temperature remains unaltered between seasons. The gray regions represent the period between dusk and dawn.

oryx recorded in Saudi Arabia, we examined a single adult male (body mass estimate = 85 kg) from a free-ranging population near Richmond, in the Karoo region of South Africa. The advantage of including this particular individual is that the weather in the Karoo, while hot in summer (February), does not reach the temperatures recorded in summer in Saudi Arabia but matches the temperatures seen in winter in Saudi Arabia (Table 1); however, the day lengths in South Africa during the summer are similar to those seen in Saudi Arabia during the summer (Table 1).

With this individual animal we used fine-grain actigraphy recording and found that the Arabian oryx in South Africa displayed a predominantly nocturnal inactivity pattern (Fig. 3), similar to that seen in the winter recordings of oryx in Saudi Arabia (Fig. 1). During the summer recording period in South Africa, the average daily inactivity measured was

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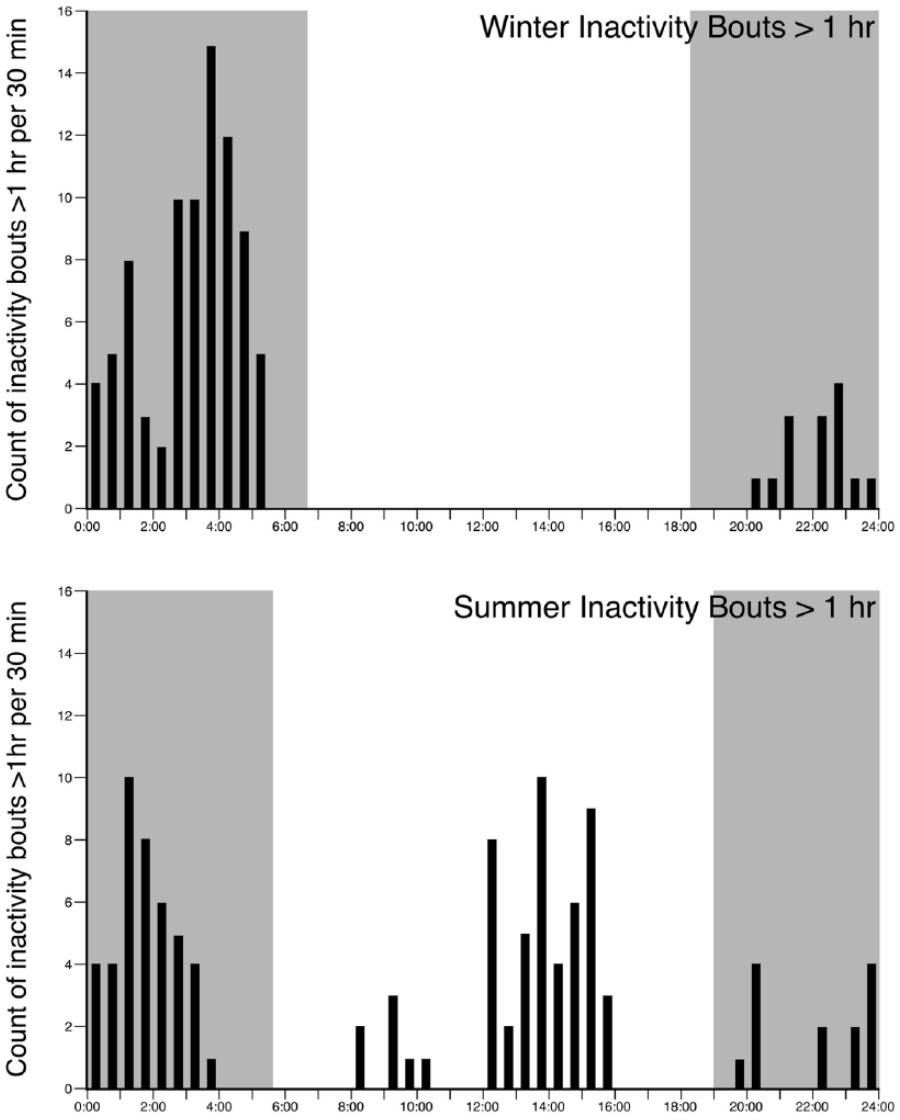


Figure 2. Frequency distribution graphs showing the number (in 30-min intervals) of inactive bouts of greater than 1 h duration during the winter and summer recording periods for the Arabian oryx measured with fine-grain actigraphy in Saudi Arabia. Note that during the winter, all bouts of inactivity greater than 1 h occurred between sunset and sunrise (gray-shaded regions), with the vast majority (97/111 bouts, 87.4%) occurring in the early morning hours between midnight and dawn. In contrast, in summer, the majority of inactive bouts (55/110 bouts, 50%) occurred during daytime, with 42/110 bouts (38.2%) occurring between midnight and dawn. The number of inactive bouts occurring between dusk and midnight was similar between seasons (winter: 14 bouts, 12.6%; summer: 13 bouts, 11.8%). These differences are statistically significant (daytime summer vs. daytime winter, Mann-Whitney, nonparametric test, $P = 9.14 \times 10^{-5}$; nighttime summer vs. nighttime winter, Mann-Whitney, nonparametric test, $P = 2.15 \times 10^{-5}$).

5.20 h (±1.7 h) (not significantly different from the average daily inactivity of the oryx measured during the Saudi winter, Mann-Whitney, nonparametric test, $P = 0.052$, but significantly less than for the oryx measured during the Saudi summer, $P = 6.04 \times 10^{-6}$). The animal displayed polyphasic inactivity with an average of 16 (±4) bouts of inactivity per day. The average daily onset of the main inactive bout occurred between 0330 and 0400 h, with this bout having an average length of 131 min, just over 2 h (±56 min). Thus, during the South African summer recording period, the single oryx displayed a nocturnal inactivity pattern, with the majority (81.6% ± 12%) of inactivity occurring during the night (Fig. 3). The only noted difference between the South African animal and the Saudi Arabian winter animals was an increase in inactivity in the late afternoon in the South African animal compared with the Saudi Arabian winter animals (Fig. 1). This may reflect some effect of increased summer temperatures but does not match the increase in summer afternoon inactivity levels observed in the Saudi Arabian summer animals.

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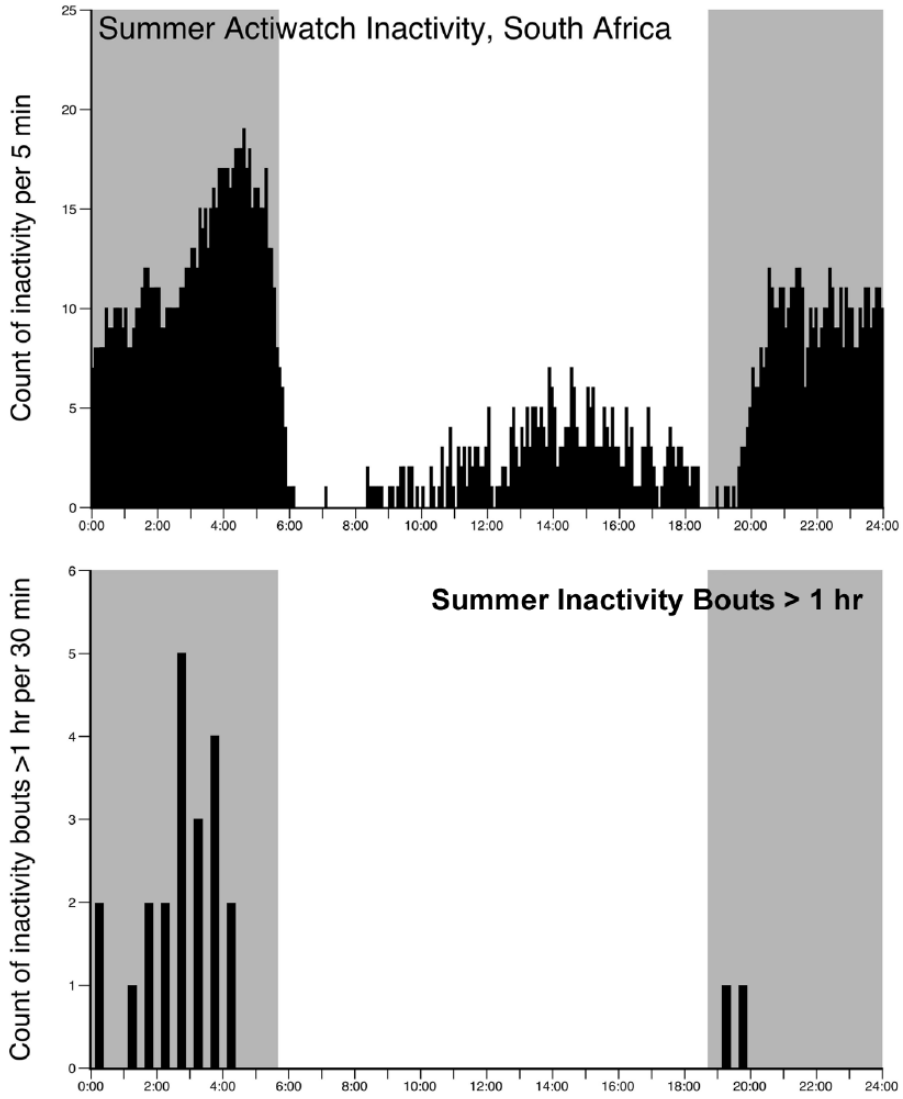


Figure 3. Upper graph illustrates the average count of inactivity for any given 5-min period scored over 28 days in the South African summer (February 2015) for a single male Arabian oryx. Note the nocturnal pattern of inactivity during this summer month, with relatively little inactivity occurring during the daytime. This contrasts with the recordings made in the summer month in Saudi Arabia (see Fig. 1). The lower graph shows the frequency distribution (in 30-min intervals) of inactive bouts of greater than 1 h duration during the South African summer recording period for the Arabian oryx measured. Note than 21 of the 23 (91.3%) bouts of inactivity greater than 1 h occurred in the period between midnight and dawn and that none occurred during the daytime, resembling closely the observations made on the Saudi Arabian oryx recorded in the winter.

DISCUSSION

The current study reveals how the Arabian oryx manages daily inactivity patterns in relation to the dramatic seasonal conditions of a hot and inhospitable environment. First, we have observed that the Arabian oryx changes from nocturnal inactivity in the cooler months to a bimodal inactivity pattern during the hotter months. Second, the timing of the majority of inactivity also changes seasonally, from the coolest parts of the winter nights to the hottest parts of the summer days. Third, through comparison with a conspecific inhabiting a different environment, we suggest that ambient temperature is the dominant environmental condition driving these changes in daily inactivity patterns. Fourth, since the timing of the oryx body temperature rhythm did not show a seasonal change, the change in inactivity patterns likely represents a "masking" effect rather than a shift in the circadian clock's endogenous oscillation.

The fine-grain actigraphy analysis revealed 2 different daily patterns of inactivity in the Arabian oryx, from nighttime inactivity in the cooler months to an additional daytime period of inactivity in the hotter

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months. Seddon and Ismail (2002) used focal 10-min observations from 0600 h to 1800 h and Hetem et al. (2012b) used a coarse grain method of actigraphy in their studies but were not able to clearly show this variation; this was because Seddon and Ismail's observations did not include the full 24-h period and Hetem et al. did not make recordings throughout the entire year (the cooler months of December to March were not recorded). While many studies report seasonal variations of daily activity patterns, termed *temporal niche switching* (reviewed by Refinetti, 2008, and Hut et al., 2012) from a range of mammalian species, fewer examples under natural conditions have been reported. Of specific interest to the current study are those examples in which seasonal changes in ambient temperature appear to drive changes in activity patterns, for example, in the degu (Kenagy et al., 2002; Vivanco et al., 2010), blind mole rat (Oster et al., 2002), and bat-eared fox (Lourens and Nel, 1990). Under certain environmental conditions, daytime activity can be induced in the golden spiny mouse (Shkolnik, 1971) and Asiatic ibex (Fox et al., 1992). It is also possible that the dromedary camel, when it becomes an adaptive heterotherm under experimental dehydration conditions (El Allali et al., 2013), may show changes in daily activity patterns that correspond to the changes in the Arabian oryx under high ambient temperatures, but this has not yet been shown (El Allali et al., 2013; Bouaouda et al., 2014). Our analysis of the Arabian oryx in the South African summer suggests that temperature is the driving environmental factor behind the seasonal change in inactivity patterns, whereas day length and rainfall appear to have little or no role.

If we focus on the timing of the main bouts of inactivity (those greater than 1 h), many of which lasted for almost 2 h in both seasons in all the oryx studied in Saudi Arabia, it is reasonable to assume that during these periods of inactivity the oryx were likely to be asleep for much of the time. In this sense, during the winter/cooler months, the majority of sleep would have occurred between approximately 0300 and 0500 h (when ambient and body temperature are at their lowest), whereas during the summer/hotter months, the majority of sleep would have occurred in 2 periods between approximately 0300-0500 h and 1230-1430 h (the latter when ambient and body temperature are at their highest). The pattern of polyphasic inactivity in the Arabian oryx, with the main inactive bout occurring mostly during the night in the winter and an overall amount of inactivity being approximately 5 h, represents a typical inactive-sleep pattern in terms of timing, phasing, and duration for an artiodactyl of approximately 80 kg body mass (e.g., Campbell and Tobler, 1984; Siegel, 2005). In contrast, the timing of inactivity observed in the summer is unlike that observed in any artiodactyl studied to date, with a major inactive bout during the hottest part of the daytime and an increase of 1.5 h per day spent inactive. This contrasts with many other mammals, including humans, which are more inactive in the winter than the summer (e.g., Erriksson et al., 1981; Everts et al., 2004; Lariviere and Messier, 2009; Yetish et al., 2015). Because during slow-wave sleep, the body and brain temperature of all mammals studied to date decrease significantly (reviewed in Kräuchi and Deboer, 2010), we speculate that the daytime shift of inactivity-sleep in the Arabian oryx may be an adaptive response to increased ambient temperatures, taking advantage of the thermoregulatory physiology of sleep to lessen the impact of the summer heat.

Our observations in the Arabian oryx demonstrate a seasonal alteration in the phase relationship between inactivity and the body temperature rhythm. How this changing phase relationship is brought about by the neural systems involved in the control of behavioral and physiological rhythmicity is unknown. It is possible that ambient temperature might act on the downstream circuitry of circadian control, such as the ventral subparaventricular zone of the hypothalamus (Saper et al., 2005), and thus modify the timing of inactivity, and presumably sleep, although this remains to be investigated in more detail.

ACKNOWLEDGMENTS

This project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award Number 11-ENV1918-02 (A.N.A., O.B.M., N.C.B. and P.R.M.). The authors are extremely grateful to Prince Bander bin Saud Al-Saud, President of the Saudi Wildlife Authority (SWA), for his unlimited and enthusiastic support to undertake these studies on the Arabian oryx managed by the SWA. We are also grateful for the tremendous help provided by our colleagues at the Prince Saud Alfaisal Research Center in Taif, Saudi Arabia, especially Mr. Ahmed Boug, Dr. Saud Anagariyah, Mr. Ghazi Raziman, Mr. Raed Aljuaid, and Mr. Ali Aljuaid. We also extend our sincerest thanks to Mr. Jan Pickard at Ratelfontein, South Africa, who allowed us to use one of his Arabian oryx in South Africa.

CONFLICT OF INTEREST STATEMENT

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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-III-

Temporal niche switching in Arabian oryx (*Oryx leucoryx*): Seasonal plasticity of 24 h activity patterns in a large desert mammal.

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Physiology and Behavior. 2017 177:148-154

Physiology & Behavior 177 (2017) 148–154

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Temporal niche switching in Arabian oryx (*Oryx leucoryx*): Seasonal plasticity of 24 h activity patterns in a large desert mammal

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ARTICLE INFO

Keywords:
Arabian oryx
Circadian rhythms
Activity
Temperature
Photoperiod
Temporal niche switching

ABSTRACT

The Arabian oryx, a moderately large mammal that inhabits a harsh desert environment, has been shown to exhibit seasonal variations in activity and inactivity patterns. Here we analyzed the continuous year-round activity patterns of twelve free-roaming Arabian oryx under natural conditions from two varying desert environments in Saudi Arabia using abdominally implanted activity meters. We simultaneously recorded weather parameters at both sites to determine whether environmental factors are responsible for temporal niche switching as well as the seasonal structuring and timing of this behavioural plasticity. Our results demonstrate that Arabian oryx undergo temporal niche switching of 24 h activity patterns at a seasonal level and exhibit distinct nocturnal/crepuscular activity during summer, diurnal activity during winter and intermittent patterns of behaviour during the transitional seasons of autumn and spring. In addition, the oryx exhibited inter- and intra-seasonal variations in the temporal budgeting of 24 h activity patterns. Strong relationships with both photoperiod and ambient temperatures were found and in some instances suggested that increasing ambient temperatures are a primary driving force behind seasonal shifts in activity patterns. These adaptive patterns may be dictated by the availability of food and water, which in turn are strongly influenced by seasonal climate variations. Overall, the adaptive responses of free-roaming Arabian oryx in such harsh and non-laboratorial conditions provide a framework for comparing wild populations as well as aiding conservation efforts.

1. Introduction

Extreme environments such as sandy deserts, the arctic tundra, and high altitude mountains strongly affect the circadian rhythms of species, including locomotion, resting, reproductive cycles and foraging based on their unique climate and biome type. These extreme environments allow the exploration of the adaptive and plastic behaviour employed in order to survive. One aspect that requires attention is how species manage the timing of their daily activity patterns throughout the changing seasons of the year, to enable them to obtain food, water, shelter, and successfully reproduce, in harsh environments.

In thermally challenging environments such as deserts, the survival rate of free-roaming mammals is low due to highly variable ambient temperatures, minimal rainfall, intense solar radiation and low primary productivity [11,36]. Xerocoles (desert animals) must therefore preserve a balance between energy expenditure and water loss and thus typically employ temporal activity budgeting throughout the 24 h period along with other morphological and physiological mechanisms. The budgeting of activity and inactivity over a day in response to environmental factors such as ambient temperature or proximate *Zeitgebers* such as photoperiod varies amongst mammals. Such variations are seen in the phasing of these activities into nocturnal, diurnal and crepuscular daily activity patterns or phenotypes [35]. These phenotypes balance both benefit and cost to the species' survival but overall allow for behavioural adaptations like avoidance of excessive heat, foraging strategies, reduction of predator risk and prevention of water loss to be at an optimum.

Certain conditions and environmental factors do not allow for such clear definitions of the temporal niche of an animal, and phenotypic plasticity can be observed in this continuum, termed “temporal niche switching”. In general the temporal niche an animal occupies and its corresponding temporal activity budgets over a 24 h period are produced through the interactions between the endogenously generated internal clock of a species (suprachiasmatic nucleus) and its responses

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http://dx.doi.org/10.1016/j.physbeh.2017.04.014
Received 24 January 2017; Received in revised form 13 April 2017; Accepted 14 April 2017
Available online 19 April 2017
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to the external environment [7]. The external variables can be photic (light-dark cycle) or non-photic in nature. In the case of temporal niche switching, the changing variables of an animal's environment such as seasons, food restrictions or temperature cycles may mask the effect of light and strongly dictate the preference of a certain temporal niche in order to produce the greatest chance of survival [54].

Temporal niche switching has been observed in both vertebrates and invertebrates (see review in [29]), although most of the research has been laboratory based [35]. Examples to date occurring under natural conditions include wolves (*Canis lupus*) [27] and migratory birds [6,34] which, during extensive travel and migrations switch from being nocturnal and diurnal to the opposite phasing respectively. Lourens and Nel [24] reported Bat-eared foxes (*Otocyon megalotis*) switching between nocturnal-summer phasing to diurnal-winter phasing along the Cape West coast of South Africa in relation to prevailing temperatures and corresponding prey availability.

Detailed studies of desert environments and naturalistic temporal niche switching in mammals are very limited, particularly with regards to larger mammals, and only include the Arabian oryx (*Oryx leucoryx*). The Arabian oryx is found primarily within the deserts of the Arabian peninsula and Middle East, this species has been able to successfully survive the extreme conditions of such harsh arid-zones owing to numerous adaptive behaviours [16,31,37,43] common to all species of oryx [20,21,42,45–49,51]. Arabian oryx were originally suggested to possibly show altered daily activity patterns between the hotter and cooler months (April – November) of the Saudi Arabian desert [17,37]; however, the winter and early spring months were not investigated (December – March). The altered patterns observed through coarse-grain actigraphy included seasonal flexibilities in activity, with crepuscular patterns in the cooler spring and early summer months, but nocturnal patterns in the hotter summer months. The daytime inactivity and excess heat avoidance during the hotter months was further linked to cooler microclimate selection, in the form of shade seeking [17].

The seasonal alteration in daily patterns was further investigated using fine-grain actigraphy, targeting specifically inactivity patterns during the hottest (July) and coldest (February) months in the Arabian Desert [10]. Temporal niche switching was observed between winter-diurnal and summer-crepuscular phasing in relation to seasonal shifts in the light-dark cycle and extreme ambient temperatures. Additional investigation into a free-roaming Arabian oryx living in the Karoo desert of South Africa (semi-arid, fewer climatic extremes than the Arabian Desert) during the summer month of February (Southern Hemisphere) revealed similar nocturnal inactivity to that of the Saudi Arabian winter month of February, indicating a possible masking effect of ambient temperature on inactivity [10]. Seasonally, the oryx investigated in Saudi Arabia exhibited no change in the phasing of their body temperature rhythm, only in the amplitude, suggesting a seasonal phase shift in the relationship between body temperature and inactivity patterns. Current data on the Arabian oryx indicate a seasonal shift in activity patterns related strongly to extreme ambient temperatures and appears to represent temporal niche switching [10,17,37]; however, no examination of the year-long 24 h temporal activity budgets and general activity phasing in relationship to seasonal environmental changes in the Arabian Desert has been conducted thus far.

The current study thus set out to non-invasively observe, under fully natural conditions, the behavioural patterns of Arabian oryx evolutionarily adapted for survival in the demanding deserts of Saudi Arabia. We investigated the 24 h temporal activity budgets and phasing profiles of free-roaming Arabian oryx continuously for one year (all four seasons) in two separate natural areas of varied size and location within the Arabian Desert. Understanding the conditions required for temporal niche switching to occur naturally and how Arabian oryx manage their activity daily and seasonally under such extreme environmental and physiological constraints provides a strong basis for future comparisons between wild mammal populations and aids conservation efforts.

2. Materials and methods

2.1. Animals and environments

Six adult Arabian oryx (2 male; 4 female (not pregnant, not lactating), mean body mass 72.7 ± 12.9 kg) were used for year-round coarse-grain activity measurements in the Mahazat as-Sayd Protected Area, Saudi Arabia (MS, see Supplementary Fig. S1). A second group of six adult Arabian oryx (2 male; 4 female (not pregnant, not lactating), mean body mass 79.8 ± 11.0 kg) were used for year-round coarse-grain activity measurements in the Uruq *B*ani Ma'arid Protected Area, Saudi Arabia (UBM, Fig. S1). The two separate environments and subject groups were selected based on availability and varied environmental parameters providing for varying degrees of climatic extremes. The recording period lasted from February 2014 through to January 2015 and totalled 365 days of recording. Ethical clearance for the current study was obtained from the University of the Witwatersrand Animal Ethics Committee (clearance certificate number 2015/09/38/C) and all animals were treated according to the guidelines of this committee, which parallel those of the National Institute of Health (NIH) for the care and use of animals in scientific experimentation.

2.2. Recording of weather conditions

Two permanent weather stations set up within the MS Protected Area (22.2°N, 41.9°E, altitude 1000 m) and the UBM Protected Area

Table 1
Monthly-seasonal groupings and recorded average measurements of meteorological parameters from February 2014 through to January 2015 in the Mahazat as-Sayd Protected Area (MS) and the Uruq Bani Ma'arid Protected Area (UBM) of Saudi Arabia.

		Mean air temp (°C)		Max air temp (°C)		Min air temp (°C)		Rainfall (mm)		Photoperiod	Sunrise/Sunset	
Season	Month	MS	UBM	MS	UBM	MS	UBM	MS	UBM	Saudi Arabia	Saudi Arabia	
Winter	December	22.7	16.8	25.9	22.9	12.0	10.4	1.5	0.0	10h38m	06:30	17:10
	January	17.0	16.6	23.8	22.5	9.9	10.5	0.0	0.3	10h47m	06:38	17:26
	February	19.3	18.7	26.1	24.6	11.6	12.7	0.0	0.0	11h20m	06:28	17:45
Spring	March	23.3	24.2	30.2	29.9	16.0	18.0	0.0	3.3	11h59m	06:00	18:05
	April	28.5	27.8	34.9	34.3	20.7	20.3	3.3	7.1	12h43m	05:30	18:15
	May	30.2	31.1	36.8	36.8	22.6	24.4	8.4	0.0	13h19m	05:12	18:30
Summer	June	33.4	32.9	39.7	39.3	25.4	25.3	0.0	0.0	13h38m	05:05	18:42
	July	33.0	34.5	39.5	40.0	25.5	28.1	0.0	0.0	13h30m	05:15	18:45
	August	33.9	34.1	40.5	39.9	26.1	27.0	0.0	0.0	13h00m	05:28	18:25
	September	32.3	31.4	37.3	37.4	24.2	24.4	0.0	0.0	12h19m	05:40	17:55
Autumn	October	29.8	26.4	34.4	32.7	19.0	19.1	0.0	0.0	11h36m	05:52	17:26
	November	25.3	21.7	28.4	27.7	14.4	15.4	4.8	0.0	10h58m	06:10	17:07

(19.2°N, 45.4°E, altitude 800 m) recorded weather parameters throughout the year (Table 1). These weather stations provided a reading of ground temperature, air temperature, air pressure, relative humidity, wind speed, wind direction, rainfall and solar radiation every 15 min throughout the recording period. Sunrise and sunset times as well as civil twilights were obtained from various freely accessible databases. The months of the year were grouped into seasons according to the Saudi Arabian seasonal calendar and previous climate studies [2,3] with the exception of autumn being from October–November, not September–November based on the Saudi Arabian dry season occurring from June to September (summer) [2].

2.3. Anaesthesia of the Arabian oryx

All animals were remote injected using a Dan-Inject dart gun (Daninject, Børkop, Denmark) with etorphine hydrochloride (Captivon™ 98, Wildlife Pharmaceuticals Ltd., White River, South Africa; 19 μg/kg), Ketamine (Ketaminol® Vet., MDS Animal Health, Intervet International B. V., Boxmeer, The Netherlands; 0.3 mg/kg), Midazolam (Midazolam, Wildlife Pharmaceuticals Ltd., White River, South Africa; 0.13 mg/kg), and Medetomidine (Zalopine 10 mg/ml, Orion Pharma, Espoo, Finland). After induction of anaesthesia, all animals received oxytetracycline (Terramycin LA, Pfizer, Brazil; 20 mg/kg intramuscular) and ketoprofen (Ketovet, Vetmedim Animal Health, Cantho City, Vietnam). After the procedure, anaesthesia was reversed using naltrexone hydrochloride (Naltrexone, APL, Kungens Kurva, Sweden; 40 mg intramuscular) and atipamezole hydrochloride (Antisedan, Orion Pharma; 2 mg intramuscular).

2.4. Surgery for coarse-grain actigraphy

Following recumbency, the animals were moved to a shaded area for surgery. The animals were then placed on a stretcher, lifted and weighed with a large scale. Following aseptic preparation, a small vertical incision (< 8 cm in length) was made through the skin in the left paralumbar fossa and the muscle layers of the abdominal wall were separated using blunt dissection. The implant, which was sterilized within a container with formalin pellets for 48 h prior to implantation, was inserted, untethered, into the intraperitoneal cavity, the incisions closed, and liberally sprayed with Necrospray (Bayer Healthcare, Germany). Once covered in the biologically inert wax (SasolWax 1276, 140 Sasol, Johannesburg, South Africa), the implant measured 30 mm in diameter and 80 mm in length and weighed approximately 60 g. The data logger measured activity every 10 min (AIC001: Abdominal implant, Africa Wildlife Tracking, South Africa). A satellite collar with a GPS unit was attached to the neck of each animal (iridium satellite collar, Africa Wildlife Tracking, South Africa). The data measured every 10 min by the logger was then transferred to the collar (via radio telemetry, on an hourly basis) and then uploaded to the satellite along with the GPS data. Following reversal of the anaesthesia, the animals were monitored in holding pens with access to food and water, before being released into the Mahazat as-Sayd Protected Area and the Uruq Bani Ma'arid Protected Area for the year-long recording period. The animals were not recaptured following the year-long recording period, due to the extreme isolation and size of the environments.

2.5. Data analysis

The data for the twelve oryx were analyzed individually by month (the first 28 days of each month) and then pooled for analysis by season for each experimental area separately. Sampling intervals were scored active or inactive when any level of movement was detected within each 10 min sampling interval or zero movements, respectively.

Adapted from Pépin et al. [33] the activity budget or mean proportion of time spent active was calculated through dividing each 24 h day into temporal windows or periods. These temporal periods were morning (1 h before – 1 h after sunrise), daytime (from 1 h after sunrise until 1 h before sunset), evening (from 1 h before sunset – 1 h after sunset) and lastly nighttime (from 1 h after sunset to 1 h before sunrise).

The Diurnality index (D-index), adapted from Hoogenboom et al. [18] and Daan et al. [9], was calculated in order to determine the concentration of daytime activity in records per individual animal per hour. The equation used is $D = (C_d - C_n)/(C_d + C_n)$. In this case C_d and C_n represent the activity counts per hour during the daytime and nighttime respectively. The D-index ranges between −1 and +1 and represents dominance for either nocturnality or diurnality respectively. A D-index at or around 0 indicates an equal distribution of activity between day and night and tends to crepuscularity [9]. Daytime and nighttime periods were defined using civil twilights from freely accessible databases.

2.6. Statistical analyses

All statistical tests were conducted separately for each experimental area. All values reported in the results section are means with standard deviation (X ± SD). For the four-period time budget analysis, all proportion ratios were arc-sine square root-transformed in order to fit fully general linear models. A three-way ANOVA (type III) with post-hoc (Tukey HSD) pairwise comparisons between season, period and animals was used to assess the effects of the seasons on the activity budgets during each time period with animals as a random independent variable, thereby accounting for the repeated measurements obtained from each animal.

For the analysis of the D-index, all values were converted to positive values between 0 and 1 for linear transformations of the data ($D' = (0.5 * D) + 0.5$) and then arc-sine square root-transformed (in order to run general linear models). A two-way ANOVA (type III) with post-hoc Tukey HSD was used in order to assess the relative effects of season on the group D-index fluctuations with animals as a random independent variable. The same was performed for months on group D-index fluctuations.

To determine the relationship between photoperiod and ambient air temperatures with year-long activity measures, multiple regressions (Pearson correlation) were conducted between D-index and the corresponding minutes of daylight and mean 24 h air temperature. Both linear and curvilinear regression fit lines were employed where appropriate for all plots. Bi-weighted Loess non-parametric smoothing curves (50% of points to fit) were applied to all regression models in order to graphically represent the rhythms of the data. All statistical analyses were conducted using IBM SPSS Statistics software version 23 and PAST 3 [14].

3. Results

3.1. Seasonal variation of four-period time budgets

The MS oryx presented with significant differences in the mean 24 h proportion of time spent active between seasons, time periods and animals (ANOVA: 3-way, $N = 6$, see Table 2 for values). Significant differences inter-seasonally as well as intra-seasonally were observed for the daily mean proportion of time spent active during the four time periods (see Fig. 1 and Table 3). Throughout the year, independent of time period effects (Post-hoc Tukey HSD), the 24 h mean proportion of time spent active was significantly higher during spring-time (43 ± 9%) compared to other seasons (autumn, $P = 0.001$; summer & winter, $P < 0.001$), followed by autumn (40 ± 9%) (summer & winter, $P < 0.001$) and lastly equally distributed for summer and winter (38 ± 8%, $P = 0.963$). Throughout the year, independent of seasonal effects (Post-hoc Tukey HSD), the mean 24 h proportion of time spent active during the four periods was highest during the evenings

Table 2
Seasonal variation in four-period time budgets for Arabian oryx in the MS ($N = 6$) and UBM ($N = 6$) experimental areas using a three-way ANOVA (type III) to assess the effects of season, period, animal and their interactions. Df_1 is degrees of freedom for the hypothesis, while df_2 for the errors.

		Season			Time period			Animal			Interaction		
		df_1; df_2	F	P	df_1; df_2	F	P	df_1; df_2	F	P	df_1; df_2	F	P
4-period	MS	3; 15.0	0.668	0.584	3; 15	2.104	0.143	5; 2.5	1.998	0.121	45; 7948	2.902	< 0.001
	UBM	3; 14.1	9.272	0.001	3; 15.7	46.439	< 0.001	5; 15.4	4.496	0.010	42; 7300	4.018	< 0.001

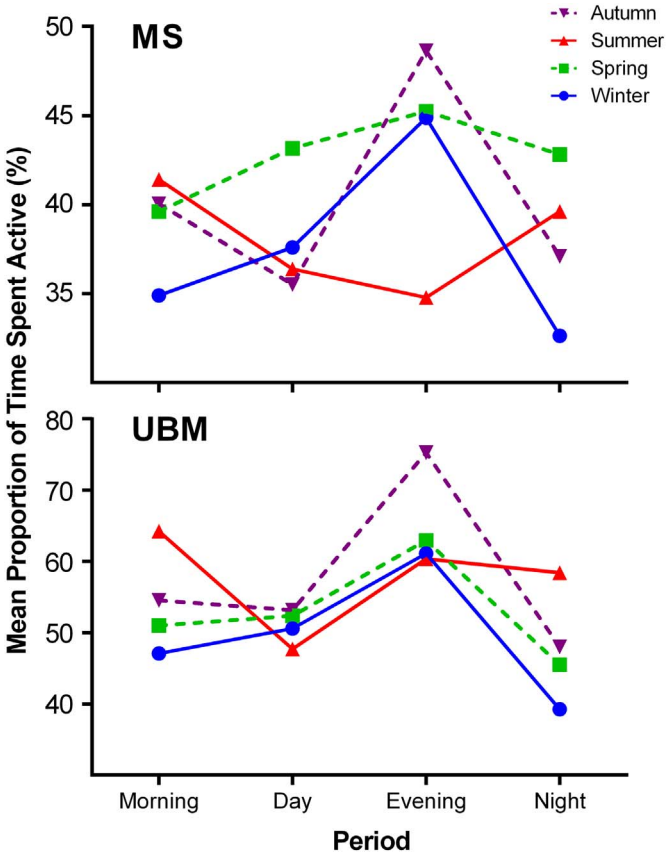


Fig. 1. Graphs showing the mean proportion of time spent active by Arabian oryx as a percentage for autumn (purple dashed line, inverted triangle markers), summer (red solid line, triangle markers), spring (green dashed line, square markers) and winter (blue solid line, circle markers) during the morning, daytime, evening and nighttime periods in each experimental area (MS & UBM). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(43 ± 10%, $P < 0.001$) and equally distributed between the mornings (39 ± 10%) (daytime, $P = 0.790$; nighttime, $P = 0.788$), daytime and nighttime (38 ± 7%, 38 ± 8% respectively, $P = 1.000$). (See Table 3.)

The UBM oryx presented with significant differences in the mean 24 h proportion of time spent active between seasons, time periods and animals (ANOVA: 3-way, $N = 6$, see Table 2 for values). Similarly to the MS oryx, the UBM oryx showed significant differences inter-seasonally as well as intra-seasonally for the daily mean proportion of time spent active during the four time periods (see Fig. 1 and Table 3).Throughout the year, independent of time period effects, the 24 h mean proportion of time spent active was greatest during summer and autumn (58 ± 12%, $P = 0.976$), followed by spring (53 ± 12%, $P < 0.001$) and lowest during winter (50 ± 15%, $P < 0.001$). Throughout the year, independent of seasonal effects, the mean 24 h proportion of time spent active during the four periods was highest during the evenings (65 ± 15%, $P < 0.001$), at 54 ± 13% during the mornings ($P < 0.001$) and closely distributed between daytime and nighttime (51 ± 9%, 48 ± 11% respectively, $P = 0.072$).

3.2. Seasonal and monthly variation of the Diurnality index and environmental relations

The daily D-index fluctuations varied significantly for the MS group between seasons (ANOVA: 2-way, $P = 0.001$, $N = 6$) and months (ANOVA: 2-way, $P < 0.001$, $N = 6$) (see Supplementary Table S1 for further values). Seasonally, the D-indexes for spring (D = 0.019 ± 0.09) and autumn (D = 0.013 ± 0.08) were not different ($P = 0.956$, Tukey HSD), while summer (D = − 0.027 ± 0.08) and winter (D = 0.084 ± 0.08) presented with significant differences ($P < 0.001$). A negative D-index (nocturnal) was observed for the months of May, July and September (late spring and partial summer); a positive D-index (diurnal) for November through to March (late autumn to early spring) with the highest values observed during February and March (late winter and early spring); and a D-index near zero (crepuscular) for April, June, August and October (mid-spring, mid-summer and early autumn). In the MS group, a sharp drop from a

Table 3
Average 24 h time active and average proportions of time spent active for Arabian oryx during the morning (1 h before – 1 h after sunrise), daytime (1 h after sunrise –1 h before sunset), evening (1 h before – 1 h after sunset) and night-time (1 h after sunset – 1 h before sunrise) periods during the winter, spring, summer and autumn of February 2014 to January 2015 in the MS and UBM experimental areas, Saudi Arabia.

Season	Morning (%)	Daytime (%)	Evening (%)	Night-time (%)	Average 24 h time active (hours)
MS					
Winter	0.35 ± 0.07 ^{A,*}	0.38 ± 0.05 ^{B}	0.45 ± 0.08^{C}	0.33 ± 0.05 ^{A,*}	8.6 ± 2.8 *
Spring	0.40 ± 0.11 ^{A}	0.43 ± 0.07 ^{B,ǂ}	0.45 ± 0.09 ^{B}	0.43 ± 0.09 ^{B,ǂ}	10.3 ± 2.8 ^{ǂ}
Summer	0.41 ± 0.09 ^{A}	0.36 ± 0.07 ^{B}	0.35 ± 0.09 ^{B,*}	0.40 ± 0.07 ^{A}	9.1 ± 3.0
Autumn	0.40 ± 0.09 ^{A}	0.36 ± 0.05 ^{B}	0.49 ± 0.09^{C}	0.37 ± 0.04 ^{AB}	9.0 ± 3.1
UBM					
Winter	0.47 ± 0.14 ^{A,*}	0.51 ± 0.10 ^{B}	0.61 ± 0.17^{C}	0.39 ± 0.10 ^{D,*}	10.8 ± 3.1 *
Spring	0.51 ± 0.11 ^{A}	0.52 ± 0.07 ^{A}	0.63 ± 0.14 ^{B}	0.45 ± 0.10^{C}	12.1 ± 2.9
Summer	0.64 ± 0.10 ^{A,ǂ}	0.48 ± 0.09 ^{B,*}	0.60 ± 0.12^{C}	0.58 ± 0.06^{C,ǂ}	13.0 ± 2.7
Autumn	0.55 ± 0.11 ^{A}	0.53 ± 0.06 ^{A}	0.75 ± 0.10 ^{B,ǂ}	0.48 ± 0.06^{C}	12.7 ± 2.0

Values are given as mean ± S.D. Values in rows with the same alphabetic letter are similar, while values in rows followed by a different letter differ significantly intra-seasonally between time periods (ANOVA: $P < 0.05$). Values in columns followed by an asterisk (*) indicate the significantly lowest value and values followed by a cross (^{ǂ}) symbol indicate the significantly highest value in a given time period inter-seasonally (ANOVA: $P < 0.001$).

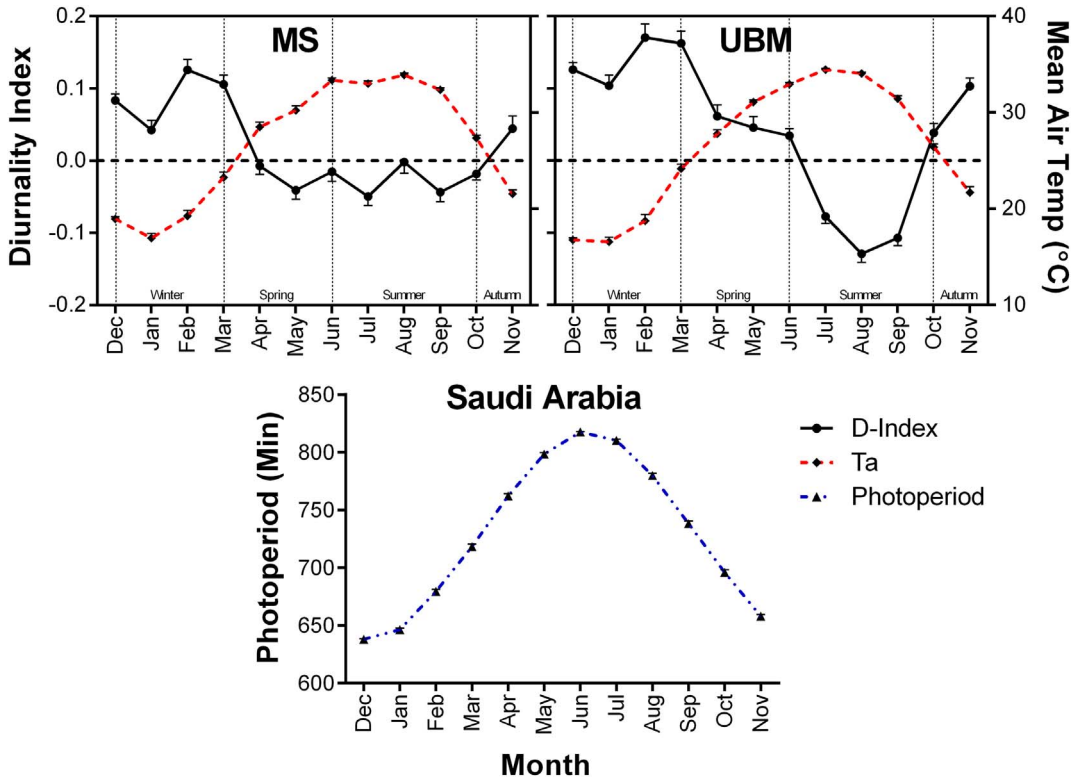


Fig. 2. Graphs showing the D-index (black solid line, circle markers) throughout the 12 months of the year in each experimental area (MS & UBM) and the corresponding mean 24 h air temperature (red dashed line, diamond markers, upper graphs) and minutes of daylight (blue dashed line, triangle markers, lower graph) recorded from the nearby weather stations. Dashed horizontal lines (upper graphs) represent the midline showing equal distribution of activity between day and nighttime, with D-indexes below indicating nighttime dominance and D-indexes above diurnal dominance. Dashed vertical lines (upper graphs) divide the relevant months into seasons of the year. Error bars show standard error of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

positive D-index to a D-index of zero (crepuscular) in early spring (March) can be related to a sharp increase in mean air temperature and increased photoperiod (Fig. 2).

The daily D-index fluctuations varied significantly for the UBM group between seasons (ANOVA: 2-way, $P < 0.001$, $N = 6$) and months (ANOVA: 2-way, $P < 0.001$, $N = 6$) (see Supplementary Table S1 for further values). As with the MS group, D-indexes were not different between the intermediate seasons of spring ($D = 0.090 \pm 0.1$) and autumn ($D = 0.071 \pm 0.07$, $P = 0.279$, Tukey HSD), but significantly different between summer ($D = -0.070 \pm 0.08$) and winter ($D = 0.133 \pm 0.08$, $P < 0.001$). A negative D-index was observed for July, August and September (mid to late summer); positive D-indexes for November through to March (late autumn to early spring) similar to the MS oryx; and a D-index near zero for April, May, June and October (mid to late spring, early summer and early autumn). In the UBM area spring D-indexes remained positive until early summer (June), when mean air temperature thresholds move toward 35 °C and D-indexes immediately drop below zero and remain negative until late summer to early autumn (July–October) (Fig. 2).

For both oryx groups, as minutes of daylight and mean 24 h air temperatures increased throughout the year, the D-index decreased congruently (Pearson correlation regression: $P < 0.001$) (see Supplementary Fig. S1). For both oryx groups, ambient temperatures exhibited moderately stronger relationships to the D-index compared to photoperiod (see values in Fig. S1). The UBM oryx D-index values exhibited a sharp decrease when daily photoperiod reached 700 min (spring) and then plateaued around 770 min (mid-summer) (Fig. S1C). In both oryx groups D-index values exhibited a sharp decrease when mean 24 h air temperatures exceeded 25 °C; however the MS oryx D-index began increasing around 35 °C whilst the UBM D-index continued decreasing.

4. Discussion

Owing to several factors and restrictions, the majority of previous studies examining the activity patterns of a species have done so in isolated locations and mostly under controlled conditions (e.g. food ad libitum, artificial temperature and lighting, captive bred animals etc.) for short periods at a time [35]. The current study is the first account of continuous year-round activity recordings (all seasons and all months examined in succession) in the Arabian oryx under completely natural conditions with no control over environmental conditions and with no interference beyond initial surgery. The study subject groups were also large, free-roaming, of mixed sex and were examined in two separate locations which varied significantly with regard to size and environmental conditions. The current study revealed that the Arabian oryx exhibit seasonal plasticity in their temporal niche preferences under fully natural conditions in relation to external environmental factors in order to survive the harsh desert climate.

4.1. Seasonal 24 h activity time budgets in the Arabian oryx

During the summer, maximum daytime air temperatures reach upper ranges (mean of 45 °C) and precipitation (mean of 0.0 mm) and relative humidity (mean of 11.6%) drop to lower ranges with a subsequence decrease in food biomass and quality [41,55]. In a possible response to these changing environmental and biomass variables, increased time spent foraging by oryx may be required so as to maintain energy balance and this possibly translates into the higher mean proportion of activity observed compared to the winter months during which maximum daytime air temperatures are lower (mean: 24 °C), precipitation (mean: 1.5 mm) and relative humidity (mean: 37%) are higher and food biomass and quality is toward the upper extent [46,55]. Additionally, the increased mean time spent active during

the summer in both areas is shifted to twilight and nighttime periods and not during the daytime possibly in order to avoid excess daytime heat load [17]. The changes in activity proportions observed for Arabian oryx between summer and winter seasons and the possible shift in foraging lengths and timing requires further study examining feeding activity patterns and diet content across the various seasons of the year.

Alternatively, the shifts in activity proportions and phasing of activity observed during summer and winter (mentioned later) may be attributed to seasonal changes in photoperiod leading to phenomena known as positive and negative masking [28,39]. In this case increased levels of light may lead to increased activity, and less light to decreased activity in possible complement with additional circadian mechanisms. These phenomena are usually dependent on a species and its phasing phenotypes (more light leads to increased activity in diurnal animals, but decreased activity in nocturnal animals) however, no previous studies have examined this type of masking on species that exhibit temporal niche switching under natural conditions. In the case of the Arabian oryx, further examination of the light/dark cycle and its relationship to activity could possibly provide interesting clues as to the effects of photoperiod and circadian rhythms in extreme conditions.

During the cold winter season, morning (mean: 12 °C) and nighttime (mean: 16 °C) temperatures were at their lowest and it appears oryx in both areas respond by exhibiting the lowest activity during these periods in a typical diurnal mammalian manner. From the morning until evening the oryx exhibit increasing activity reaching a maximum at sunset (evening), which then drops afterwards as it gets dark and air temperatures drop rapidly. Similar patterns were observed for the autumn months; however, higher morning and evening activity were observed compared to winter, possibly as daylight hours were longer and mean air temperatures warmer, allowing for extended foraging time.

The spring climate strongly supports diurnal activity patterns when considering that spring has the most annual rainfall (wet season is between November and April, [2]) and thus provides the highest biomass quality and quantity [55] coupled with mid-range ambient temperatures allowing for extended foraging time without excessive heat load or loss.

MS oryx summer activity exhibited nocturnal type phasing in agreement with Hetem et al. [17], possibly to avoid excess heat load and prevent evaporative water loss in response to increased ambient temperatures and photoperiod (increased solar radiation). UBM oryx summer activity exhibited more of a crepuscular type phasing in agreement with July activity patterns observed by Davimes et al. [10]. Such patterns are typical of most northern free-ranging ungulate species like Spanish ibex (*Capra pyrenaica*) [1], roe deer (*Capreolus capreolus*) [5], rocky mountain elk (*Cervus elaphus*) [12] and most caribou [25] and reindeer species, *Rangifer* sp. [8]. The bimodal activity peaks typical of northern ungulates has been linked to predator avoidance [22,32]; however, Arabian oryx in the UBM and MS areas do not have any natural predators and thus most likely employ this behaviour in response to daytime heat load and solar radiation avoidance. Additionally, in the more arid and climatically hostile UBM environment, evening and nighttime periods represent favourable foraging times, as relative humidity is highest, allowing flora to retain high water contents [46,55]. Arabian oryx are reported to be independent of surface water during the year and attain the majority of the necessary fluids from metabolic water in the flora they digest [30,46,50]. The digestibility of desert plants is usually dictated by the water content and thus seasonal precipitation and relative humidity strongly affect these factors [55].

4.2. Seasonal and monthly variation of the Diurnality index and environmental relations in Arabian oryx

It appears that a strong connection between both photoperiod and ambient temperature dictates the temporal niche preferences for activity patterns in free-roaming Arabian oryx. Increases in either the minutes of daylight and mean 24 h air temperatures translate into a decreased D-index, moving from diurnal activity in the months with a cooler climate and shorter photoperiod to nocturnality in the months with a hotter climate and longer photoperiod, with intermediate patterns during the transitional periods. These transitional seasons reflect ideal climate patterns in the desert with increased rainfall, water availability, relative humidity and biomass quality as well as mean air temperatures within the mammalian thermo-neutral zone [15,26].

Equal and weaker effects on the concentration of daytime activity (D-index) were observed for both photoperiod and temperature in the MS area oryx compared to the UBM oryx, indicating a possible masking effect by temperature on light and the subsequent output of activity patterns. However, without further examination of additional outputs of the circadian clock (e.g. body temperature and melatonin) no further suggestions can be made. In the UBM area however, moderately stronger relationships to temperature compared to photoperiod can be observed, indicating a slight dominance to non-photic changes driving activity patterns during the extreme seasons. These niche phenotypes and their seasonal switching can thus possibly be attributed to the direct stresses that temperature places on desert species like the availability of water, biomass quantity and quality and thermal loads in conjunction with alternating photoperiod.

Additionally, it is less likely that factors other than environmental proximate factors, such as reproductive cycles, sociality and territorial behaviours would be principally effective in driving activity patterns throughout the year. Arabian oryx have been shown to breed opportunistically and without seasonality in wild populations with calving occurring throughout the year [4,13,40,43,44,53,56]. It appears that male oryx exhibit testosterone cyclicity as an adaptive response to the variable ovarian cycles of female oryx as a means of energy conservation, and overall reproductive behaviours are heavily subjected to, and generally determined by, food and water availability as well as environmental conditions [4,38]. As with breeding and reproductive strategies, the variable social structure of the Arabian oryx also appears to be influenced by various environmental cues [19,52]. Furthermore, the benefit of social flexibility around extreme desert conditions seemingly outweighs the cost [23,52].

As Lourens and Nel [24] indicated with the bat eared foxes in South Africa, proximate factors such as food availability and environmental conditions strongly influence the activity of a species in natural conditions. They attribute prevailing temperatures and subsequent prey-availability to the winter-diurnal and summer-nocturnal phasing observed in their study. In the case of the Arabian oryx, the seasonal time budgeting of activity and phasing profiles may be dictated by water and food availability [15,31,37,43], which in turn is highly influenced by seasonal shifts in ambient temperature.

5. Conclusion

Overall, our long-term, continuous and detailed observations of two groups of free-roaming Arabian oryx living in two distinct locations within the Arabian Peninsula Desert demonstrates temporal niche switching of activity patterns at a seasonal level. The adaptive plasticity in activity patterns amongst the numerous morpho-physiological adaptations is quite notable and in conjunction with the Arabian oryx's previously threatened conservation status merits consideration. Inclusively understanding the basic responses of a species to extreme environmental parameters and the subsequent optimized evolutionary adaptations is of vital importance for both comparisons amongst wild populations and aiding deliberated conservation. As mammals continue to occupy extreme environments throughout the world, further plasticity of temporal niche preferences in response to climate and climate change may be uncovered.

Supplementary data to this article can be found online at http://dx.

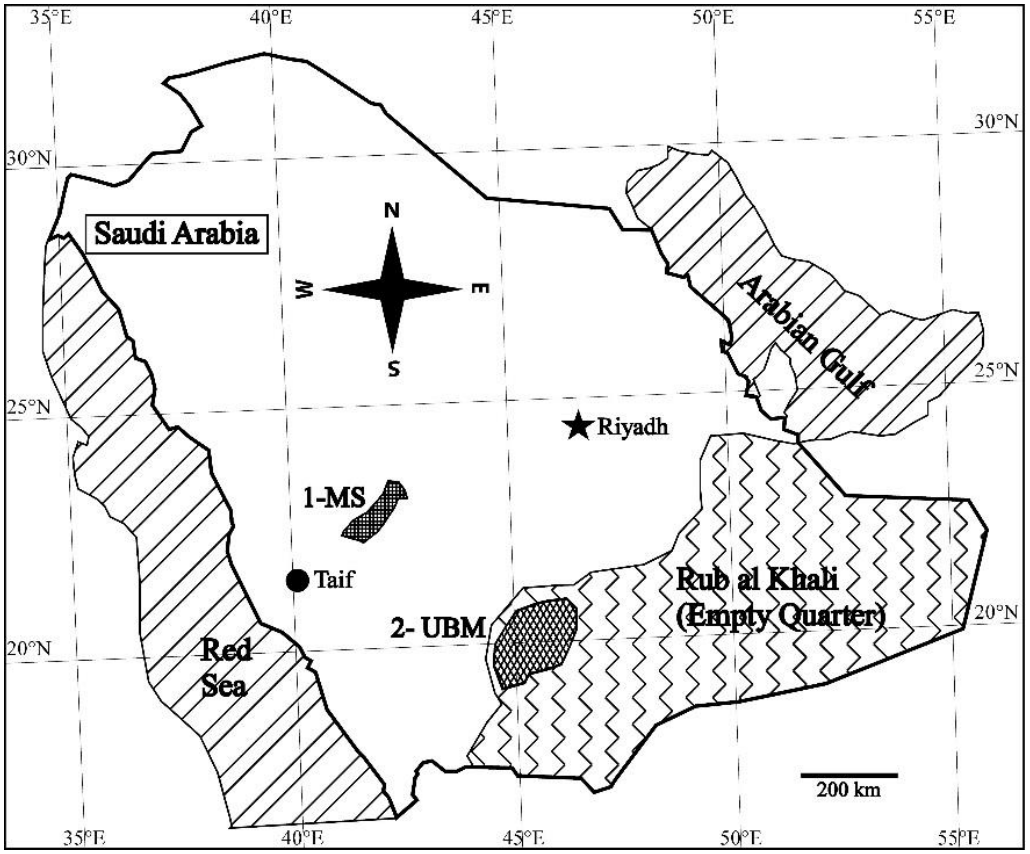
doi.org/10.1016/j.physbeh.2017.04.014.

Declaration of interest statement and acknowledgments

All authors declare no competing interests. This project was funded by the South African National Research Foundation (NRF) (94233, IFR150115112130, 64956, 87888) (J.G.D., P.R.M., N.C.B. and N.G) and the Deanship of Scientific Research Chairs at the King Saud University through the research group project number RGP_020 (A.N.A., O.B.M.). The authors are extremely grateful to Prince Bander bin Saud Al-Saud, former President of the Saudi Wildlife Authority (SWA), for his unlimited and enthusiastic support to undertake these studies on the Arabian oryx managed by the SWA. We are also grateful for the tremendous help provided by our colleagues at the National Wildlife Research Center in Taif, Saudi Arabia, especially Mr. Ahmed Boug, Dr. Saud Anagariyah, Mr. Khairy Ismail, Mr. Ghazi Raziman, Mr. Raed Aljuaid and Mr. Ali Aljuaid.

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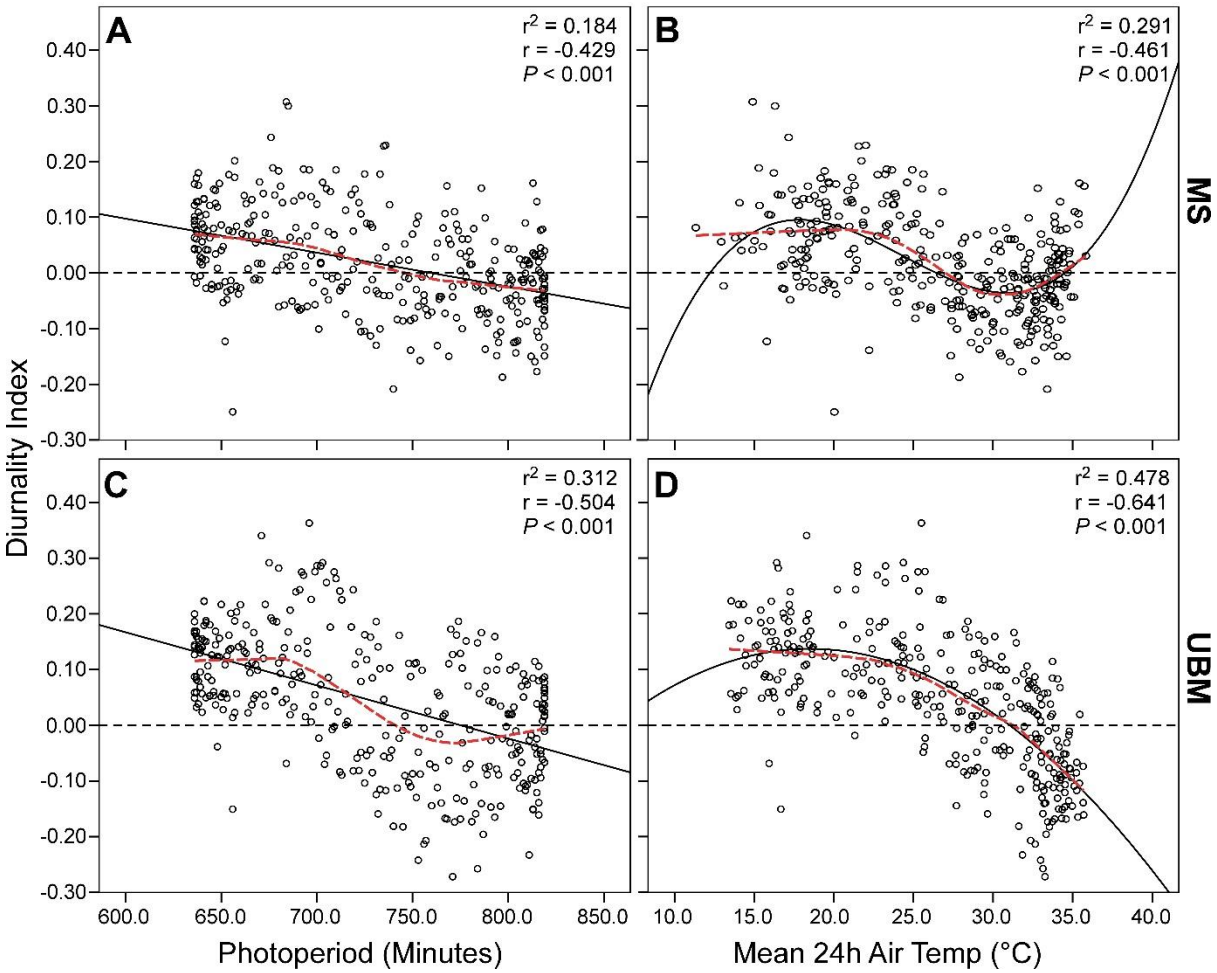
Supplementary Fig. S1. Geographical map showing experimental areas (hatched areas). Area 1, the Mahazat as-Sayd Protected area (MS), covers a 2200 km^2 area northeast of Taif, Saudi Arabia (22.2°N, 41.8°E). Area 2, the Uruq Bani Ma'arid Protected Area (UBM), covers a 12,658 km^2 area within the Rub al Khali desert (Empty Quarter), Saudi Arabia (19.3°N, 45.3°E).

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Physiology & Behavior, Volume 177, 2017, 148–154

http://dx.doi.org/10.1016/j.physbeh.2017.04.014



Supplementary Fig. S2. Group D-index plotted against (A & C) minutes of daylight (photoperiod) and against (B & D) mean 24 h air temperature for MS and UBM oryx. All graphs use data averaged for six Arabian oryx in the Mahazat as-Sayd Protected Area (MS) and six Arabian oryx in the Uruq Bani Ma'arid Protected Area (UBM) throughout the entire recording period (n = 336). R-squared (R^2), Pearson correlation (r) and significance (p) values are included for each plot in the top right corner of each graph. Dashed horizontal lines represent the midline showing equal distribution of activity between day and nighttime, with D-indexes below indicating nighttime dominance and D-indexes above, diurnal dominance. Corresponding linear and curvilinear regression fit lines are included in each plot as solid black lines. Red dashed lines represent bi-weighted Loess smoothing curves (50% of points required to fit) for non-parametric, realistic visualization of data patterns.

Table S1: Seasonal and monthly variation in D-index for Arabian oryx in the MS (N = 6) and UBM (N = 6) experimental areas using two-way ANOVAs (type III) to assess the effects of season, animal and their interactions as well as month, animal and their interactions. Df_1 is degrees of freedom for the hypothesis, while df_2 for the errors.

	Season			**Animal**			**Interaction**		
	df_1; df_2	F	P	df_1; df_2	F	P	df_1; df_2	F	P
MS	3; 15.0	9.833	0.001	5; 15.3	4.980	0.007	15; 1987	4.371	<0.001
UBM	3; 14.2	32.044	<0.001	5; 14.3	2.653	0.068	14; 1825	6.763	<0.001
	Month			**Animal**			**Interaction**		
MS	11; 55.0	6.297	<0.001	5; 55.0	5.430	<0.001	55; 1939	3.903	<0.001
UBM	11; 49.0	20.043	<0.001	5; 49.0	3.348	0.011	49; 1782	4.463	<0.001

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Physiology & Behavior, Volume 177, 2017, 148–154

http://dx.doi.org/10.1016/j.physbeh.2017.04.014

-IV-

Seasonal variations in sleep of free-ranging Arabian oryx (Oryx leucoryx) under natural hyper-arid conditions

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Sleep. 2018 (In Press)

Seasonal variations in sleep of free-ranging Arabian oryx (*Oryx leucoryx*) under natural hyper-arid conditions

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Short running title: Sleep in Free-ranging Arabian oryx

Abstract

Study objectives: The Arabian oryx lives under hyper-arid conditions in the Arabian Desert and exhibits temporal niche switching of activity patterns at a seasonal level. The objective of the current study was to provide a polysomnographic-based study of sleep in free-roaming Arabian oryx in their natural habitat to determine whether extreme seasonal climate variations resulted in changes in sleep patterns and physiology associated with the seasonal switching of temporal niches.

Methods: Electroencephalography, nuchal electromyography, actigraphy and subcutaneous temperature were recorded in free-roaming Arabian oryx in the Mahazat as-Sayd Protected Area, Kingdom of Saudi Arabia during winter and summer.

Results: Total daily sleep time in winter was 6.69 h and 3.77 h in summer. In winter, oryx exhibited nocturnal sleep typical of artiodactyls of around 60 kg body mass. In summer oryx, slept mostly during the day and subcutaneous temperature was seen to rise during sleep, but not as rapidly as the rises observed in ambient air temperature. REM sleep formed a very small percentage of total sleep time, especially so in the summer.

Conclusions: The unusual sleep patterns and physiology during summer appears to be related to high ambient air temperatures that affect both intrinsic and extrinsic factors necessary for survival. The Arabian oryx appears to use sleep physiology as an adaptive thermoregulatory mechanism in the hot summer months.

Key words: Arabian oryx; NREM sleep; REM sleep; temporal niche switching; electrophysiology; actigraphy; temperature.

Statement of Significance

The current study provides electrophysiological sleep recordings in free-roaming Arabian oryx under natural conditions. Arabian oryx exhibit typical artiodactyl nocturnal sleep patterns and physiology during the cooler winter months, but these patterns and physiology change dramatically during the hot summer months. These changes to sleep patterns and physiology in the summer months appear to be driven by the increased ambient air temperatures experienced, and the Arabian oryx appears to employ the standard physiology of mammalian sleep to derive a novel mechanism of thermoregulation.

1. Introduction

The Arabian oryx (*Oryx leucoryx)*, is a medium-sized desert dwelling artiodactyl, that was found throughout the hyper-arid desert conditions of the Arabian Peninsula, but is now mostly restricted to protected areas of the region. Arabian oryx exhibit numerous behavioural and morphophysiological responses to high temperatures including enhanced selective brain cooling[1] and adaptive heterothermy. [2-4] Additionally, Arabian oryx undergo extensive seasonal changes in the amplitude of their daily body temperature rhythms[3-6] and have been shown to exhibit temporal niche switching of activity/inactivity patterns under natural conditions. [5,7-9] The oryx change from a nocturnal inactivity pattern during the cooler winter months to a bimodal inactivity pattern during the hotter summer months. [5] The timing of the major inactive bouts shifts seasonally from the coolest periods of the winter nights to the hottest periods of the summer days. A full year study of free-roaming Arabian oryx in the deserts of Saudi Arabia confirmed that oryx exhibit a seasonal plasticity and switch between diurnal activity patterns during winter and nocturnal/crepuscular patterns during summer, with intermittent patterns during autumn and spring. [9] Significant inter- and intra-seasonal variations were noted in the temporal budgeting of 24 h activity patterns in relation to changing weather patterns, photoperiod lengths and possibly food and water availability. It appears that the temporal niche switching exhibited by the Arabian oryx is dictated by the availability of food and water, which in turn is primarily driven by seasonal shifts in ambient temperatures and rainfall. [5,9]

Comparative polysomnographic studies are mostly limited to captive or zoological species, apart from recent studies on wild sloths, [10,11] pectoral sandpipers, [12] barn owls, [13] ostriches (semi-wild conditions), [14] and great frigatebirds. [15] Critical differences between captive and free-roaming animals include: (1) animals in captivity are often provided food and water *ad libitum*, thereby reducing or removing the need for foraging; and (2) captive

animals do not need to remain vigilant towards predators. [16] Thus, captive animals will have more time available for sleep, and appear to sleep longer than their wild conspecifics. [10,16] Polysomnographic studies on captive animals thus raise concerns that sleep durations and patterns reported do not truly reflect those found in wild, free-roaming animals. [10,17-19]

Descriptions of the activity-based phenomenon of temporal niche switching are rare (see reviews in [20,21]), especially under natural conditions. [5,22-24] No prior mammalian studies have examined temporal niche switching in terms of the physiological parameters of sleep, thus the polysomnographic study of sleep in species that exhibit temporal niche switching (see [12,15] for examples in avians) may provide insights into how sleep patterns are influenced by hyper-arid conditions (especially shifts in temperature) and determine the potential plasticity of sleep patterns. Thus, the aim of the current study was to provide a polysomnographic-based sleep study of the Arabian oryx to determine whether exposure to the hyper-arid desert climate has led to any novelties regarding sleep architecture, patterns or physiology in this species.

2. Materials and Methods

2.1. Animals used and experimental groups

In the current study, a total of 6 adult Arabian oryx (4 males, 2 females – not pregnant, not lactating; mean body mass 62.7 ± 5.6 kg) were used. The animals were divided into two groups. Group 1 comprised three adult Arabian oryx (2 male, 1 female; mean body mass 61.7 ± 8.5 kg) in which polysomnography (PSG) (electroencephalogram, EEG and electromyogram, EMG), subcutaneous temperature (Tsc) and fine-grain actigraphy (ACT) were recorded in the Mahazat as-Sayd Protected Area, northeast of Taif, Kingdom of Saudi Arabia (22°12′12″N, 41°49′36″E; 2200 km^2 area) during January 2016 (winter season). Group 2 consisted of three adult Arabian oryx (2 male, 1 female; mean body mass 63.7 ± 1.5

kg) in which identical parameters, as those for group 1, were recorded during June 2016 (summer season). Due to PSG equipment malfunctioning, data could not be retrieved from all devices and as such the PSG data analysis was reduced to n = 1 (case #1: male, group 1) and n = 2 (cases #2 and #3: male, group 2). However, ACT and Tsc data was obtained from all six animals for the entire recording period and are presented (see limitations of study section). Ethical clearance for the current study was obtained from the University of the Witwatersrand Animal Ethics Screening Committee (clearance certificate number 2014/53/D), and all animals were treated according to the guidelines of this committee, which parallel those of the National Institutes of Health (NIH) for the care and use of animals in scientific experimentation.

2.2. Anaesthesia protocol

The current study follows similar protocols for anesthesia and surgeries in the Arabian oryx as previously described. [5-6] All animals were remotely injected using a Dan-Inject dart gun (Daninject, Børkop, Denmark) with etorphine hydrochloride (Captivon™ 98, Wildlife Pharmaceuticals Ltd., White River, South Africa; 19 µg/kg), ketamine (Ketaminol® Vet., MDS Animal Health, Intervet International B. V., Boxmeer, The Netherlands; 0.3 mg/kg), midazolam (Midazolam, Wildlife Pharmaceuticals Ltd., White River, South Africa; 0.13 mg/kg), and medetomidine (Zalopine 10 mg/ml, Orion Pharma, Espoo, Finland; 5 µg/kg). All animals received oxytetracycline (Terramycin LA, Pfizer, Brazil; 20 mg/kg) and ketoprofen (Ketovet, Vetmedim Animal Health, Cantho City, Vietnam; 2 mg/kg). After the procedure, anaesthesia was reversed using naltrexone hydrochloride (Naltrexone, APL, Kungens Kurva, Sweden; 40 mg) and atipamezole hydrochloride (Antisedan, Orion Pharma; 2 mg). After the recording period, the animals were again immobilized using a similar drug combination, and

the implants were removed. Again, oxytetracycline and ketoprofen was administered and the animals were allowed to recover in a shaded pen prior to release to their normal environment.

2.3. Neurologger® and iButton® implantation

Following recumbency after darting, the animals were moved to a shaded area for surgery. The implantation sites for the Neurologger® (the left side of the neck and the scalp behind the horns) were shaved and washed with chlorhexidine disinfectant (CHX, 0.5% chlorhexidine digluconate in 75% alcohol, Kyron Laboratories Pty Ltd.). Sterile drapes were taped in place over the respective surgical sites to isolate the disinfected area. Under aseptic conditions, an incision (approximately 10 cm in length) was made at the left lateral aspect of the neck, and a subcutaneous pocket extending approximately 15 cm ventrally from the incision site was created for the implantation of the Neurologger ® unit (model: Neurologger 1, Evolocus LLC). The Neurologgers® were fitted with two Lithium ion batteries (Lithium Primary Battery, SW-AA11, 3.6 V, Tekcell, Vitzrocell Co. Ltd.) and memory included an onboard 8GB micro SD card. The Neurologger® had a mass of 78 g and approximate dimensions of 66 × 36 × 10 mm (mass and dimensions include wax covering and batteries). A midsagittal incision was made over the skull and the skin reflected to expose the part of the skull overlying the cerebral cortex. A subcutaneous tunnel was made to join the pocket on the left side of the neck to the incision site on the skull in order to run the electrode wires from the Neurologger® to the skull. Using a cordless Dremel drill, five 2-mm-diameter holes were made in the dorsal aspect of the cranium for electrode placement. The first hole was drilled posterior to the lambdoid suture, for the placement of the indifferent electrode, while two holes were drilled approximately 2 cm apart and 2 cm lateral to the sagittal suture over both the left and right cortices for the placement of the EEG electrodes (LiFY Colorflex Li-HF 0.06mm2; Conductor configuration: Cu 30 x ø0.05mm; Insulation: PVC; electrode

impedance approximately 1.1Ω) The electrodes were placed in such a manner that the tips rested firmly on the surface of the dura mater and were secured in place with dental cement. The electrodes were presumably placed over the visual cortex based on the location of visual cortex in other Artiodactyl species. For the EMG recordings, two electrodes (±2 cm apart) were sutured into the dorsal nuchal musculature. All skin incisions were sutured following implantation. Bioelectric signals from the cerebral cortex and nuchal musculature were recorded at 500 Hz (6 channels including: 1 EMG, 2 EEG and 3 for accelerometer x, y and z planes). The recording periods were approximately 10 days. All skin incisions were sutured following implantation.

One iButton® data logger (DS1922L, Maxim Semiconductors, Dallas, USA) that measured subcutaneous temperature (Tsc) (data acquisition rate set at 5-min intervals) was inserted within the same subcutaneous pocket used for the Neurologger® (left lateral aspect of the neck) prior to the suturing of the sites. For recording Tsc, the device was calibrated against an accurate thermometer in an insulated water bath, providing an accuracy of ≤ 0.06°C.

The Neurologger® and iButton® units were coated in a biologically inert wax (SasolWax 1276, Sasol, Johannesburg, South Africa) and sterilized within a container with formalin pellets for 48 h prior to implantation.

2.4. Actiwatch implantation

Following similar sterilization methods and aseptic techniques as described above, a subcutaneous pocket was created on the right lateral aspect of both the neck and hindleg for the implantation of the Actiwatches. The Actiwatch Spectrum (Philips Respironics) was used in the current study and had a mass of 25 g and approximate dimensions of 35 × 35 × 12 mm (mass and dimensions include wax covering). Each Actiwatch, factory calibrated, was

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). An incision (less than 5 cm in length) was made at each of the respective implantation sites, and a subcutaneous pocket extending approximately 10 cm ventrally from the incision site was created. The Actiwatch, sterilized within a container with formalin pellets for 48 h prior to implantation, was inserted into the respective pockets, and the incisions sutured and liberally sprayed with Necrospray (Bayer Animal Health). Following reversal of the anaesthesia, the animals were monitored in holding pens for 2 days with access to food and water, before being released into a 1 x 2 km enclosure within the Mahazat as-Sayd Protected area for the 10 day recording period (no further food or water provided; animals grouped together within enclosure). After the recording period, the implanted animals were re-anesthetized, and all of the implanted devices were retrieved.

2.5. Recording of weather conditions

A permanent weather station within the Mahazat as-Sayd Protected Area (22.2˚N, 41.9˚E, altitude 1000 m) recorded weather parameters during the recording period (Table 1). This weather station provided a reading of ground temperature (Tg), air temperature (Ta), air pressure, relative humidity, wind speed, wind direction, rainfall and solar radiation every 15-min throughout the recording period. Sunrise and sunset times were obtained from a freely accessible database (https://www.timeanddate.com/sun/saudi-arabia/riyadh) providing archival data for daily sunrise/sunset, day length, astronomical twilight, nautical twilight and civil twilight times.

2.6. Data analysis

The recorded data on the Neurologger® micro SD was converted to float32 format and imported into Version 7.02a of the Spike 2 software (Cambridge Electronic Designs, UK) for visual scoring and analysis. Due to PSG device malfunctioning, only 48h of data was considered for each animal per season (ACT data was unaffected, see limitations of study section). The first 48h post-surgery was treated as habituation and data subsequently excluded. Prior to scoring, DC remove and smooth channel processes were applied to all channels. The PSG data was scored in 1-min epochs as: 1) wake, characterized by low-voltage, high-frequency EEG and high-voltage EMG; 2) NREM, characterized by high-voltage, low-frequency EEG and EMG lower in voltage amplitude than waking; or 3) REM, characterized by low-voltage, high-frequency EEG and an almost atonic EMG (Figs. 1A, B, C and D). A state was only assigned to an epoch if the particular state occupied at least 50% of the epoch. Adapted from the analysis undertaken by Voirin et al.[11] and for ease of comparison, the following variables were calculated and averaged across the recording period for each individual oryx: the percent time spent in each state (wake, NREM sleep, and REM sleep) per 24 h, the percent of total sleep time (TST) in REM sleep, the percent time spent in each state during the light period (sunrise to sunset) and during the dark period (sunset to sunrise). Additionally, the mean duration of episodes and the number of episodes per state for the 24 h, light and dark periods were calculated. Since the sample size of Arabian oryx for winter (n = 1) and summer (n = 2) were considered too small for statistical comparisons, only mean values for PSG data are reported. An episode was defined as a sequence 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 seasonally for all animals. Sleep bout duration frequencies for the 24 h, light, and dark periods were determined for winter and summer (Fig. S1). A sleep bout was defined as a period lasting at least 10 minutes

(two consecutive 5-min sleep bouts without waking) and included both NREM and REM epochs. The number of REM sleep episodes and their occurrence relative to Tsc minimum (nadir) was determined for winter and summer.

The average NREM sleep EEG delta power (slow wave activity (SWA), 0.5 – 4 Hz power density) was calculated from NREM sleep bouts early and late in the longest consolidated sleep periods for both seasons. 4 s artifact free epochs (REM epochs also excluded) were utilized and SWA was estimated using the Spike 2 software (total power of frequency in band, FFT number 512) (Fig. 1E). The power spectrum for each of the defined states was calculated with the Spike 2 computer program (Fig. 1F) (Hanning window, FFT number 512, sampling frequency 500 Hz, segment length 1.024 s; see [25]). State transition probabilities per 24 h were determined based on all animals studied for winter and summer (Fig. 1G). All statistical analyses, where possible, were conducted using IBM SPSS Statistics software version 23, PAST 3[26] and GraphPad Prism 6.

Maxim Integrated's 1-wire software tools® was used to retrieve the temperature data recorded from the iButtons® and then exported to Microsoft Excel for analysis. The animals from each group were analyzed individually and the data were then pooled by season. Tsc from each 5-min epoch was averaged across the recording period, and the data were then pooled to create an overall average Tsc per 24 h per season. The subcutaneous and ambient temperature differentials (ΔTsc = Tsc at end of bout – Tsc at start of bout, ΔTa = Ta at end of bout – Ta at start of bout) between sleep bout onset and offset per season were determined for all sleep bouts lasting more than 20 min. Similarly, the temperature differentials were determined for wake bouts of 60 min following the major sleep bouts. The rate of change in Tsc and Ta per minute during major sleep and wake bouts was then determined for both seasons (Tsc Rate of Change = ΔTsc (˚C) / bout length (minutes), Ta Rate of Change = ΔTa (˚C) / bout length (minutes)).

Phillips Respironics Actiware 5 was used to retrieve the recorded data from each of the implanted actiwatches. The raw data was exported to Microsoft Excel where it was manually scored and analysed. Data obtained from the neck and leg actigraphs were scored concurrently in 1-min 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 percent 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 per season (winter: n = 3, summer: n = 3) over the same recording days used for PSG. Total time spent active/inactive was determined for the 24 h, light and dark periods per season for all 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 inactive bouts seasonally for all animals.

3. Results

3.1. Actigraphy patterns seasonally

Significant differences were observed for time inactive per 24 h, with winter oryx inactive 12.98 ± 1.66 h (mean ± SEM) and summer oryx inactive 6.84 ± 1.42 h (unpaired, two tailed t-test, $p < 0.001$). Time spent inactive during the light period was similar between winter and summer, with winter oryx inactive 4.33 ± 2.41 h and summer oryx inactive 5.61 ± 1.74 h (unpaired, two tailed t-test, $p > 0.05$). Time spent inactive during the dark period was significantly different between winter and summer with winter oryx inactive 8.65 ± 1.93 h and summer oryx inactive 1.22 ± 1.74 h (unpaired, two tailed t-test, $p < 0.001$) (See Table 2 for number and duration of ACT state episodes for winter and summer oryx over 24 h and during the light and dark periods).

When using 5-min modal times for all animals, the average daily onset of the main inactive bout (longest consecutive period of inactivity) occurred around 03h00 during winter, whilst during summer the onsets occurred around 09h40 or around 12h25. The average length of the main inactive bout was 127 ± 19 min during winter, whilst in summer it lasted 115 ± 50 min. Additionally in summer, oryx exhibited a third main inactive bout with an onset around 15h00 and a length of 90 ± 37 min. Thus, during the winter recording period, 68.21% (± 13.46%) of inactivity occurred during the dark period compared with 16.77% (± 13.20%) during summer (unpaired, two tailed t-test, $p < 0.001$). During the light period, winter oryx were inactive 31.80% (± 13.46%) compared with 83.23% (± 13.20%) during summer (unpaired, two tailed t-test, $p < 0.001$). Overall, the current findings of inactivity timing resemble previous reports, [5] with winter oryx exhibiting a primarily nocturnal inactivity pattern (major inactive bout occurring during the cooler part of the night) and summer oryx exhibiting a primarily diurnal inactivity pattern (major inactive bouts occurring during the hottest parts of summer days) (Fig. 2).

3.2. Temperature rhythms between seasons

Tsc rhythms between seasons exhibited similar daily timing patterns with an early morning trough at sunrise and a peak at 15h00 followed by a dip at sunset across all animals. Winter mean Tsc was 32.92 ± 0.05˚C and was significantly lower than the summer mean Tsc of 38.29 ± 0.02˚C (unpaired, two tailed t-test, $p<0.001$; Fig. 2). The range of Tsc varied highly between seasons. Winter oryx exhibited an average daily variation of 8.93˚C in Tsc, while summer oryx exhibited an average daily variation of 3.23˚C in Tsc. Overall, Tsc rhythms exhibited no seasonal change in the timing, only amplitude, and thus resemble previous reports. [3-5]

3.3. Polysomnographic recordings of Animal case #1: Winter

24 h TST observed was 6.69 ± 1.21 h (mean ± SEM) with 17.31 ± 1.93 h spent in waking, 6.42 ± 1.84 h in NREM sleep and 0.28 ± 0.21 h in REM sleep (Fig. 3 and Table 3). TST observed during the light period was 2.18 ± 1.44 h with 8.82 ± 2.42 h spent in waking, 2.10 ± 2.31 h in NREM sleep and 0.08 ± 0.18 h in REM sleep. TST observed during the dark period was 4.51 ± 1.84 h with 8.49 ± 3.09 h spent in waking, 4.32 ± 3.00 h in NREM sleep and 0.19 ± 0.32 h in REM (for number and duration of state episodes over 24 h, light and dark periods see Fig. 3 and Table 2). REM sleep when expressed as a percentage of TST was 4.18 ± 0.51% over 24 h, 3.67 ± 3.68% during the light period, and 4.21 ± 0.25% during the dark period (Fig. 4) (See Fig. 5 for total state times, episode numbers and episode durations intra-seasonally for winter during the light and dark periods). When using 5-min modal times, the average daily onset of the main sleep bout (longest consecutive period of NREM and REM sleep combined) occurred between 03h30 and 04h00 and lasted 110 ± 14 min. This oryx appeared to exhibit predominantly nocturnal sleep phasing, resembling previous inactivity timing reports, [5] with the majority of NREM and REM sleep occurring during the dark period (coolest parts of the winter nights) (dark period: 67.41 ± 14.87%, light period: 32.58 ± 14.87%, of 24 h TST) (Fig. 6 and 7).

3.4. Polysomnographic recordings of Animal cases #2 and #3: Summer

24 h TST observed was 3.77 ± 0.78 h (mean ± SEM) with 20.23 ± 1.45 h spent in waking, 3.69 ± 1.41 h in NREM sleep and 0.08 ± 0.07 h in REM sleep (values are averages of both oryx, Fig. 3 and Table 3). TST observed during the light period was 3.13 ± 1.21 h with 10.87 ± 2.16 h spent in waking, 3.05 ± 2.10 h in NREM sleep and 0.08 ± 0.11 h in REM sleep. TST observed during the dark period was 0.64 ± 0.70 h, with 9.36 ± 1.32 h spent in waking and 0.64 ± 1.32 h in NREM sleep. No REM sleep was observed during the dark

period for either oryx (for number and duration of state episodes over 24 h, light and dark periods see Fig. 3 and Table 2). REM sleep when expressed as a percentage of TST was 2.12 ± 0.70% over 24 h and 2.56 ± 1.53% during the light period (Fig. 4) (See Fig. 5 for total state times, episode numbers and episode durations intra-seasonally for summer during the light and dark periods). When using 5-min modal times, the average daily onset of the main sleep bout occurred either around 08h00 or 12h30 and lasted 135 ± 21 min. Summer oryx appear to exhibit predominantly diurnal sleep phasing, resembling previous inactivity reports, [5] with the majority of NREM and all of REM sleep occurring during the light period (sunrise until early afternoon) (dark period: 16.98 ± 22.53%, light period: 83.02 ± 22.53%, of 24 h TST) (Figs. 6 and 7).

3.5. Variations between winter and summer sleep

The number of sleep bouts and the duration of these bouts were similar between winter and summer for the 24 h, light and dark periods and overall appears polyphasic (Fig. S1). Sleep bouts with longer durations were more frequent during summer, particularly during light hours, while shorter sleep bouts were more frequent during winter especially during dark hours. NREM sleep-related SWA examined early (between 19h00 – 21h00 in winter; between 06h00 – 09h00 in summer) and late (between 03h00 – 05h00 in winter; between 10h00 – 14h00 in summer) into the longest consolidated sleep periods for both seasons exhibited differences. During winter and summer, SWA was higher early compared to late into the longest sleep periods [winter median SWA (v^2 x 10^{-3}): early – 8.50, late – 6.16; summer median SWA: early – 13.45, late – 8.33; Fig. 1E]. SWA during summer exhibited higher median power compared to winter during both early and late into the longest sleep periods (Fig. 1E). Between winter and summer, oryx exhibited variation in vigilance state transition probabilities, namely NREM – REM and REM – wake transitions. The

probability of REM – NREM transitions was 30.6% of the time during winter and 20% during summer. The probability of REM – wake transitions was 69.5% of the time during winter and 80% during summer (Fig. 1G).

When comparing PSG and ACT data over the same recording days for time spent in state (% of the hour) both methods show similarity during winter and summer periods (Pearson correlation, $p<0.001$; Fig. S2). PSG and ACT data show similar patterns per season and between individual oryx (Figs. 6 and 7). Overall, similar patterns of nocturnal inactivity/sleep during winter and diurnal inactivity/sleep during summer can be observed for all animals (including oryx for which PSG data could not be retrieved) and thus resemble a previous report. [5]

3.6. Relationship between sleep and temperature

During winter, 67% (14/21 episodes) of the time spent in REM sleep occurred during the night (20h00-01h00) and prior (02h00 – 05h00) to Tsc min (28.7 ± 0.2˚C at 06h00) (Figs. 8A and B). During summer, 88% (15/17 episodes) of REM sleep occurred after Tsc min (36.5 ± 0.1˚C at 05h00) and ended around noon (05h00-12h30) when Ta reached peak values (40.2 ± 0.1˚C). During winter, Tsc at the onset and offset of the main sleep bout was 33.2˚C and 32.0˚C (03h30), respectively (Ta start: 5.4˚C, Ta end: 3.9˚C). During summer, the Tsc at the onset and offset of the main sleep bout was 36.5˚C and 37.2˚C (08h00) and 38.5˚C and 39.1˚C (12h30), respectively (08h00: Ta start: 31.9˚C, Ta end: 35.7˚C; 12h30: Ta start: 39.6˚C, Ta end: 40.5˚C).

When examining the rate of change of Tsc and Ta during sleep bouts greater than 20 minutes, seasonal differences were noted (Fig. 9, Table 4). During winter sleep bouts of greater than 20 minutes during the dark period, when most sleep in winter occurred (67.41% of TST, see above), Tsc was observed to decrease at a rate of -0.0125°C/min, while Ta

decreased at a similar rate of -0.0110°C/min. During summer sleep bouts of greater than 20 minutes during the light period, when most sleep in summer occurred (83.02% of TST, see above), Tsc was observed to increase at a rate of 0.0023°C/min, while Ta increased at a much faster rate of 0.0178°C/min (Table 4). Thus, during sleep in the light period in summer Tsc increased at a rate almost 8 times slower than Ta while the oryx were asleep. In contrast, in the one hour following a light period sleep bout in summer, when the oryx were awake, Tsc increased at a rate of 0.0076°C/min, while Ta increased at a rate of 0.0016°C/min (Table 4), indicating that following sleep, Tsc increased at a rate of 4.75 times faster than Ta. Thus, during the light period sleep bouts in summer, the rate of increase in Tsc was substantially lower than the rate of increase of Ta, but when awake, the rate of increase in Tsc was substantially higher than the rate of increase of Ta (Fig. 9). This indicates that sleep bouts during the light period in summer slows the overall rate of increase of body temperature (Fig. 9).

4. Discussion

The current study documents polysomnographic and associated recordings in a freely roaming artiodactyl species that inhabits very harsh, hyper-arid, desert conditions. The recordings revealed that the Arabian oryx exhibits typical mammalian sleep-wake states[27] that can be readily defined as wakefulness, NREM and REM sleep. In addition, the Arabian oryx exhibits sleep patterns that vary seasonally confirming previous reports of seasonal temporal niche switching and varying activity/inactivity patterns. [5,7-9] The temporal variation of electrophysiologically defined sleep patterns between seasons exhibited by Arabian oryx appears to be related to thermoregulation in response to the challenges faced with high ambient temperatures during the summer.

Limitations of the current study

Arabian oryx were brought to the edge of extinction through excessive hunting, the last naturally occurring populations being eliminated in the Nafud and the Rub al Khali deserts in the Kingdom of Saudi Arabia during the 1970's. [28] Since then, Arabian oryx numbers have been restored through captive breeding programmes and they are now assigned "vulnerable" status on the IUCN Red List (June 2011). [29] This vulnerable status is reflected in the primary limitation of the current study, which is the small sample size of oryx from which we obtained comprehensive PSG recordings, with an n of 1 in winter and an n of 2 in summer. While we attempted to have an n of 3 for each season, equipment failure resulted in a smaller sample size than intended. Although the sample size for the PSG data is small, it does correlate significantly with the timing patterns of the ACT data for all six animals. In the case of ACT data, an underestimation of wakefulness compared with PSG will always be present as quiet waking can be scored and interpreted as inactivity. Moreover, the combination of ACT and PSG data in the current study is very similar to the seasonally defined patterns of activity and inactivity described in previous studies of the Arabian oryx. [5,7-9] In the case of any potential effects of rumination on sleep and EEG patterns, no prior information exists for Arabian oryx and no examination was conducted within the current study. In other Artiodactyl species, no specific EEG pattern was associated for the onset or offset of rumination during sleep states and no correlations between EEG and rumination were reported. [30] Due to the invasive nature of the current study, the vulnerable conservation status of the Arabian oryx, as well as ethical and governmental approval limitations due to the conservation status of the oryx, the experimental procedures unfortunately could not be repeated to increase the sample size. This will obviously reduce the interpretive strength of the results obtained, thus, in the ensuing discussion, while we generalize our data, we are

cognizant of the fact that the results we are discussing should be considered as case studies and we temper our conclusions appropriately.

Arabian oryx sleep in winter

Our analysis indicates that during the winter, the Arabian oryx obtains the majority of its sleep (~67%) during the coolest hours of the early morning prior to sunrise. During winter, the oryx recorded had a total sleep time of 6.69 h, with REM occupying 4.18% of TST, the remainder of sleep being NREM. This total amount of sleep, the timing of sleep, the polyphasic nature of sleep, the observation that sleep consists of readily identifiable NREM and REM states, with no unusual sleep states being observed, all indicate that the Arabian oryx, being a herbivorous mammal with a body mass of around 60 kg, sleeps, in the winter, similarly to what might be predicted from previous studies of mammalian species, albeit captive studies, of similar size, diet and phylogenetic affinities. [18,27,31,32] In addition, during sleep in the winter, the subcutaneous temperature of the oryx was seen to decrease at a rate similar to the decrease in ambient air temperature, and this is also a typical feature of mammalian sleep. [33-35] The one specific difference is that in the Arabian oryx REM occupies only 4.18% of TST, which is substantially less than the ~13 – 30% of TST being REM reported in other artiodactyl species. [32]

Arabian oryx sleep in summer

In contrast to what might be described as a typical sleep pattern for an artiodactyl in the winter, in the hot summer months sleep in the Arabian oryx was substantially different. While the typical sleep states of NREM and REM were observed, rather than sleeping during the dark period, the majority of this polyphasic sleep (~83%) occurred during the light period. During the summer we observed that the Arabian oryx slept an average of 3.77 h per day,

with REM occupying 2.12% of TST. Thus, in the summer, the oryx slept mostly during the light period, had substantially less sleep than in the winter (around 2.92 h/day less), and the percentage of TST occupied by REM was approximately half, while the actual amount of REM decreased from 16.8 min/day in winter to 4.8 min/day in summer. In addition, during the sleep bouts in the summer, the subcutaneous temperature was observed to rise, although not as rapidly as the ambient temperature rose. Thus, similar to many other mammals, the TST in summer was less than that observed in winter[36]; however, the timing of sleep in the light period, the substantially lowered amount of REM sleep, and the increase in body temperature during sleep, are not features that can be described as typical of artiodactyl sleep physiology.

Why is summer sleep so unusual in the Arabian oryx?

As outlined above, there are several features of the patterns and physiology of sleep that change in the summer when compared to the winter in the Arabian oryx. While it is typical for mammals to reduce daily TST in summer, the Arabian oryx has approximately half the amount of sleep per day in summer compared to winter (3.77 h/day compared to 6.69 h/day), which is a far larger reduction in daily TST than generally observed in other species. [36,37] This dramatic reduction in TST may be related to the decline in seasonal biomass quality and quantity during the summer as a result of markedly reduced precipitation, and the relative contribution of certain flora species, like perennial grasses, to the oryx diet. Thus, more time must be allocated for active foraging and grazing in the summer, and less time is available for sleep. [2,9,38]

A second major variation is the allocation of the majority of sleep to the light period in summer compared to the dark period in winter. Thus, the Arabian oryx appear to be actively foraging at night, which may help mitigate the effects of exposure to the hot sun

during the day. However, in addition, night time humidity is higher during the summer than the winter, providing plants with a higher water content and thus allowing the oryx to gain access to necessary hydration from metabolic water in their diet. [9,39-42]

The third major variation observed in summer sleep for the oryx is the reduction in the amount of time spent in REM sleep, from 16.8 min/day in winter to 4.8 min/day in summer. Indeed, it is clear that, irrespective of the season, the amount of time spent in REM sleep per day by the Arabian oryx is substantially less than that observed in other artiodactyls. [32] It is well documented that during mammalian REM sleep the temperature of the core CNS increases (e.g. [34]); however, Arabian oryx are always exposed to high ambient air temperatures compared to many other mammals, even in the winter (see Table 1). Given that the Arabian oryx possesses enhanced selective brain cooling compared to close relatives, [1] and their exposure to consistently warm conditions, it is possible that REM sleep times are reduced in this species, especially so in the summer, to avoid overheating, or unnecessary heating, of the brain.

The last major variation observed in the summer sleep physiology of the Arabian oryx compared to the winter is the rise in subcutaneous temperature during summer sleep. When sleeping in the winter, the subcutaneous temperature of the oryx decreases, which is typical for mammals, but winter sleep occurs during the dark period when ambient air temperatures are also dropping at a similar rate. In contrast, summer sleep occurs during the light period when ambient temperatures are rising; however, during this daytime summer sleep, the rate of increase in subcutaneous temperature is approximately 8 times slower than the rate of increase of ambient air temperatures. Immediately following a daytime summer sleep bout, when the oryx are awake, the rate of increase in subcutaneous temperature is approximately 4.75 faster than the rate of increase in ambient air temperature. Thus, overall body temperature in the awake oryx is strongly influenced by ambient air temperature, but while

sleeping, the body temperature of the oryx appears to be substantially less influenced by ambient air temperatures. Thus, the oryx may be making use of the normal physiology of mammalian NREM sleep, where body temperature normally drops, to mitigate, or delay, increases in body temperature (Fig. 9). By mitigating or delaying the inevitable rise in body temperature in the summer by sleeping, the Arabian oryx may be using daytime summer sleep as a novel mechanism for thermoregulation, preventing their body temperatures rising beyond levels that their physiology can accommodate.

As core body temperature begins to decrease in the daily phase the occurrence of sleep is most probable, however it is rather uncommon for sleep to transpire during the rising phase[35,43-45] or under extreme ambient heat and heat stress[46-48] and this is most likely when ambient temperature supersedes body temperature in oryx during the summer. Through an underlying circadian rhythm, core body temperature decreases during normal sleep onset and the sleep that follows further promotes this effect. [49,50] Changes in peripheral skin temperature are the primary driver of this effect[51] and have been subsequently associated with the timing of melatonin secretion and thus the timing of sleep. [52] The extreme ambient temperature cycle in the desert would strongly affect both peripheral skin and core temperature (both possibly under different regulation) and has been suggested to advance circadian core body temperature and REM sleep. [50] Such phase advancing effects of temperature on internal circadian mechanisms may dictate or drive the seasonal timing of sleep in the Arabian oryx.

During both seasons SWA appears to decrease as the sleep period progresses (early vs. late into the longest sleep periods) indicating that sleep may be homeostatically regulated in the oryx. [53] Additionally, during summer, SWA is higher overall compared to during winter, as was similarly observed for the spectral power of all vigilance states (Figs. 1E and F). Previous studies have shown that active cooling of brain temperature in rats, cats and

humans can lead to decreased EEG wave amplitude and frequency[35,54-57]; however, whether active warming of the brain leads to an increased amplitude in EEG patterns, like that observed during summer, is not well described or understood and no further comments can be made. The high ambient temperatures, and associated body, brain and core temperatures in the oryx, during summer may restrict NREM and REM sleep to the point of reducing sleep quality i.e. manifesting as sleep deprivation, and thus leading to the increased SWA observed. As both sample size and recordings were limited (see limitations of study section) our conclusions, in all instances, are speculative at best and further investigations are warranted.

Conclusion

The Arabian oryx, which inhabits the hyper-arid desert environment of the Arabian Peninsula, shows an unusual seasonal variation in sleep. While sleep in the cooler winter months can readily be described as typical for an artiodactyl of around 60 kg body mass, sleep in the hotter summer months shows significant variations. It would appear that all the variations noted in these summer sleep patterns and physiology are directly related to the increased ambient air temperatures, which not only affect the individual animal's thermoregulatory physiology, but the time of the day, when nocturnal activity is most likely to be beneficial to survival through the ingestion of plants that have more water due to higher nocturnal humidity levels. Lastly, the Arabian oryx appears to be using the standard physiology of sleep to mitigate or delay increases in body temperature as a novel thermoregulatory mechanism to prevent overheating. Thus, the Arabian oryx presents an interesting case of using the physiology of sleep in an adaptive way to ensure survival in an environment where most large mammals would rapidly perish.

Acknowledgments:

All authors declare no competing interests. This project was funded by the South African National Research Foundation (NRF) (94233, IFR150115112130, 64956, 87888) (J.G.D., P.R.M., N.C.B. and N.G) and the Research Chairs Program, Deanship of Scientific Research Chairs at the King Saud University (A.N.A., O.B.M.). The authors are extremely grateful to Prince Bander bin Saud Al-Saud, former President of the Saudi Wildlife Authority (SWA), for his enthusiastic support to undertake these studies on the Arabian oryx managed by the SWA. We are also grateful for the tremendous help provided by our colleagues at the National Wildlife Research Center in Taif, Saudi Arabia, especially Mr. Ahmed Boug, Dr. Saud Anagariyah, Mr. Khairy Ismail, Mr. Ghazi Raziman, Mr. Raed Aljuaid and Mr. Ali Aljuaid. We also extend our sincerest thanks to Mr. Martin Haupt from African Wildlife Tracking for assistance with the Neurologger® assembly used in the study.

Disclosure Statement

Financial Disclosure: none.

Non-financial Disclosure: none.

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Figure and Table Legends

Table 1: Weather parameters measured during the recording periods in January and June 2016 in the Mahazat as-Sayd Protected Area, Saudi Arabia.

Table 2: Mean number and duration of episodes of each defined state for the 24 h, light and dark periods using polysomnography (PSG) and actigraphy (ACT).

Table 3: Total state times for the 24 h, light and dark periods.

Table 4: Rates of change in subcutaneous (Tsc) and ambient air (Ta) temperatures during sleep and wake bouts for the 24 h, light and dark periods during summer and winter.

Figure 1: Polysomnographic recordings from Arabian oryx (*Oryx leucoryx*). **(A)** EEG recording (120 s) showing a period of non-rapid eye movement sleep (NREM) transitioning into rapid eye movement sleep (REM). NREM sleep was characterized by a low frequency, high amplitude EEG signal accompanied by a high frequency, low amplitude EMG signal. REM sleep was characterized by a high frequency, low amplitude EEG signal, resembling that of waking, accompanied by a near atonic EMG signal with irregular spikes. **(B)** Recording (120 s) showing a transition from REM sleep to wake characterized by an abrupt increase in EMG signal frequency and amplitude with no visible change in EEG signal to that of prior REM sleep. **(C)** Expanded views of 20 s recording from segment (A) showing NREM sleep, and **(D)** segment (B) showing the transition from REM sleep to wakefulness, at higher temporal resolutions. **(E)** NREM sleep EEG delta power (SWA; 0.5 – 4 Hz power) during the longest consolidated sleep bouts early and late in the sleep period for winter and summer oryx (median and quartiles for the median). (**F)** The spectral power and associated frequency band characteristics of waking, NREM and REM sleep in the Arabian oryx during both the winter and summer periods. **(G)** State transition probabilities in the Arabian oryx during 24 h based on the data from all animals studied for both the winter and summer

periods. The format of this figure (A and B) is modelled on Fig. 3 of Voirin et al.[11] for ease of comparison between studies.

Figure 2: Graphs illustrating the average count of inactivity per hour scored over 6 days using actigraphy in the winter month (upper graph, January 2016, Saudi Arabia, pooled from the 3 animals recorded) and the summer month (lower graph, June 2016, Saudi Arabia, pooled from the 3 animals recorded). The average ambient (Ta, solid lines) and subcutaneous (Tsc, dashed lines) temperatures, recorded every 15 (Ta) and 5 (Tsc) min, across the recording period are overlain on the activity graphs (blue for winter, red for summer). The gray regions represent the dark periods between sunset and sunrise.

Figure 3: The average number of hours, average number of episodes and average episode duration per state for the 24 h (first column), light (second column) and dark (third column) periods for winter (black bars) and summer (grey bars). The results represented are the grouped averages for all animals studied. Error bars show standard error of the mean. The format of this figure is modelled on Fig. 4a of Voirin et al.[11] for ease of comparison between studies.

Figure 4: REM sleep as a percentage of total sleep time for the 24 h, light and dark periods for winter (black bars) and summer (grey bars). The results represented are the grouped averages for all animals studied. Error bars show standard error of the mean. The format of this figure is modelled on Fig. 4b of Voirin et al.[11] for ease of comparison between studies.

Figure 5: The average number of hours, average number of episodes and average episode duration in each state intra-seasonally for winter (left column) and summer (right column) for the light (grey bars) and dark periods (black bars). The results represented are the grouped averages for all animals studied. Error bars show standard error of the mean. The format of this figure is modelled on Fig. 6 of Voirin et al.[11] for ease of comparison between studies.

Figure 6: Time spent per hour (%) in wake (red line, circle markers), non-rapid eye movement (NREM) sleep (blue line, square markers), and rapid eye movement (REM) sleep (green dashed line, diamond markers), recorded with polysomnography (PSG) (upper graphs), for winter (left) and summer (right) Arabian oryx. The corresponding time spent per hour (%) active (orange line, circle markers) and inactive (purple line, square markers), recorded with actigraphy (ACT), for the same oryx as recorded for PSG (middle graphs), and the oryx only recorded using ACT (lower graphs) during winter (left column) and summer (right column). Horizontal bars above graphs represent light (grey, sunrise to sunset) and dark (black, sunset to sunrise) periods. Dashed vertical lines represent sunrise and sunset per recording period. Data reflect all 2 days for each Arabian oryx. Subcutaneous temperature (Tsc - black dashed line, triangle markers) and ambient temperature (Ta - grey dashed lines, inverted triangles) is plotted on the secondary y-axis and reflects averages for all Arabian oryx for all days per recording period. The format of this figure is modelled on Fig. 5 of Voirin et al.[11] for ease of comparison between studies.

Figure 7: Individual hypnograms (upper graphs) illustrating wake, NREM and REM sleep states across 24 h and corresponding raw activity (middle graphs; leg activity – red line, neck activity – blue line) and Tsc (lower graphs) traces for winter (left column, case #1 oryx) and summer (right column, case #2 oryx) periods. The gray regions represent the dark periods between sunset and sunrise. The black arrows in the lower graphs indicate the time of the major sleep bouts per season as described.

Figure 8: **(A)** Time spent per hour (%) in REM sleep (upper graphs) and the corresponding ambient (Ta - grey dashed lines, inverted triangles) and subcutaneous (Tsc - black dashed line, triangle markers) temperature rhythms (lower graphs) for winter (left column) and summer (right column) Arabian oryx. Horizontal bars above graphs represent light (grey, sunrise to sunset) and dark (black, sunset to sunrise) periods. Dashed vertical lines represent

sunrise and sunset times. Data reflect 2 days for each Arabian oryx. **(B)** The number of REM sleep episodes shown as a function of their time of occurrence relative to the time of the minimum subcutaneous temperature (Tsc Min) within the cycle during winter (left graph) and summer (right graph).

Figure 9: The average ambient (Ta, red dashed lines) and subcutaneous (Tsc, black solid lines) temperatures, recorded every 15 (Ta) and 5 (Tsc) min, across the recording period for winter and summer. Ta is plotted on the secondary y-axis. The results represented are the grouped averages for all animals studied per season over the 2 day recording period. The horizontal black bars represent the time of the major sleep bouts per season as described. Dashed vertical lines represent sunrise and sunset in each recording period. Tsc for winter and summer are shown on different y-axis scales so as to best represent the data shown and reinforce the shifts observed in temperature related to the major sleep bouts.

Figure S1: Frequency of sleep bouts and subsequent durations for the 24 h, light and dark periods for winter (black bars) and summer (grey bars). The results represented are the grouped averages for all animals studied.

Figure S2: Correlations of time spent per hour (%) in total sleep time (PSG - NREM and REM sleep combined) against inactivity (ACT) for winter (upper graphs) and summer (lower graphs) Arabian oryx during the recording period. Data points represent hourly means across all recording days (48 h) for the winter oryx (PSG: N=1; ACT: N=3) and both summer oryx (PSG: N=2; ACT: N=3). R-squared (R^2), Pearson correlation (r) and significance (p) values are included for each plot. Corresponding linear regression fit lines are included in each plot as solid black lines.

Table 1: Weather parameters measured during the recording periods in January and June 2016 in the Mahazat as-Sayd Protected Area, Saudi Arabia.

Mahazat as-Sayd Protected Area	**Winter** January (25^{th}-31^{st}) 2016	**Summer** June (16^{th}-22^{nd}) 2016
24h air temperature (˚C)		
Highest recorded	22.6	41.5
Maximum	18.6 ± 2.5	40.9 ± 0.6
Mean	12.5 ± 4.7	34.1 ± 5.1
Minimum	6.5 ± 4.0	25.3 ± 1.8
Lowest recorded	2.5	23.3
Light period mean	14.3 ± 4.7	35.9 ± 5.2
Dark period mean	11.0 ± 4.2	31.5 ± 3.5
24h ground temperature (˚C)		
Highest recorded	27.6	50.9
Maximum	24.0 ± 2.8	49.6 ± 1.0
Mean	16.7 ± 5.0	38.4 ± 6.8
Minimum	11.1 ± 3.3	29.6 ± 1.3
Lowest recorded	8.4	28.5
Light period mean	18.9 ± 5.4	41.0 ± 7.4
Dark period mean	14.8 ± 3.7	34.9 ± 3.3
Rainfall (mm)	4.1	0.0
Time of sunrise	06h40	05h00
Time sunset	17h40	18h45

Light and dark period mean temperatures are according to sunrise and sunset times.
All values are mean ± S.D.

Table 2: Mean number and duration of episodes of each defined state for the 24 h, light and dark periods using polysomnography (PSG) and actigraphy (ACT).

		Winter			Summer		
		24 h	Light	Dark	24 h	Light	Dark
	Mean number of episodes						
PSG	Wake	113.5 ± 20.5	49.0 ± 15.0	65.5 ± 5.5	65.3 ± 9.8	53.8 ± 9.7	12.0 ± 4.5
	NREM	117.5 ± 19.5	49.0 ± 15.0	68.0 ± 4.0	66.0 ± 10.3	55.0 ± 10.4	11.0 ± 4.5
	REM	10.5 ± 1.5	3.5 ± 3.5	7.0 ± 2.0	4.3 ± 1.5	4.3 ± 1.5	0.0 ± 0.0
ACT	Active	19.2 ± 2.3	9.8 ± 2.2	9.3 ± 1.3	24.0 ± 1.7	19.3 ± 2.0	4.7 ± 1.3
	Inactive	20.0 ± 2.2	9.3 ± 2.3	10.7 ± 1.2	23.2 ± 1.8	19.2 ± 2.0	4.0 ± 1.5
	Mean duration of episodes (min)						
PSG	Wake	9.6 ± 2.3	12.3 ± 5.0	7.8 ± 0.7	20.2 ± 3.6	13.9 ± 3.5	180.0 ± 140.2
	NREM	3.3 ± 0.1	2.5 ± 0.4	3.8 ± 0.2	3.4 ± 0.5	3.3 ± 0.6	3.1 ± 1.7
	REM	1.6 ± 0.2	0.7 ± 0.7	1.8 ± 0.4	0.8 ± 0.3	0.8 ± 0.3	0.0 ± 0.0
ACT	Active	35.2 ± 7.0	58.4 ± 20.3	24.5 ± 3.2	42.5 ± 2.5	25.2 ± 2.3	259.3 ± 112.8
	Inactive	44.0 ± 5.0	32.3 ± 4.2	55.3 ± 8.0	20.2 ± 3.7	20.2 ± 3.7	19.2 ± 9.5

Mean values are based on 1 min scoring for all animals per season. Subsequently light and dark mean values are according to sunrise and sunset times (winter: light-11h and dark-13h; summer: light-14h and dark-10h). All values are percentage mean ± S.E.M.

Table 3: Total state times for the 24 h, light and dark periods.

				24h Average				Light				Dark			
Season	**Animal ID**	**Body mass (kg)**	**Sex**	**Total wake time***	**Total sleep time***	**Total Non-REM***	**Total REM***	**Total wake time***	**Total sleep time***	**Total Non-REM***	**Total REM***	**Total wake time***	**Total sleep time***	**Total Non-REM***	**Total REM***
Winter	C	53.0	Male	72.1 ± 1.9	27.9	26.8 ± 1.8	1.2 ± 0.2	80.2 ± 2.5	19.8	19.1 ± 2.4	0.7 ± 0.2	65.3 ± 2.7	34.7	33.2 ± 2.5	1.5 ± 0.4
Summer	A	65.0	Male	81.5 ± 2.4	18.5	18.2 ± 2.3	0.4 ± 0.1	71.9 ± 3.4	28.1	27.4 ± 3.3	0.6 ± 0.1	94.8 ± 2.1	5.2	5.2 ± 2.1	0.0 ± 0.0
	C	62.0	Male	87.1 ± 1.7	12.9	12.6 ± 1.6	0.3 ± 0.1	83.4 ± 2.6	16.6	16.2 ± 2.5	0.5 ± 0.2	92.4 ± 1.6	7.6	7.6 ± 1.6	0.0 ± 0.0
Summer Mean	-	63.5	-	84.3 ± 1.5	15.7	15.4 ± 1.4	0.3 ± 0.1	77.6 ± 2.2	22.4	21.8 ± 2.1	0.6 ± 0.1	93.6 ± 1.3	6.4	6.4 ± 1.3	0.0 ± 0.0

* Values are based on 1 min scoring and are percentages of 24h for each animal as well as the species mean. Subsequently light and dark values are percentages of light and dark hours according to sunrise and sunset times (winter: light-11h and dark-13h; summer: light-14h and dark-10h). All values are percentage mean ± S.E.M.

Table 4: Rates of change in subcutaneous (Tsc) and ambient air (Ta) temperatures during sleep and wake bouts for the 24 h, light and dark periods during summer and winter.

		Winter		Summer	
	Period	Tsc rate (Δ˚C/min)	Ta rate (Δ˚C/min)	Tsc rate (Δ˚C/min)	Ta rate (Δ˚C/min)
Sleep bouts > 20 min	24 h	-0.0035	-0.0037	-0.0004	0.0079
	Light	0.0213	0.0161	0.0023	0.0178
	Dark	-0.0125	-0.0110	-0.0083	-0.0221
Wake bouts (60 min)	Post sleep bouts > 20 mins	0.0172	0.0213	0.0076	0.0016

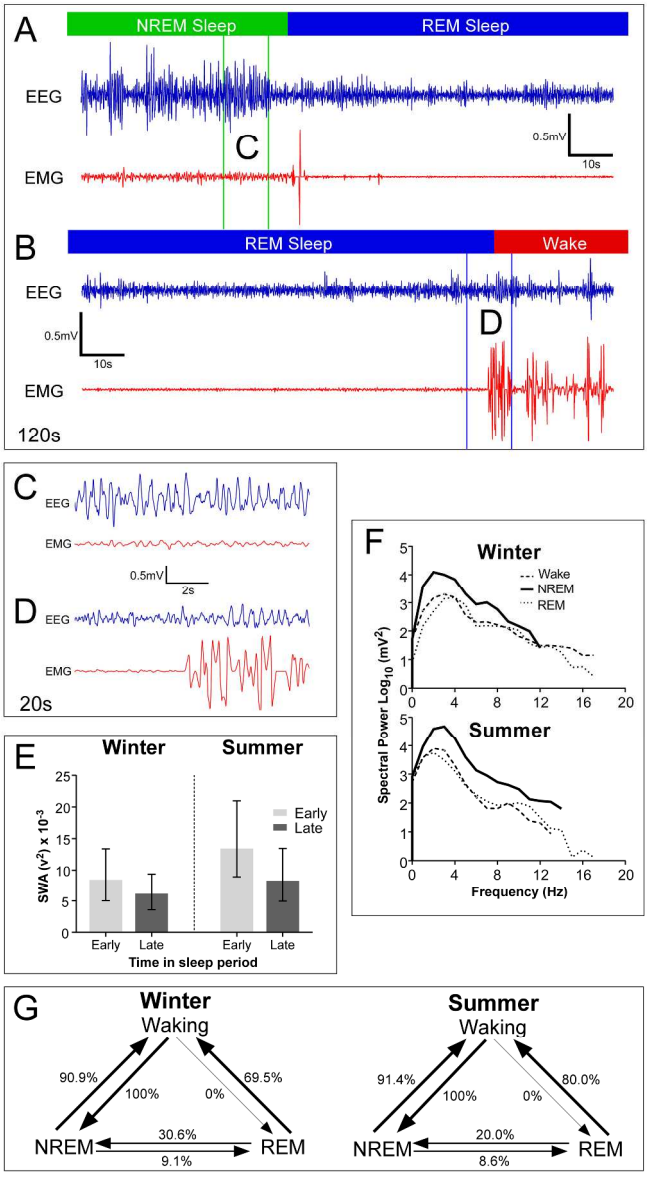


Figure 1: Polysomnographic recordings from Arabian oryx (Oryx leucoryx). (A) EEG recording (120 s) showing a period of non-rapid eye movement sleep (NREM) transitioning into rapid eye movement sleep (REM). NREM sleep was characterized by a low frequency, high amplitude EEG signal accompanied by a high frequency, low amplitude EMG signal. REM sleep was characterized by a high frequency, low amplitude EEG signal, resembling that of waking, accompanied by a near atonic EMG signal with irregular spikes. (B) Recording (120 s) showing a transition from REM sleep to wake characterized by an abrupt increase in EMG signal frequency and amplitude with no visible change in EEG signal to that of prior REM sleep. (C) Expanded views of 20 s recording from segment (A) showing NREM sleep, and (D) segment (B) showing the transition from REM sleep to wakefulness, at higher temporal resolutions. (E) NREM sleep EEG delta power (SWA; 0.5 – 4 Hz power) during the longest consolidated sleep bouts early and late in the sleep period for winter and summer oryx (median and quartiles for the median). (F) The spectral power and associated frequency band characteristics of waking, NREM and REM sleep in the Arabian oryx during both the winter and summer periods. (G) State transition probabilities in the Arabian oryx during 24 h based on the data

from all animals studied for both the winter and summer periods. The format of this figure (A and B) is modelled on Fig. 3 of Voirin et al.11 for ease of comparison between studies.

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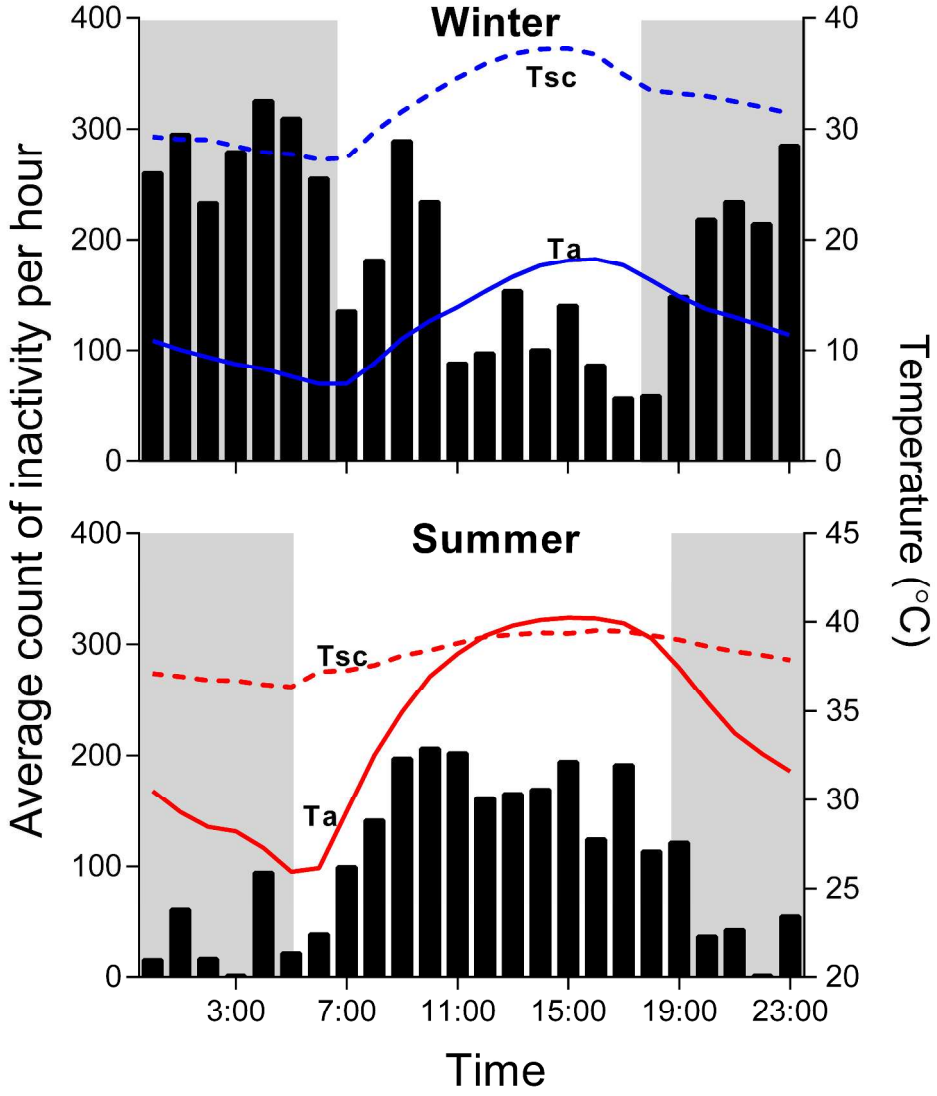


Figure 2: Graphs illustrating the average count of inactivity per hour scored over 6 days using actigraphy in the winter month (upper graph, January 2016, Saudi Arabia, pooled from the 3 animals recorded) and the summer month (lower graph, June 2016, Saudi Arabia, pooled from the 3 animals recorded). The average ambient (Ta, solid lines) and subcutaneous (Tsc, dashed lines) temperatures, recorded every 15 (Ta) and 5 (Tsc) min, across the recording period are overlain on the activity graphs (blue for winter, red for summer). The gray regions represent the dark periods between sunset and sunrise.

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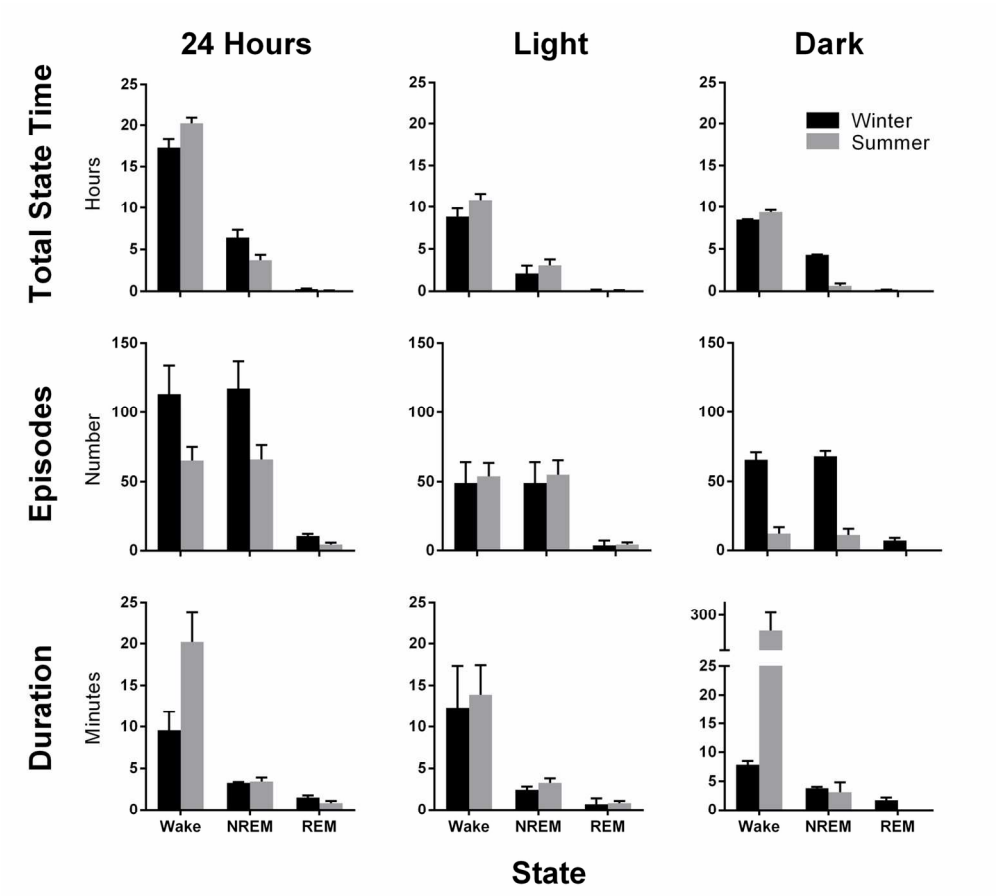


Figure 3: The average number of hours, average number of episodes and average episode duration per state for the 24 h (first column), light (second column) and dark (third column) periods for winter (black bars) and summer (grey bars). The results represented are the grouped averages for all animals studied. Error bars show standard error of the mean. The format of this figure is modelled on Fig. 4a of Voirin et al.[11] for ease of comparison between studies.

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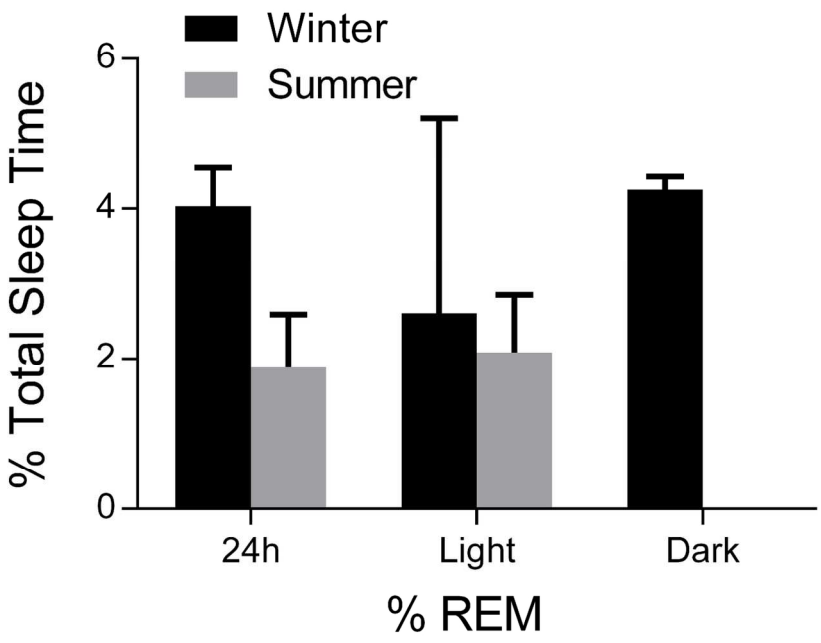


Figure 4: REM sleep as a percentage of total sleep time for the 24 h, light and dark periods for winter (black bars) and summer (grey bars). The results represented are the grouped averages for all animals studied. Error bars show standard error of the mean. The format of this figure is modelled on Fig. 4b of Voirin et al.[11] for ease of comparison between studies.

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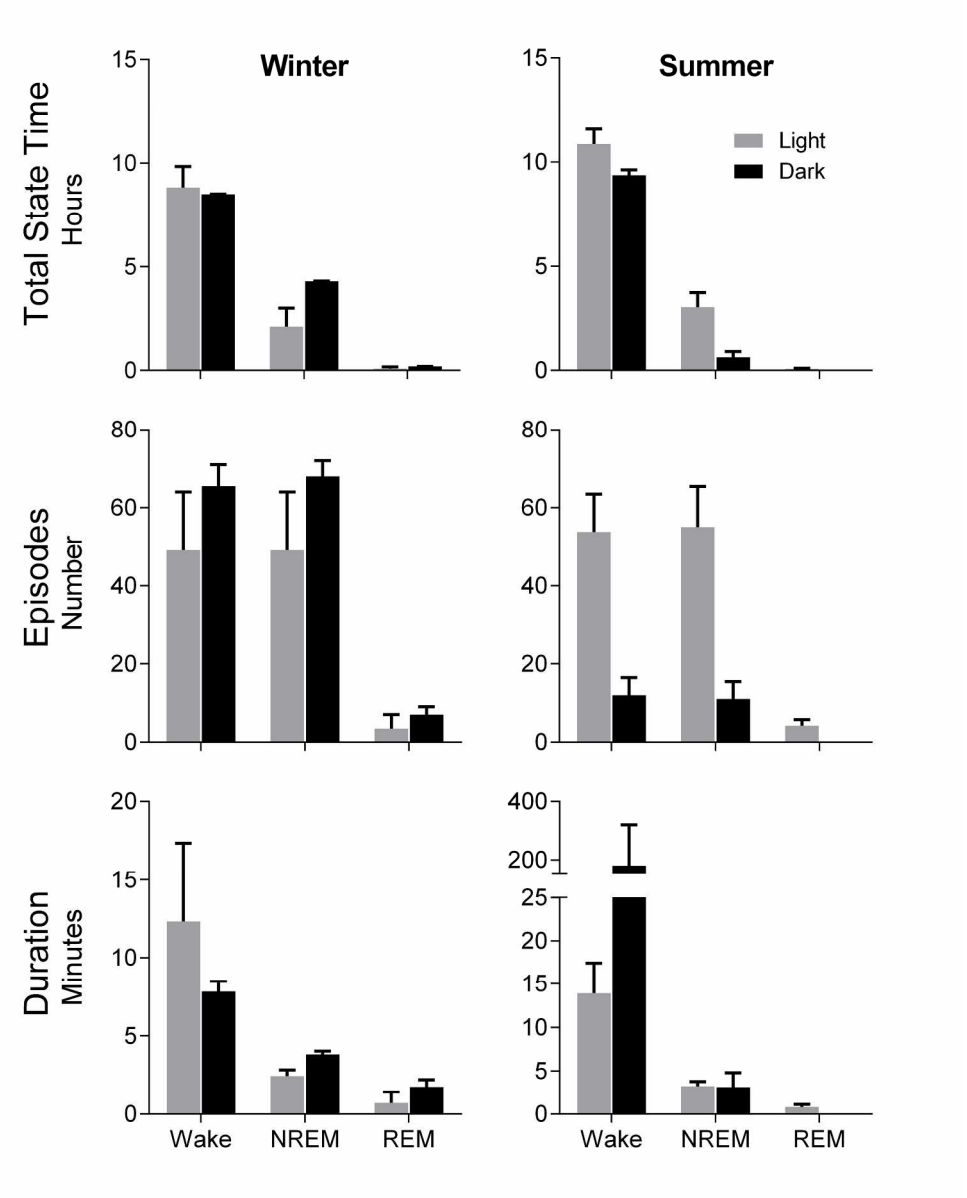


Figure 5: The average number of hours, average number of episodes and average episode duration in each state intra-seasonally for winter (left column) and summer (right column) for the light (grey bars) and dark periods (black bars). The results represented are the grouped averages for all animals studied. Error bars show standard error of the mean. The format of this figure is modelled on Fig. 6 of Voirin et al.[11] for ease of comparison between studies.

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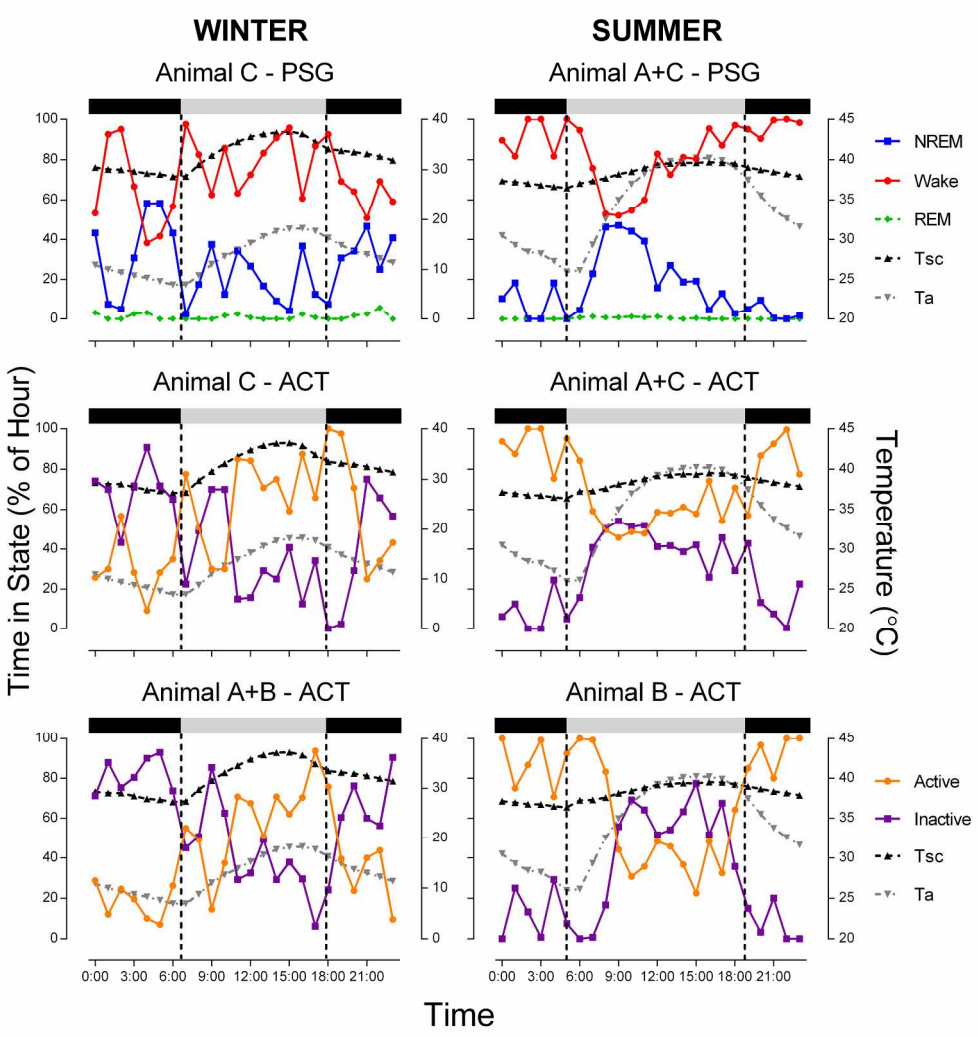


Figure 6: Time spent per hour (%) in wake (red line, circle markers), non-rapid eye movement (NREM) sleep (blue line, square markers), and rapid eye movement (REM) sleep (green dashed line, diamond markers), recorded with polysomnography (PSG) (upper graphs), for winter (left) and summer (right) Arabian oryx. The corresponding time spent per hour (%) active (orange line, circle markers) and inactive (purple line, square markers), recorded with actigraphy (ACT), for the same oryx as recorded for PSG (middle graphs), and the oryx only recorded using ACT (lower graphs) during winter (left column) and summer (right column). Horizontal bars above graphs represent light (grey, sunrise to sunset) and dark (black, sunset to sunrise) periods. Dashed vertical lines represent sunrise and sunset per recording period. Data reflect all 2 days for each Arabian oryx. Subcutaneous temperature (Tsc - black dashed line, triangle markers) and ambient temperature (Ta - grey dashed lines, inverted triangles) is plotted on the secondary y-axis and reflects averages for all Arabian oryx for all days per recording period. The format of this figure is modelled on Fig. 5 of Voirin et al.11 for ease of comparison between studies.

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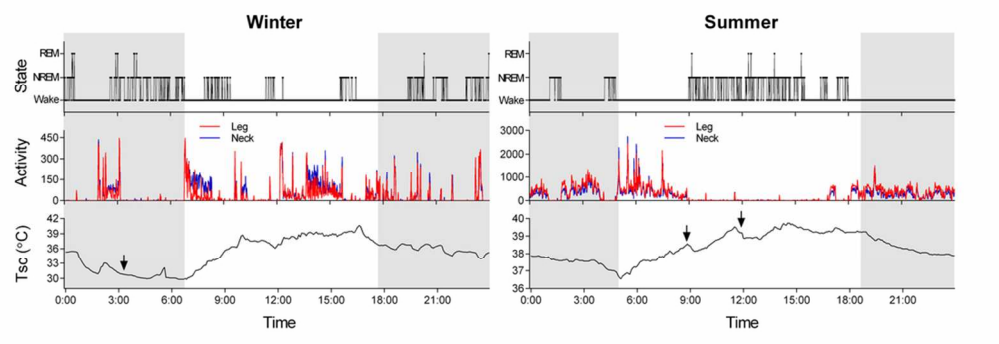


Figure 7: Individual hypnograms (upper graphs) illustrating wake, NREM and REM sleep states across 24 h and corresponding raw activity (middle graphs; leg activity – red line, neck activity – blue line) and Tsc (lower graphs) traces for winter (left column, case #1 oryx) and summer (right column, case #2 oryx) periods. The gray regions represent the dark periods between sunset and sunrise. The black arrows in the lower graphs indicate the time of the major sleep bouts per season as described.

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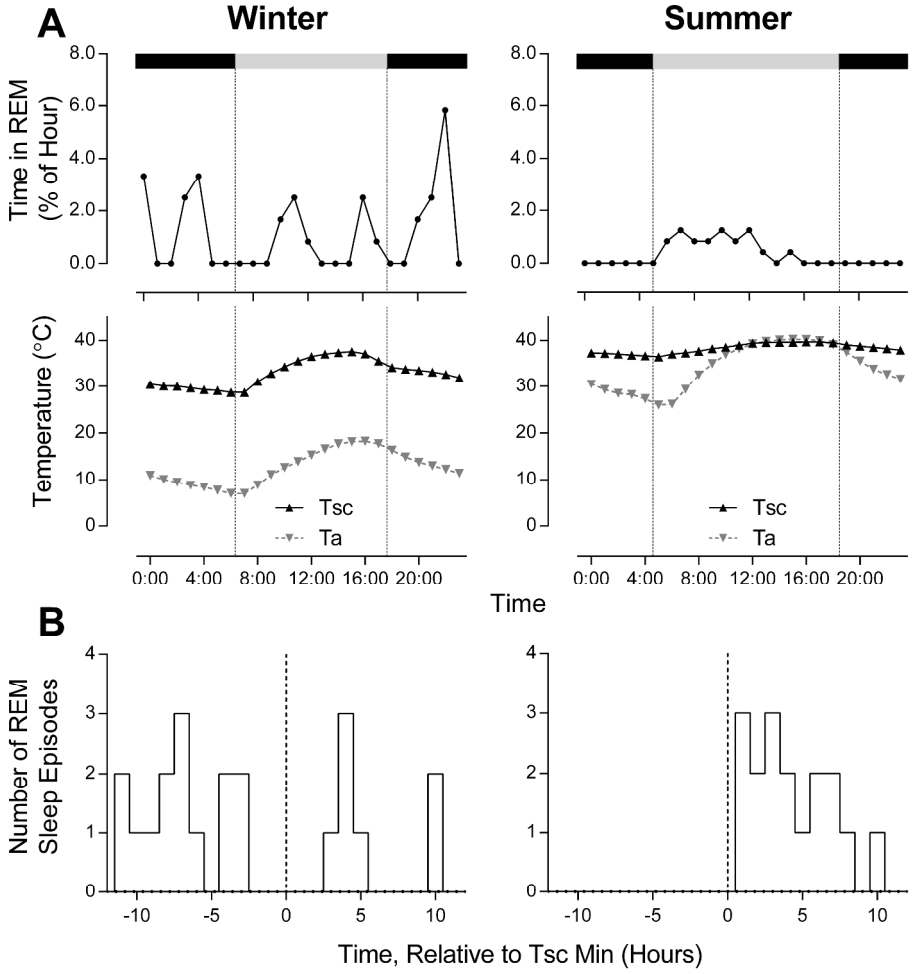


Figure 8: (A) Time spent per hour (%) in REM sleep (upper graphs) and the corresponding ambient (Ta - grey dashed lines, inverted triangles) and subcutaneous (Tsc - black dashed line, triangle markers) temperature rhythms (lower graphs) for winter (left column) and summer (right column) Arabian oryx. Horizontal bars above graphs represent light (grey, sunrise to sunset) and dark (black, sunset to sunrise) periods. Dashed vertical lines represent sunrise and sunset times. Data reflect 2 days for each Arabian oryx. (B) The number of REM sleep episodes shown as a function of their time of occurrence relative to the time of the minimum subcutaneous temperature (Tsc Min) within the cycle during winter (left graph) and summer (right graph).

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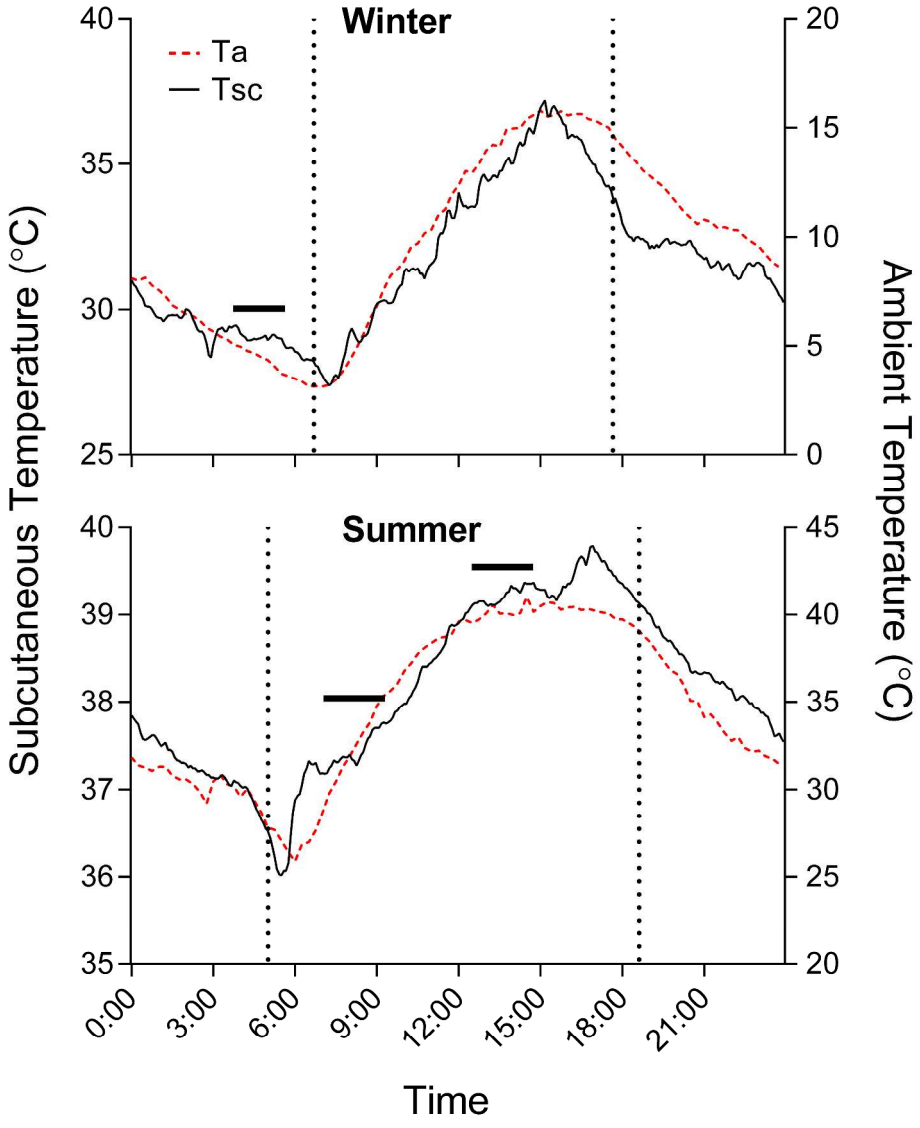
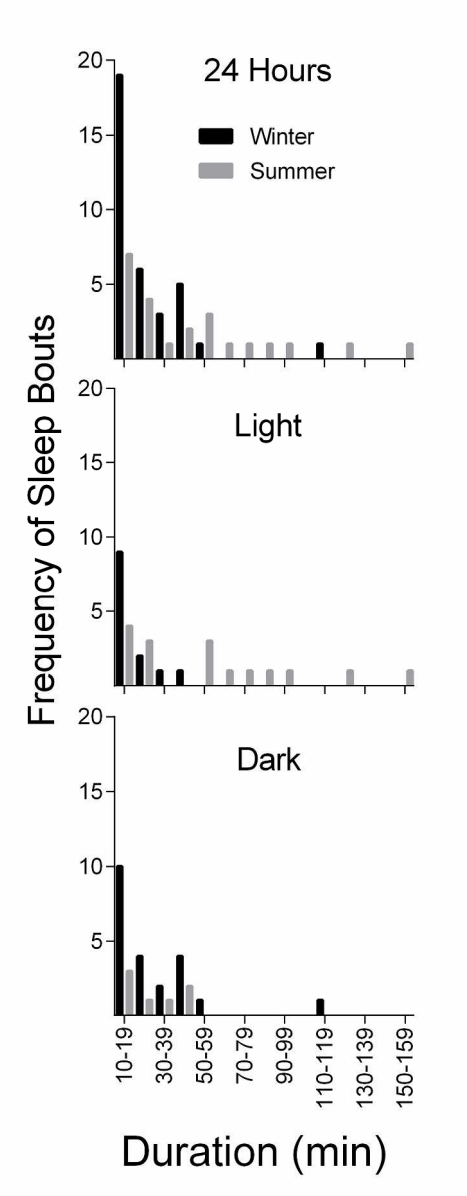
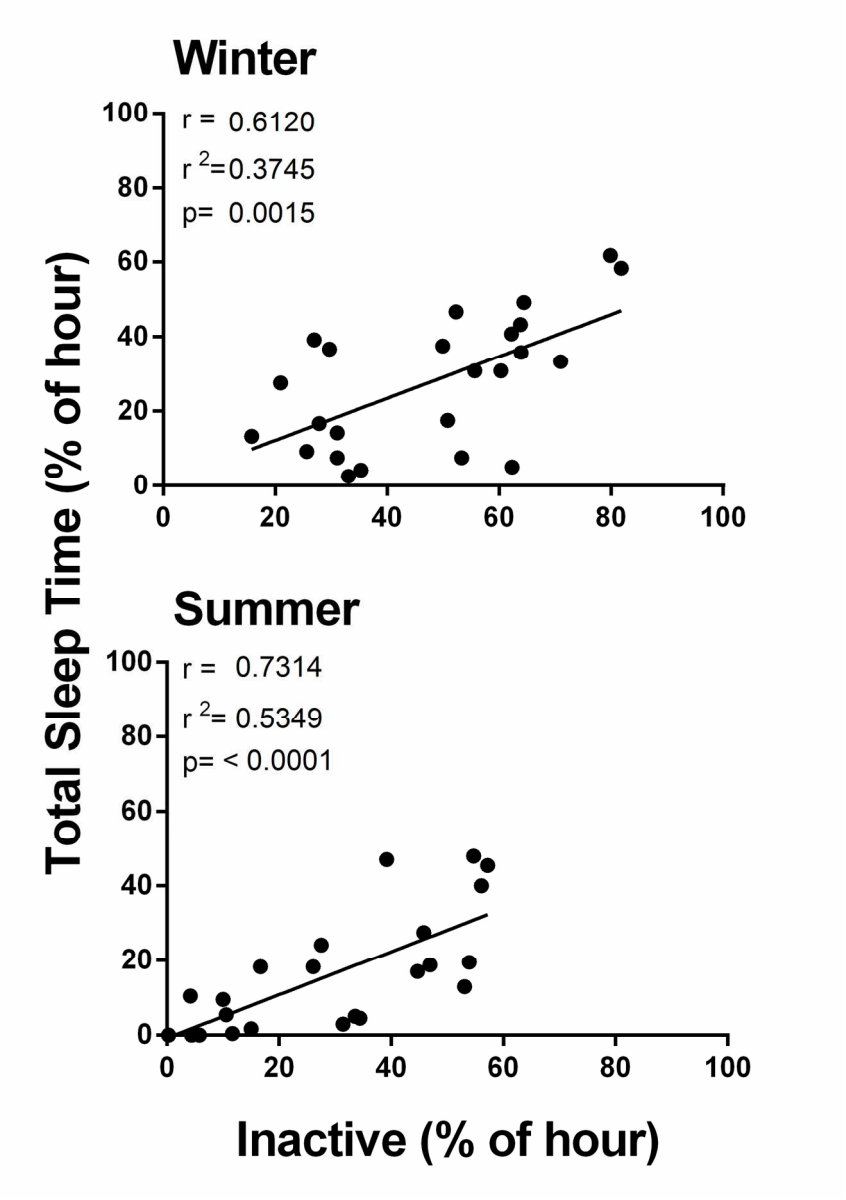


Figure 9: The average ambient (Ta, red dashed lines) and subcutaneous (Tsc, black solid lines) temperatures, recorded every 15 (Ta) and 5 (Tsc) min, across the recording period for winter and summer. Ta is plotted on the secondary y-axis. The results represented are the grouped averages for all animals studied per season over the 2 day recording period. The horizontal black bars represent the time of the major sleep bouts per season as described. Dashed vertical lines represent sunrise and sunset in each recording period. Tsc for winter and summer are shown on different y-axis scales so as to best represent the data shown and reinforce the shifts observed in temperature related to the major sleep bouts.

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Conclusion

1. Concluding Chapter

The Arabian oryx represents a species rather unique in its biology and ecology. Extensive research has been accumulated over many years to provide an impressive and in-depth understanding of its life history, behaviour and physiology. The continued conservation of wild, free-roaming Arabian oryx combined with the preservation of its habitat have allowed for further research endeavors which may eventually assist in maintaining well-established populations existing successfully in the deserts of the world. The restoration of the Arabian oryx and its continued conservation and preservation efforts throughout Saudi Arabia is a primary initiative and core programme for the National Commission for Wildlife Conservation and Development (NCWCD). Concurrently, habitat protection projects within the former range of the Arabian oryx and captive breeding programmes are managed at the National Wildlife Research Center (NWRC) (Ostrowski et al., 1998). To date, several free-ranging populations of Arabian oryx have been successfully reintroduced and sustained in multiple sites across Saudi Arabia and the Middle East.

The current thesis provides a thorough examination of the neuroanatomy, physiology and behaviour related to the sleep-wake cycle of the Arabian oryx. The work contributes to the expansion of previous literature relating to the Arabian oryx and is in agreement with other research efforts of the same nature as described in Part I. We describe novel findings related to sleep and behaviour in the Arabian oryx. The following sections discuss, conclude and provide a brief synthesis of the results contained within each of the published articles within the context of data derived from other studies and the field as a whole.

Article I

The purpose of Article I was to identify and describe the neurochemical organization and morphology of the nuclei implicated in the control of the sleep-wake cycle in the brain of the Arabian oryx. As previously mentioned there is a large void describing neuroanatomical

characteristics for the Cetartiodactyl superorder. Previous work in the Artiodactyl order has studied the giraffe, sheep, cow and pig (Iqbal et al., 2001; Bux et al., 2010; Ettrup et al., 2010; Dell et al., 2012; Mahady et al., 2017) whilst in the Cetaceans the Harbour porpoise, Minke whale and hippopotamus have been examined (Dell et al., 2016a, b, c). Of the 290 extant species of Cetartiodactyls, less than 2.5% have been studied with regards to the nuclei implicated in the control of the sleep-wake cycle. Prior to Article I, no complete description was available for any Artiodactyl species, especially a non-domestic species.

Typical methodologies such as immunohistochemistry and stereology were used to allow for the specific identification of the neuronal organization, subsequent morphology and stereological parameters within neurotransmitter systems of the brain. Used in combination the two techniques are considered the gold standard methodology for diagnostic and research based comparative neuroanatomical studies (Gundersen, 1985; Janson and Møller, 1993; West, 1993; Aika et al., 1994; Mayhew and Gundersen, 1996; Ramos-Vara, 2005, Dell et al., 2012; Takei et al., 2014). The Arabian oryx brain was examined in depth and the entire complement of sleep related nuclei was identified and described. Stereological counts and volumetric measurements were provided for neurons within the hypothalamus, locus coeruleus and the LDT and PPT nuclei. Immunohistochemical analysis was provided for all relevant sleep areas within the basal forebrain, hypothalamus and pons, and included descriptions for cholinergic, serotonergic, orexinergic and catecholaminergic systems as well as the GABAergic neurons and terminal networks using calbindin and calretinin.

A planned management cull of Arabian oryx at the NWRC provided a unique opportunity to retrieve two adult female Arabian oryx brains for use in the study. It is highly unlikely that further analysis on Arabian oryx brain tissue will be permissible as the conservation status of the Arabian Oryx limits accessibility to biological samples. Analyses revealed that the sleep related nuclei in the Arabian oryx brain follow the typical mammalian organizational plan and morphology (e.g. Manger, 2005; Bhagwandin et al., 2013; Maseko et al., 2013) with some additional order-specific features previously observed in the Cetartiodactyls (Bux et al., 2010; Dell et al., 2012, 2016a, b, c; Mahady et al., 2017), as well as two species-specific features unique to the Arabian oryx.

The order-specific features include: 1) presence of the parvocellular subdivision of the orexinergic hypothalamic main cluster in the Arabian oryx brain, which is similar to that previously described for the Cetartiodactyls (Dell et al., 2012, 2016a, b, c) and African

elephant (Maseko et al., 2013); and 2) the neuronal morphology of the LDT and PPT; exhibiting size differences more similar to that of the Goettingen mini pig (Mahady et al., 2017) of the Artiodactyl order, than to that of the Cetaceans (Dell et al., 2016c). Secondly, a major goal of the study was to determine whether the extreme environment of the Arabian oryx has led to any evolutionary novelties in the sleep related nuclei. As mentioned, two features appear species-specific: 1) the dorsal division of the catecholaminergic anterior hypothalamic group (A15d), previously unreported in any Artiodactyl; and 2) the catecholaminergic tuberal cell group (A12), notably more augmented than previously observed for any other mammal.

The order- and species-specific nuclei play important roles within the sleep-wake cycle as well as other vital physiological processes. The presence of the orexinergic hypothalamic parvocellular subdivision seems typical within the Cetartiodactyl order and appears, in combination with the orexinergic system as a whole, to have reached its highest complexity level in terms of evolution across mammals within this superorder (Dell et al., 2012). The appetitive drive, and related behaviours associated with the orexinergic system (Sakurai et al., 1998; Ida et al., 1999) and the nuclear cluster within the hypothalamus are possibly of most importance to the Cetartiodactyls (Dell et al., 2012). Time budgeting of behaviours like feeding and foraging are generally controlled by appetite and satiety, and are regulated within the sleep-wake cycle by circadian rhythms. The evolution of the parvocellular subdivision and orexinergic system within the Arabian oryx brain is likely associated with noted behaviours, especially considering the necessities of successful foraging in an extremely arid and food depauperate environment.

The variable size differences observed for the cholinergic LDT and PPT nuclei in the Arabian oryx brain also appears in other Cetartiodactyls studied (Bux et al., 2010; Dell et al., 2016c; Mahady et al., 2017) and to a report for the rock hyrax (*Procavia capensis*) (Gravett et al., 2009). The associated function of these nuclei has been primarily linked to REM sleep (Shouse and Siegel, 1992; Siegel, 2004; van Dort et al., 2015) and may be related to the unambiguous REM features (*somnus innominatus*) exhibited by the rock hyrax (Gravett et al., 2012). Such morphological variation with the LDT and PPT nuclei may be involved in the highly plastic sleep phenomenology observed for the Arabian oryx. As no other examination of sleep physiology in any other species of Cetartiodactyl with such features has been conducted to date, further study is required to provide a better understanding.

The presence of the catecholaminergic A15d nucleus in the brains of most mammals (Smeets and González, 2000), but general absence within the Artiodactyls (Leshin et al., 1996; Iqbal et al., 2001; Bux et al., 2010), indicates variable occurrence of this nucleus. Considering its associated functions with osmoregulation (van Vulpen et al., 1999) and seasonal reproduction control (Smeets and González, 2000), it is possible that its presence within the Arabian oryx brain is again related to necessity, and has evolved as a beneficial trait in response to an extreme environment. A similar observation was made regarding the A12 nucleus and its apparent augmentation within the Arabian oryx brain notable because of its role in milk production, metabolism and immuno-regulation (Zoli et al., 1993; Tillet, 1994). Furthermore, the susceptibility of the A12 nucleus to external stressors (Demarest et al., 1987; Tucker et al., 1991) appears as specialized and perhaps more evolved towards the ecology of the Arabian oryx.

Conclusively, the majority of the sleep related neuronal systems in the brain of the Arabian oryx follow the typical mammalian organizational plan and exhibits predictable Cetartiodactyl specific features. In addition, the variation and presence of the A12 and A15d nuclei, respectfully, is more likely related to osmoregulation and energy conservation physiology than sleep-wake control. The complete description of the sleep-wake system neuroanatomy in the Arabian oryx brain will allow for direct comparison amongst members of the Cetartiodactyl superorder in any future research. It will additionally provide a neuroanatomical atlas in the event of any further study of the brain, especially one addressing the SCN and circadian rhythms which requires further examination.

Article II

Article II addresses how the Arabian oryx manages inactivity patterns at a seasonal level (the hottest and coldest month) under natural conditions in the Arabian Desert. The work of Seddon and Ismail (2002), Ostrowski et al. (2003), and Hetem et al. (2012a, b) observed altered daily activity patterns between cooler and hotter months. We sought to understand how seasonal variations in an extreme environment effect inactivity patterns in a resident species. Using fine-grain actigraphy (two actiwatches, 1 min resolution), the 24 h inactivity patterns of Arabian oryx were measured in addition to Tb during summer and winter months under natural conditions in the Mahazat as-Sayd Protected Area (MS), Saudi Arabia.

The main findings allude to a clear shift from nocturnal inactivity during winter to a bimodal inactivity pattern (mostly diurnal) during summer. The actual timing of the major inactive bouts also shifted from the coldest part of the winter nights to the hottest part of the summer days. By comparison with a conspecific Arabian oryx living in the Karoo, South Africa, it was determined that Ta and not light (photoperiod) appears to be the dominant *Zeitgeber* or proximate factor driving this seasonal shift of inactivity patterns. Several studies have demonstrated shifts in activity patterns related to changes in Ta (Lourens and Nel, 1990; Kenagy et al., 2002, 2004; Oster et al., 2002; Vivanco et al., 2010), although few represent examples under natural conditions, or with large mammalian species. Our study showed that no apparent shift in the timing of Tb rhythms between seasons accompanied the shift in inactivity patterns suggesting that a masking effect may be responsible for these altered behaviours rather than an endogenous shift at the level of the circadian clock.

At this junction, an in-depth analysis of output variables such as plasma melatonin, cutaneous and core Tb, and locomotor rhythms in Arabian oryx, under experimental conditions, would provide an interesting investigation for entrainment by Ta. As demonstrated by El Allali et al. (2013) in their study on camels, there are several variables that need to be examined to prove that daily Ta cycles are able to entrain the circadian clock and not an effect of masking. Such steps firstly include a verification of Tb as an endogenous rhythm and secondly, a controlled series of Ta fluctuation experiments. Obtained results may definitively demonstrate Ta as a dominant *Zeitgeber,* able to entrain seasonal rhythms in the Arabian oryx similarly to camels (El Allali et al., 2013). A mobile harness device that could continuously measure plasma melatonin levels in unrestricted, free-roaming Arabian oryx under natural conditions would provide expedient and interesting results in the above mentioned hypothetical examinations. Such a study could track activity pattern switching naturally in the context of circadian rhythms and further elucidate the phenomenon of temporal niche switching.

Although no physiological sleep was recorded, the inactivity patterns (used as a proxy for sleep) indicate that the Arabian oryx exhibit a polyphasic-type sleep, with approximately 5 h total inactive time during winter and 6.5 h during summer. The subsequent results obtained in Article IV using PSG indicated 6.69 h TST in winter and 3.77 h in summer. These differences highlight the limitations of actigraphy and the ability to accurately infer sleep times; however, the sample size and recording lengths in Article IV can be considered insufficient for direct comparisons between ACT and PSG. Further improvement in the methodology and device settings may improve the accuracy of ACT; however, PSG will

always be considered the gold standard method for examining TST and therefore should be included simultaneously in such ACT studies in order to tease apart specific states in behaviour and sleep physiology.

The specific timing, phasing and duration of the reported inactivity during winter in Article II appears to conform to that reported for most Artiodactyl species with similar mass (Campbell and Tobler, 1984; Siegel, 2005), whilst during summer these architectural features of inactivity appear unique to the Arabian oryx. Similar seasonal features however may be discovered in future studies in other desert species and thus such studies are highly encouraged. The summer time behaviour of Arabian oryx, in conjunction with shade seeking (Stanley Price, 1989; Seddon and Ismail, 2002; Hetem et al., 2012a), was speculated to be an adaptive response to increasing Ta possibly utilizing the thermoregulatory actions related to sleep.

The results that we obtained for the summer month of July indicate crepuscular activity patterns which are in contrast to the nocturnal activity patterns reported by Hetem et al. (2012a) for the same month. These differences may be related to the methodology used as well as differences in weather patterns. In our study we utilized fine-grain actigraphy (two actiwatches, 1 min resolution) whilst Hetem et al. (2012a) utilized coarse-grain actigraphy (one actiwatch, 5 min resolution). As such the results produced in our study may provide a more accurate representation of oryx activity during July in the Arabian Desert; however, differences related to climate should also be considered.

Concerning future conservation efforts, understanding the behavioral patterns of the Arabian oryx in relation to rising temperatures and the impact thereof on thermoregulatory ability will be important. If Ta continues to climb, oryx and other similar desert species may seek cooler habitats with new risks to ecological balance. Risks *inter alia* include predation, intra and inter-species competition, novel parasites and decreased resource availability (Bartholomew, 1963; Huey, 1991; Bakken, 1992; Hetem, 2010; Fuller et al., 2016).

The findings of our study provide confirmation of temporal niche switching at a seasonal level with regards to inactivity patterns in the Arabian oryx. Article IV applies the new found information to confirm the behavioural, activity-based switching patterns in relation more directly to electrophysiological sleep. The results described in article II prompted us to further conduct a year-long study under varied conditions to examine the activity budgets of Arabian oryx in relation to temperature and light.

Article III

The purpose of the study described in article III was to investigate the behavioural patterns of wild Arabian oryx under natural conditions in two discrete environments, continuously, over a year-long period in the deserts of Saudi Arabia. The major investigations were the 24 h time budgeting of activity patterns and the phasing profiles throughout the year, specific to each of the four seasons. The data were obtained using coarse-grain actigraphy (one intra-abdominal actiwatch) with simultaneous weather recordings for the entire period. The two experimental areas used included the MS (same site as for Article II) and the Uruq Bani Ma'arid Protected Area (UBM), an area having not been previously used for any long term study of the Arabian oryx. The reasoning to using two sites was to determine whether varied geographic size (habitat size), climatic conditions and biomass availability would alter the inter-seasonal behavioural patterns.

The MS is located northeast of Taif in the Kingdom of Saudi Arabia (22.2˚N, 41.8˚E) and covers 2200 km^2. The MS is the same area used previously to study the Arabian oryx and was one of the first and most successful reintroduction sites for captive Arabian oryx (Williams et al., 2001; Ostrowski et al., 2002, 2003; Hetem et al., 2010, 2012a, b, c; Ismail et al., 2011). The MS is an open steppe desert without any permanent sources of drinking water, and is considered the current and historical habitat for Arabian oryx in Saudi Arabia (Pallas, 1777). It was designated as a protected area in 1988 and all domestic livestock and predators are excluded from the area. Other species protected within the MS include the Houbara bustards (*Chlamydotis undulata macqueenii*) and the Arabian sand gazelle (*Gazella subgutturosa marica*) (Child and Grainger, 1990; Ismail et al., 2011). Occasional reports of Arabian wolf (*Canis lupus*) have been noted; however, they do not threaten Arabian oryx and usually prey on sand gazelle or smaller species (Cunningham and Wronski, 2010; Ismail et al., 2011). The region's climate exhibits extremely hot summers with mild winters and very low yearly rainfall. Severe droughts have been occasionally documented in this region and have led to high mortality rates and very low birth rates in Arabian oryx (Islam et al., 2007, 2010; Cunningham, 2009; Ismail et al., 2011). The vegetation of the region contains mostly perennial grasses, small acacia trees and maeru trees (Mandaville, 1990; Fischer et al., 1998; Williams et al., 2001; Ostrowski et al., 2002).

The UBM covers a 12 658 km^2 area within the Rub al Khali desert (Empty Quarter) (19.3˚N, 45.3˚E) in the Kingdom of Saudi Arabia (Meigs, 1953; Mésochina et al., 2003). The Rub al

Khali desert itself spans over 600 000 km^2 and is the world's largest sandy desert (Besler, 1982; Goudie, 2002; Atkinson et al., 2013). The UBM has far lower annual rainfall than that of the MS and more extreme Ta shifts between seasons (Child and Grainger, 1990; Dunham, 1997; Islam et al., 2011). Overall the vegetation and biomass quality is sparser and substantially lower year-round when compared with MS. The rainfall in this area is highly unpredictable and several prolonged droughts have been documented leading to reduced vegetation growth and subsequently decreased intrinsic population growth of oryx (Bedin, 2001; Mésochina et al., 2003). Arabian oryx were reintroduced to the UBM in 1995 and by 2001 the population had increased from 139 to 220 animals (Ostrowski and Bedin, 2001; Mésochina et al., 2003). Both environments pose typical arid zone hardships; however, the geographic, climatic and ecological differences provided for a unique contrast in terms of behavioural study.

Of the initial twenty Arabian oryx used for Article III's study, four of the ten oryx used in the UBM site were killed by poachers and one oryx from the MS area died during the year-long recordings. Several of the implants also had to be replaced or failed to work for the entire period. In lieu of the difficulties faced, our study produced the first long term description, encompassing all four seasons, of 24 h activity budgets and phasing profiles for free-roaming Arabian oryx under natural conditions in the UBM and MS areas of Saudi Arabia. The major findings reveal that oryx exhibit seasonal plasticity in their temporal niche preferences in relation to external environmental factors; a form of temporal niche switching at a seasonal level. Using the diurnality index (Hoogenboom et al., 1984; Refinetti, 2008; Daan et al., 2011), our results demonstrated that the oryx show nocturnal/crepuscular activity during summer, diurnal activity during winter and interchanging/intermittent patterns between spring and autumn. Qualitative differences were noted between oryx belonging to the MS and UBM areas in terms of seasonal activity budgets and also between the relationship of activity to Ta and photoperiod. These differences may be related to habitat size, climate and biomass quantity and quality and requires further investigation. A statistical comparison of the activity budgets between the two study sites would have also been beneficial and could be a point of improvement for future work. It appears that Ta rather than the light-dark cycle is more dominant in driving the temporal niche switching observed in our study.

The conclusion of this work was that the highly plastic, seasonally-sensitive, behavioural patterns of free-roaming Arabian oryx may be driven by the availability of food and water, which themselves are heavily influenced by seasonal climate changes. These conclusions are

merely speculative as food biomass was not measured and thus leaves room for future examinations. The data produced, in combination with the long-term study of Hetem et al. (2012a) and the most recent work of Streicher et al. (2017) investigating year-long Tb patterns within the same locations as Article III, will provide a useful framework of which the behaviour of wild populations of Arabian oryx can be compared in lieu of climate change and its possible effects on future biomass availability. Results would aid in conservation efforts examining seasonal resource management and vital requirement coverage for future reintroduction endeavors of arid zone species.

Article IV

The study described in Article IV was developed to address the physiological sleep of wild, unrestricted Arabian oryx during summer and winter under natural conditions in Saudi Arabia. As temporal niche switching of activity and inactivity patterns was described and confirmed for Arabian oryx at a seasonal level in Article II and III, the study was further expanded to confirm the phenomenon in terms of sleep physiology. The data obtained from this study was measured through a neurologger unit that recorded EEG and EMG. We simultaneously recorded fine-grain actigraphy and subcutaneous body temperature (Tsc, using iButtons) from the oryx, and also included weather data from the Mahazat as-Sayd Protected Area.

Comparative sleep studies under natural, unrestricted conditions is underrepresented in the field of sleep research (Rattenborg et al., 2017). As described by Aulsebrook et al. (2016) there are barriers to understanding the functions of sleep and by direct quotation, “Sleep is treated by ecologists as an unassuming behaviour, instead of a diverse neurophysiological state, while neuroscientists commonly misjudge and undervalue the critical discernments accessible by the inclusion and value of ecology and evolutionary history”. Aulsebrook et al. (2016) goes on to further suggest that a multidisciplinary approach would provide a more comprehensive method for understanding sleep and its adaptability as a waking behaviour and essential component of fitness.

The overall outcome of Article IV was threefold. Firstly, it provides a complete polysomnographic (PSG)-based study of sleep in a wild, unrestricted Cetartiodactyl species. Secondly, the results show that Arabian oryx have varied sleep architecture patterns under the

extreme desert climate between the seasons. Thirdly, the study provides a confirmation of prior reports of seasonal activity-based temporal niche switching on a physiological level.

As mentioned in Part I, four earlier studies to date have examined physiological sleep in wild species under natural conditions (Rattenborg et al., 2008; Lesku et al., 2011; Scriba et al., 2013a, b; Voirin et al., 2014). These prior studies, in addition to more recent studies of a similar nature with different methodologies, provide a framework for conducting such naturalistic studies and highlight relevant technologies and questions (Rattenborg et al., 2017). Such questions include inter-individual variation in sleep durations, individual plasticity in sleep durations, and the costs and benefits of such variations in relation to the functions of sleep (Rattenborg et al., 2017).

Article IV represents the first attempts to examine natural sleep patterns under an extreme environment, the hyper-arid Arabian Desert, in which Ta appears to play a major role in the sleep of Arabian oryx. Such a study represents the first attempts to quantify physiological sleep in such an environment, where survival is limited and behaviour is dictated by specific environmental conditions. The results provide a framework for comparing sleep with similar arid zone species and further demonstrate individual plasticity in sleep durations and timing of sleep. Such behaviour highlights the costs and benefits related to surviving such extreme conditions and still maintaining the necessary process of sleep.

Our recordings demonstrate that Arabian oryx exhibit the typical mammalian sleep states defined as wakefulness, non-REM and REM sleep; and that between winter and summer, the duration and timing spent in these states differed. The average sleep quota for Arabian oryx (when combining winter and summer total sleep time) places the Arabian oryx within reported ranges for other studied Artiodactyl species (Immelmann and Gebbing, 1962; Ruckebusch and Morel, 1968; Ruckebusch, 1972; Bell and Itabisashi, 1973; Campbell and Tobler, 1984; Tobler and Schwierin, 1996); however, the reduction in total sleep time from winter to summer is greater than reported for other species (Borbély and Tobler, 1996; Yetish et al., 2015). Direct comparisons across the order are somewhat constrained as all previous studies include domesticated or captive species living in temperate climates, and appear to lack consistency or consensus within the same species; additionally, lighting conditions are often not specified (Campbell and Tobler, 1984).

The Arabian oryx show mostly nocturnal polyphasic sleep during winter and then appeared to fully switch to diurnal polyphasic sleep during summer without accompanied timing shifts in

Tsc, only amplitude. The seasonal difference in physiological sleep aligns with our previous reports of temporal niche switching using actigraphy (Articles II and III) and with the altered daily activity patterns between cool and hot months described by Stanley Price (1989), Seddon and Ismail (2002), Ostrowski et al. (2003) and Hetem et al. (2012a, b). Although, as mentioned earlier actigraphy does appear to overestimate total sleep time and inactive bouts may include periods of rumination. Thus, in future studies the inclusion of both PSG and ACT simultaneously is recommended in order to first establish baseline patterns and durations, especially for the inference of sleep times in ruminant species. The extent of the surgeries involved in the study did not allow for additional implantation of i-buttons within the intra-abdominal muscles; however, the lack of Tsc shifts reported seasonally also appears consistent with previous descriptions for Tb (Hetem et al., 2010; Bertelsen et al., 2017; Streicher et al., 2017). As Tb was not measured in this study it would be a great future point to include for more accurate comparisons to prior studies.

The mechanisms dictating the observed seasonal temporal niche switching and differences in sleep of Arabian oryx in their extreme desert habitat are at present unknown. It is possible that such behaviour provides a highly plastic and adaptive response to extreme temperature variation thereby mitigating the environmental effects on Tb, possibly by preventing hyperthermia, conserving energy and reducing evaporative water loss (Berger and Phillips, 1995; Revell and Dunbar; 2007). Again the specific mechanisms in which the environmental effects on Tb are possibly mitigated is unknown and in conjunction with the thermal effects observed during sleep require further study. As both body and brain temperature are known to exhibit significant decreases during NREM (specifically slow-wave sleep) (McGinty and Szymusiak, 1990; Wehr, 1992; Kräuchi and Deboer, 2010), such dramatic shifts of sleep timing during summer may exemplify such effects in terms of an adaptive response to increasing Ta and solar radiation. It does appear that a threshold exists with regard to the upper extremes of Ta in which Arabian oryx can no longer maintain high quality or efficient sleep or engage in sleep whatsoever. At this point Arabian oryx may be experiencing forms of sleep deprivation and such possible effects should be further examined especially in relation to trade-offs between energy expenditure, body water conservation and brain functioning.

The occurrence and highest probability of sleep is normally associated with the decrease of core Tb in the daily phase; however, the occurrence of sleep during the rising phase is rather uncommon (Dijk and Kronauer, 1999; Gilbert et al., 2004; Kräuchi and De Boer, 2010; Sato

et al., 2015) especially under extreme ambient heat and heat stress (Heller, 2005; Sinha & Ray, 2006; Downs et al., 2015) which is most likely to occur when Ta supersedes Tb during the hot season. The thermal physiology of NREM sleep (Kräuchi and Deboer, 2010) may be utilized during summer as a form of body and brain temperature reduction; whilst the variation observed in the duration and timing of REM sleep between seasons may relate to a thermal function of the state and brain temperature maintenance and thus requires further study (Wehr, 1992).

It has been suggested that REM sleep may function for heat conservation (Snyder, 1966; Walker and Berger, 1980; Palca et al., 1986; Wehr, 1992) and previous studies show dramatic increases in brain and core Tb during REM sleep in mammals (Synder, 1966; Palca et al., 1986; Alföldi et al., 1990; Wehr, 1992). Additionally, REM sleep propensity is usually highest when Tb is at its lowest (Wehr, 1992); inversely, the propensity is lowest at high Tb (Parmeggiani et al., 1975; Parmeggiani, 1980). The amount of REM sleep is reduced when subjected to high temperatures and typically exhibits virtual absence above 30˚C in rats (Szymusiak and Satinoff, 1981; Kräuchi and De Boer, 2010). Conversely, increasing temperature appears to increase NREM sleep pressures (Morairty et al., 1993; Kumar et al., 2009; Kräuchi and De Boer, 2010). In response to the extreme high Ta during summer in the desert, it may be that normal or extended periods of REM sleep will lead to dangerously high brain temperatures and as such, lower amounts of REM sleep with shorter durations, compared to winter, appear more prevalent in the Arabian oryx observed. Alternatively, the drastic rise in both core body and brain temperatures may lead to a favouring for diurnal sleep during summer and more typical, artiodactyl-like nocturnal sleep during winter (Kräuchi and Deboer, 2010). Such speculation, however, requires larger sample size and direct brain and core temperature recordings to be conducted in combination with polysomnography. Additionally, the possible consequences of reduced REM sleep in this species and in mammals requires further examination.

Ta effects not only the sleep centers of the brain (Parmeggiani and Rabini, 1967; Parmeggiani, 1986; Fletcher et al., 1999; Gilbert et al., 2004) but also circadian centers (Boulant, 1981; Hensel, 1981; Van Someren, 2000, 2003; Van Someren et al., 2002) by altering the circadian timing of sleep and Tb rhythms under different heat loads. Daily Ta fluctuations provide essential input to the circadian clock (Van Someren, 2003); it has been demonstrated that Ta elicits a direct effect on thermal regulation at the same time influencing Tb and the circadian rhythm of Tb (El Allali et al., 2013; Bouâouda et al., 2014).

Subsequently, the sleep-wake cycle is highly sensitive to the timing and effects of the circadian temperature cycle, notable especially under extreme Ta stress (Duffy et al., 1997; Van Someren, 2000, 2003; De Boer, 2002; Kubota et al., 2002). Bouâouda et al. (2014) discuss the process of adaptive heterothermy in the camel as being possibly circadian-related. Under heat stress and dehydration, adaptive heterothermy may change the SCN master clocks function and the synchronization to the light-dark cycle. Based on our results such an effect may be similarly occurring in the Arabian oryx at a seasonal level and thus requires further examination.

Through an underlying circadian rhythm, core Tb decreases during normal sleep onset and subsequent sleep further promotes this effect (Barrett et al., 1993; Okamoto-Mizuno and Mizuno, 2012). Changes in peripheral skin temperature are main driver of decreasing core Tb (Kräuchi et al., 2000) and have been associated with the timing of melatonin secretion and thus the timing of sleep (Kräuchi et al., 1998). The extreme Ta cycle in the desert affects both peripheral skin and core temperature, and this relationship has been suggested to advance circadian core Tb and REM sleep (Okamoto-Mizuno and Mizuno, 2012). Phase advancing effects of temperature on internal circadian mechanisms may dictate or drive the seasonal timing of sleep in the Arabian oryx. The oryx's sleep plasticity may be predictive and preemptive to reach optimal timing during each season. Such behavior is most likely highly dependent on a complex system of endogenous clocks, their synchronization to input variables, several levels of oscillators and the final circadian expression (Davidson et al., 2003; Dibner et al., 2010; Pevet and Challet, 2011; Bouâouda et al., 2014).

We experienced malfunctions with the PSG equipment in some of the Arabian oryx during data capture. Taking into consideration the nature of the experiment, the equipment and the local environmental conditions, the risk for technical difficulties was high; however, we attempted to reduce these risks by any means available to us but as a result our sample size (recovered, high-quality data) was smaller than desirable. Any chance of repeating the experiment was hindered by the conservation status of the oryx and overreaching expenses. Fortunately, the simultaneous actigraphy recordings allowed for confirmation of observed patterns in the oryx without PSG equipment and permitted the results to be treated as a form of case study. Ecologically realistic studies are necessary for the advancement of our understanding of sleep behaviours of non-domestic species. The importance of the results obtained would counterpoise experimental inadequacies such as sample size; an understanding that indefinite, uncontrollable experimental conditions may not reproduce

sample sizes typically associated with laboratory-based experiments that would allow for any data obtained to be viewed as scientifically valuable (Campbell and Tobler, 1984; Refinetti, 2008; Lesku et al., 2009; Aulsebrook et al., 2016).

Article IV provides a baseline measure of physiological sleep in the Arabian oryx and may benefit future studies examining behavioural responses of arid zone species to climate change and anthropogenic disturbance in relation to reproductive fitness, adaptive waking behaviour and may improve species conservation (Roth and Krochmal, 2015; Roth et al., 2015; Aulsebrook et al., 2016). The findings of the highly varied seasonal sleep physiology in the Arabian oryx directly addresses the issue of generalizing behavioural sleep and its correlates across strains, species or certain conditions (van Alphen et al., 2013; Aulsebrook et al., 2016; Rattenborg et al., 2017). Our findings support that sleep is a unique state for each species, probably providing multiple functions and creating necessary trade-offs for fitness while being subject to different selective pressures (Siegel, 2005; Lesku et al., 2006, 2009; Rattenborg et al., 2007; Roth et al., 2010; Steinmeyer et al., 2012; Aulsebrook et al., 2016; Stuber et al., 2016; Rattenborg et al., 2017).

2. Future Perspectives and Study

The Arabian oryx appear to employ a set of behavioural and physiological responses in particular order as temperatures rise and fall to extremes throughout the seasons in concurrence with changing biomass quality and quantity, rainfall, drought and other variables. In the extreme environment of the oryx, a daily balance must be obtained between metabolic energy, body fluid and thermoregulatory homeostases (Hetem, 2010; Fuller et al., 2016). Considering that the major hardship faced by Xerocoles is obtaining water, the management of body fluid homeostasis and its loss to evaporative means would be of primary importance. The four primary autonomic physiological and behavioural strategies employed by Arabian oryx are adaptive heterothermy, activity pattern variability, selective brain cooling and shade-seeking behaviour. In addition, there is the putative thermoregulatory role of sleep, with sleep timing and duration appearing highly adaptive and seasonally plastic in wild Arabian oryx. The relevant response order and rate may be multifaceted, adaptive and highly plastic itself, based on what may be most beneficial to survival.

Adaptive heterothermy is considered an important autonomic response during the hot months in the desert as the necessary dissipation, by evaporation, of resting metabolic and

environmental heat prevents hyperthermia (Mitchell et al., 2002; Hetem, 2010; Hetem et al., 2010; Fuller et al., 2016; Streicher et al., 2017). This necessary evaporation depletes body water stores and reserves at a dangerous rate when abundant drinking water is not available. As such, the employment of adaptive heterothermy and the transfer of heat dissipation to non-evaporative means allows for survival outside the mammalian thermo-neutral zone. Heterothermy has been considered previously as a case of dehydration-induced hyperthermia combined with starvation-induced hypothermia and not an adaptive process, although this is still under debate and requires further study (Taylor, 1969, 1970a, b; Maloiy, 1973; Finch and Robertshaw, 1979; Baker, 1989; Nijland and Baker, 1992; Silanikove, 1994; Jessen et al., 1998; Alamer, 2006; Hetem, 2010; Hetem et al., 2010). In the case of the Arabian oryx, it is not known whether such physiological response is adaptive or consequential; however, it does seem to be driven more by water deficit over ambient heat stress (Ostrowski et al., 2003; Hetem, 2010; Hetem et al, 2010) and may additionally involve intrinsic factors such as the animal's sex and behaviour (Streicher et al., 2017).

The highly plastic activity patterns exhibited by Arabian oryx appear to serve multiple roles. Firstly, the cathemerality of activity and locomotion allows for foraging time to be maintained throughout the year. The behaviour features in other ungulate species and may be compensatory to limited daytime foraging, in lieu of high ambient heat stress, resulting from increased shade-seeking behaviors (Zervanos and Hadley, 1973; Belovsky and Jordan, 1978; Stanley Price, 1989; Grenot, 1992; Hayes and Krausman, 1993; Berger et al., 1999; Dussault et al., 2004; Maloney et al., 2005, Hetem, 2010; Hetem et al., 2012a, b; Fuller et al., 2016). Secondly, the plasticity of activity and inactivity patterns on a seasonal level may play a role in mitigating the effects of increasing Ta. By shifting the timing and duration of activity, especially when foraging, oryx are able to increase shade seeking and avoid increased heat load (through solar radiation) and in doing so reduce evaporative water loss while simultaneously reducing metabolic energy use and maintaining homeostasis. These behaviours appear to occur together with shade-seeking behaviour and cooler microclimate selection to further compound the thermal, fluid and energy saving specializations.

The thermoregulatory physiology of sleep may fall under the second order response along with activity patterns; however, this is purely speculative and requires further study. The second order responses may in fact be driving the behavioural patterns and additional shade seeking. As the output of sleep features strongly within the circadian rhythms organization; it may be that the most permissible time for sleep is during specific times of the day when Ta is

within amenable ranges. The daily timing of sleep, seasonally, appears to be driven predominantly by Ta and secondly by photoperiod; this may interrelate and drive subsequent activity and rest patterns throughout the circadian cycle. Considering that the Arabian oryx do not face natural predators, especially within their protected reserves, it is most likely that climatic condition, related foraging and feeding availability and behaviour is responsible for seasonal sleep plasticity. Arabian oryx may first employ physiological sleep to reduce both brain and body temperature, especially during the daytime in summer, while still maintaining the necessary duration of sleep required for sleep homeostasis and its consolidation (Kräuchi and Deboer, 2010). The results from Article IV suggest that sleep may in fact be homeostatically regulated during both seasons; however, whether any form of sleep deprivation may be occurring during summer, in light of the substantially higher slow wave activity, compared to winter, is unknown and further study is welcomed. Once Ta reaches and exceeds a permissible range for efficient and consolidated sleep, oryx may continue resting and shade seeking behaviours throughout the afternoon and evening periods, and then successively forage through the night. As not much is currently understood about the circadian rhythms of the Arabian oryx and the thermal physiological features of sleep under natural conditions, additional study is required to further strengthen the stated observations.

The autonomic response of selective brain cooling in the Arabian oryx and amongst most of the Artiodactyls appears as a powerful modulator of environmental change and a strong acclimatization factor, provided that phenotypic plasticity amongst its capabilities and magnitude is achieved (Gillilan, 1974; Simoens et al., 1987; Mitchell et al., 2002; Hetem, 2010; Hetem et al., 2010). Selective brain cooling was originally hypothesized to protect the brain, especially the hypothalamus, from extreme rise in Tb (Schmidt-Nielsen et al., 1957; Withers, 1992; Schmidt-Nielsen, 1997; Willmer et al., 2000; Mitchell et al., 2002; Hetem, 2010, Hetem et al., 2010). The process of adaptive heterothermy, by mechanism, is associated with extreme and sudden increases in Tb; more recent studies suggest that selective brain cooling may in fact be used to attenuate thermal drive and modulate thermoregulation rendering it to be mechanistic in the rise of heterothermy and not protective against its features (Jessen, 1998; Mitchell et al., 2002; Hetem, 2010; Hetem et al., 2010).

Such a seemingly complex interrelationship amongst the survival strategies of the Arabian oryx would provide an interesting model for studying the dynamics of circadian rhythms, sleep, behaviour and physiology in light of an extreme environment undergoing dramatic seasonal variations. In order to fully test such theories and dynamic relationships, an

extensive investigation using a suite of recording equipment under natural conditions over a long-term period would need to be conducted. It may be best to begin such endeavors under controlled laboratory conditions; however, as alluded to throughout this thesis, such confinement and interference would probably alter the natural response and affect the ecological relevance of the data. A laboratorial study of the suggested type would be costly and would require extensive, invasive surgery. Perhaps in the future, as the conservation status of the Arabian oryx improves, such a study would be permitted and feasible. Meanwhile, continued work broadening the phylogenetic database and observing ecological factors in relation to sleep and circadian rhythms will support the understanding of shared and derived features and selective pressures impelling the evolutionary convergence and divergence of biological traits and their functions (Rattenborg and Martinez-Gonzalez, 2015; Aulsebrook et al., 2016).

The current viewpoint that the neuroanatomy of sleep appears predictable across species within the same order, whilst the physiology of sleep appears purely adaptive and unpredictable seems to hold true for the species studied thus far (Manger, 2005). In the case of the Arabian oryx brain, the sleep related neuronal systems appear to conform overall to the typical mammalian organizational plan and that of the Cetartiodactyl superorder; however, its behaviour and sleep physiology are remarkably plastic and unpredictable. In an attempt to merge form and function with regards to such systems and features, further laboratorial and wild based investigation within the Cetartiodactyl superorder, in combination with behavioural analyses, is required. Such a gap in knowledge between the neural correlates of such behaviours, and the underlying circadian rhythm organization and output pathways leaves much to be understood and studied. It also leads to the suggestion that each species may be uniquely adapted and specialized to its environment, both abiotic and biotic aspects, and that our attempts to define traits distinctly within a set continuum requires more flexibility and insight.

Understanding the full phenotypic evolution, genetic microevolution without speciation, as well as population dynamics of the Arabian oryx will be of major importance as global warming and climate change affect its environment and similar arid-zone dwelling species (Bradshaw and Holzapfel, 2006, 2008; Boko et al., 2007; Hetem, 2010; Fuller et al., 2016). Another caveat to this situation lies with the fact that large, long-lived species undergo long generation times, in terms of evolution, and as such are predicted to be less responsive genetically to new selective pressures (Rosenheim and Tabashnik, 1991; Fuller et al., 2016;

Pilfold et al., 2016). In such cases, the very real threat of extinction may once again be faced (Gomulkiewicz and Holt, 1995; Hetem, 2010). It is possible that the Arabian oryx is one of the most specialized and adapted large, long-lived mammalian ungulate species. With regards to desert environments, however, it is possible that similar plasticity may be uncovered in other species of the genus oryx, camel and within the 13-antelope species successfully residing within the Sahara and Arabian deserts (Mésochina et al., 2003). As increasing desertification continues around the world, it may be that these species will be unable to cope with predicted extremes as well as inherently unpredictable and uncontrollable environmental factors. They may be incapable of relocating to more temperate climates due to several restricting variables in migration corridors, thermal tolerance windows and natural resource management (Holling, 1978; Walters, 1986; Pörtner, 2002; Gilad et al., 2008; Fuller et al., 2016). In addition, it is unlikely that evolutionary adaptation will be adequate to moderate the effects of climate change in its current trajectory, although micro-evolutionary and epigenetic effects require further investigation (Bradshaw and McNeilly, 1991; Davis and Shaw, 2001; Huntley, 2007; Hetem, 2010; Fuller et al., 2016). As global temperature rises and aridity increases in such environments, the availability of vegetation will decrease (directly related to rainfall) and subsequently relay a diminution of the viable home range size of the Arabian oryx, a species heavily dependent on such factors (Stanley Price, 1989; Van Heezik et al., 2003; Gilad et al., 2008).

On a similar note, the continued use and refinement of biologgers, biotelemetry, actigraphy and neurologgers, along with other monitoring technology (Andrews, 1998; Cooke et al., 2004; Block, 2005; Wikelski and Cooke, 2006; Rattenborg et al., 2008; Hetem, 2010; Hetem et al., 2012a,b c; Scriba et al., 2013a, b; Voirin et al., 2014; Rattenborg et al., 2017) will be an important tool for understanding and predicting natural ecology and behaviour in lieu of endangerment and extinction of multiple species in times ahead. The examination and appropriate integration of the circadian rhythms, ecology, neuroanatomy and physiology of sleep within species and across taxa has the potential to illuminate on multiple questions like that of the interactions with factors such as predation risk, reproductive success, sex, biomass dependency, age, metabolism, climate and survival (Aulsebrook et al., 2016; Helm et al., 2017). To end, there is a vast and unlimited amount of information to be learnt from our animal kingdom and as such, basic and cross-disciplinary science should remain prevalent and vital to our understanding of life.

3. References

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APPENDICES

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FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Joshua Gabriel Davimes (Student number: 319626) am a student registered for the degree of Doctor of Philosophy (PhD, Anatomical Sciences) in the academic year 2018.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
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ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2015/11/44/C

APPLICANT: Dr N Gravett

SCHOOL: School of Anatomical Sciences
DEPARTMENT:
LOCATION:

PROJECT TITLE: Neuronal organisation and distribution of the systems that control the sleep-wake cycle in different species of ungulates

Number and Species

3 Wild Blue Wildebeest, 3 Wild Plain Zebra, 3 Wild Arabian Oryx

Approval was given for the use of animals for the project described above at an AESC meeting held on 2015/11/24. This approval remains valid until 2017/12/01.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

None

Signed: _____ (Chairperson, AESC) Date: 1st Dec 2015

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ (Registered Veterinarian) Date: 1 December 2015

cc: Supervisor: N/A
Director: CAS

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ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2015/09/38/C

APPLICANT: Dr N Gravett

SCHOOL: School of Anatomical Sciences
DEPARTMENT:
LOCATION:

PROJECT TITLE: Activity patterns in the Arabian Oryx (Oryx leucoryx)

Number and Species

19 Adult Arabian Oryx (wild)

Approval was given for the use of animals for the project described above at an AESC meeting held on 2015/09/29. This approval remains valid until 2017/10/28.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

None

Signed: _____ (Chairperson, AESC) Date: 10th November 2015

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ (Registered Veterinarian) Date: 11th November 2015

cc: Supervisor: N/A
Director: CAS

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ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2014/53/D

APPLICANT: Dr G Gravett

SCHOOL: Anatomical Sciences

LOCATION: Faculty of Health Sciences

PROJECT TITLE: *Actigraphy: A novel approach to study sleep in free-roaming ungulates in their natural habitat*

Number and Species

3 Blue Wildbeest
3 Zebras
3 Gemsbok
3 Arabian Oryx

Approval was given for to the use of animals for the project described above at an AESC meeting held on 23 September 2014. This approval remains valid until 22 September 2016.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

None.

Signed: _____ (Chairperson, AESC) Date: 19/01/2015

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ (Registered Veterinarian) Date: 19/01/2015

cc: Supervisor: N/A
Director: CAS

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Name of Primary Supervisor: Dr. Nadine Gravett

Signature of Primary Supervisor  Date: 14/07/2017

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ABDULAZIZ N. ALAGAILI, PhD

Signature of Co-author: 

Date: Oct 10, 2017

Article I: Davimes, J.G., **Alagaili, A.N.**, Bennett, N.C., Mohammed, O.B., Bhagwandin, A., Manger, P.R. and Gravett, N., 2017. Neurochemical organization and morphology of the sleep related nuclei in the brain of the Arabian oryx, Oryx leucoryx. *Journal of Chemical Neuroanatomy*, *81*, pp.53-70 – **2nd Author**

Article II: Davimes, J.G., **Alagaili, A.N.**, Gravett, N., Bertelsen, M.F., Mohammed, O.B., Ismail, K., Bennett, N.C. and Manger, P.R., 2016. Arabian oryx (Oryx leucoryx) respond to increased ambient temperatures with a seasonal shift in the timing of their daily inactivity patterns. *Journal of biological rhythms*, *31*(4), pp.365-374. – **Shared 1st Author**

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Article IV: Seasonal variations in sleep of free-ranging Arabian oryx (*Oryx leucoryx*) under natural hyper-arid conditions. *Sleep* (*In Press*) – **2nd Author**

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NIGEL C. BENNETT, PhD, FZS, FRSSAf, MASSAf

Signature of Co-author: Nigel Bennett

Date: 10|10|2017

Article I: Davimes, J.G., Alagaili, A.N., **Bennett, N.C.**, Mohammed, O.B., Bhagwandin, A., Manger, P.R. and Gravett, N., 2017. Neurochemical organization and morphology of the sleep related nuclei in the brain of the Arabian oryx, Oryx leucoryx. *Journal of Chemical Neuroanatomy*, *81*, pp.53-70 – **3rd Author**

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MADS F. BERTELSEN, DVM, DVSc

Signature of Co-author: 

Date: 12-10-2017

Article II: Davimes, J.G., Alagaili, A.N., Gravett, N., **Bertelsen, M.F.,** Mohammed, O.B., Ismail, K., Bennett, N.C. and Manger, P.R., 2016. Arabian oryx (Oryx leucoryx) respond to increased ambient temperatures with a seasonal shift in the timing of their daily inactivity patterns. *Journal of biological rhythms*, *31*(4), pp.365-374. – **4th Author**

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ADHIL BHAGWANDIN, PhD

Signature of Co-author: 

Date: 10/10/2017.

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Signature of Primary Supervisor  Date: 14/07/2017

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NADINE GRAVETT, PhD

Signature of Co-author: 

Date: 10/10/2017

Article I: Davimes, J.G., Alagaili, A.N., Bennett, N.C., Mohammed, O.B., Bhagwandin, A., Manger, P.R. and **Gravett, N.,** 2017. Neurochemical organization and morphology of the sleep related nuclei in the brain of the Arabian oryx, Oryx leucoryx. *Journal of Chemical Neuroanatomy*, *81*, pp.53-70. – **7th Author**

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Name of Primary Supervisor: Dr. Nadine Gravett

Signature of Primary Supervisor  Date: 14/07/2017

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JASON HEMINGWAY, PhD

Signature of Co-author: 

Date: 24/07/2017

Article III: Davimes, J.G., Alagaili, A.N., Bertelsen, M.F., Mohammed, O.B., **Hemingway, J.,** Bennett, N.C., Manger, P.R. and Gravett, N., 2017. Temporal niche switching in Arabian oryx (Oryx leucoryx): Seasonal plasticity of 24h activity patterns in a large desert mammal. *Physiology & Behavior*, *177*, pp.148-154. - **5th Author**

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KHAIRY ISMAIL, PhD

Signature of Co-author: Khairy Ismail

Date: July 17, 2017

Article II: Davimes, J.G., Alagaili, A.N., Gravett, N., Bertelsen, M.F., Mohammed, O.B., **Ismail, K.,** Bennett, N.C. and Manger, P.R., 2016. Arabian oryx (Oryx leucoryx) respond to increased ambient temperatures with a seasonal shift in the timing of their daily inactivity patterns. *Journal of biological rhythms*, *31*(4), pp.365-374. - **6th Author**

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PAUL R. MANGER, PhD

Signature of Co-author:

Date: 10/10/2017

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OSAMA B. MOHAMMED, PhD

Signature of Co-author:

Date: 10 - 10 2017

Article I: Davimes, J.G., Alagaili, A.N., Bennett, N.C., **Mohammed, O.B.,** Bhagwandin, A., Manger, P.R. and Gravett, N., 2017. Neurochemical organization and morphology of the sleep related nuclei in the brain of the Arabian oryx, Oryx leucoryx. *Journal of Chemical Neuroanatomy*, *81*, pp.53-70. – **4th Author**

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Article IV: Seasonal variations in sleep of free-ranging Arabian oryx (*Oryx leucoryx*) under natural hyper-arid conditions. (*Under Review*) – **5th Author**

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Comments by primary supervisor

All of the published and submitted works integrated within this thesis include the current PhD candidate, Joshua Gabriel Davimes, as first author; except for Article II in which he shares first authorship with Dr. Abdulaziz Alagaili.

Article II titled: Arabian oryx (*Oryx leucoryx*) respond to increased ambient temperatures with a seasonal shift in the timing of their daily inactivity patterns.

There are three primary reasons for the shared 1st authorship:

(1) The logistics of organizing the work in Saudi Arabia and effort required by Dr. Alagaili to ensure the study was successful.
(2) The primary grant funding the work was awarded to Dr. Alagaili.
(3) The majority of the field data collection was undertaken by Dr. Alagaili.

Please see the preface section for the full author contributions list regarding Article II.

Kind regards

Dr. Nadine Gravett

Name of Primary Supervisor: Dr. Nadine Gravett

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Table 8-1 in publication. Used as Table 2 in thesis.

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Figure 1 in publication. Connections of suprachiasmatic nuclei (SCN) to sleep-wake regulatory systems

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