

## RESEARCH ARTICLE

# Selective brain cooling in Arabian oryx (*Oryx leucoryx*): a physiological mechanism for coping with aridity?

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### SUMMARY

**Selective brain cooling is a thermoregulatory effector proposed to conserve body water and, as such, may help artiodactyls cope with aridity. We measured brain and carotid blood temperature, using implanted data loggers, in five Arabian oryx (*Oryx leucoryx*) in the desert of Saudi Arabia. On average, brain temperature was  $0.24 \pm 0.05^\circ\text{C}$  lower than carotid blood temperature for four oryx in April. Selective brain cooling was enhanced in our Arabian oryx compared with another species from the same genus (gemsbok *Oryx gazella gazella*) exposed to similar ambient temperatures but less aridity. Arabian oryx displayed a lower threshold ( $37.8 \pm 0.1^\circ\text{C}$  vs  $39.8 \pm 0.4^\circ\text{C}$ ), a higher frequency ( $87 \pm 6\%$  vs  $15 \pm 15\%$ ) and a higher maximum magnitude ( $1.2 \pm 0.2^\circ\text{C}$  vs  $0.5 \pm 0.3^\circ\text{C}$ ) of selective brain cooling than did gemsbok. The dominant male oryx displayed less selective brain cooling than did any of the other oryx, but selective brain cooling was enhanced in this oryx as conditions became hotter and drier. Enhanced selective brain cooling in Arabian oryx supports the hypothesis that selective brain cooling would bestow survival advantages for artiodactyl species inhabiting hot hyper-arid environments.**

Key words: brain temperature, artiodactyl, biologging, desert.

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### INTRODUCTION

Artiodactyla, an evolutionarily successful and hugely speciose order of large ungulates, with 90 extant genera, evolved during the Eocene and speciated during a warming period in the Mid-Miocene Climatic Optimum (Barnosky et al., 2003). Since they evolved in conditions that were hotter and drier than present, artiodactyls may be pre-adapted to cope with warmer and drier environments than those currently experienced (Barnosky et al., 2003; Mitchell and Lust, 2008). Traditionally, the success of artiodactyls has been attributed to the evolution of improved locomotion and the ruminant digestive tract, adaptations which allowed these ungulates to use widely dispersed forage with a high fibre content (Janis, 1989; Codron et al., 2008). Recently, however, another potential contributor to their success has been proposed: the evolution of the carotid rete and the use of selective brain cooling (Fuller et al., 2008; Mitchell and Lust, 2008). The carotid rete consists of an intertwining network of arterioles that lies within a venous sinus at the base of the brain. Venous blood cooled by evaporation from the nasal mucosa drains into the sinus and so cools the arterial blood destined for the brain, generating selective brain cooling (Baker, 1982; Mitchell et al., 1987) – the reduction of brain temperature below arterial blood temperature (IUPS Thermal Commission, 2003). The rete is absent in the closest relatives to the artiodactyls, the perissodactyls, absent in the oldest ruminant group, the Tragulidae (Fukuta et al., 2007), but is present in each of the artiodactyl clades: Ruminantiamorpha, Suinamorpha and Camelidamorpha. Recent systematic analysis of the Artiodactyla

places the Ruminantiamorpha/Suinamorpha/Camelidamorpha divergence before the appearance of the Tragulidae within the Ruminantiamorpha (Spaulding et al., 2009), making it likely that the absence of the rete in Tragulidae has been a secondary loss. Thus, the carotid rete in the Artiodactyla lineage probably evolved at about the same time as the rumen, during the mid-Miocene, about 20 Mya.

Selective brain cooling was originally hypothesized to primarily protect the brain during rises in body temperature (Baker, 1979; Mitchell et al., 1987). However, recent studies support an alternative concept, namely that mammals possessing a carotid rete may employ selective brain cooling to attenuate the drive for evaporative cooling in arid conditions. Selective brain cooling reduces the drive from temperature-sensitive neurones in the hypothalamus on evaporative heat dissipation and conserves body water by switching body heat loss to non-evaporative avenues (Jessen et al., 1994; Mitchell et al., 1997; Fuller et al., 1999; Maloney et al., 2002; Mitchell et al., 2002). Abolition of selective brain cooling, which can occur rapidly, allows evaporative heat loss to be restored in thermal emergencies, such as flight from a predator (Jessen, 1998; Mitchell et al., 2002). So, selective brain cooling would have survival advantages for artiodactyls, not available to perissodactyls, in environments in which water resources are limited and unpredictable (Mitchell et al., 2002).

If the evolution of the carotid rete indeed promoted thermoregulatory flexibility and thus facilitated artiodactyl invasion of arid zones during the highly seasonal post-Eocene world (Mitchell

and Lust, 2008), selective brain cooling may be a key adaptation allowing artiodactyls to cope with the increasing aridity and heat stress that is occurring with climate change (Fuller et al., 2008). However, not all artiodactyl species exhibit the same capacity for selective brain cooling. The absence of the rete in primitive artiodactyls, such as the mouse deer [*Tragulus javanicus*, *Tragulus napu* (Fukuta et al., 2007)], might be expected to decrease the water economy of these species and restrict them to habitats where water scarcity is not an issue. Indeed, the mouse deer is restricted to climatically stable forest environments (Whittow et al., 1977). By contrast, enhanced selective brain cooling may be expected in artiodactyl species inhabiting hot hyper-arid environments. Arabian oryx (*Oryx leucorox*) inhabit one of the hottest deserts in the world, in Saudi Arabia, and survive most of the year, including the hottest summer months, without access to drinking water. To investigate the status of their selective brain cooling, we employed implanted data loggers to measure brain and carotid arterial blood temperatures of five free-living Arabian oryx in their natural habitat. Brain and carotid blood temperature have not been measured in Arabian oryx previously but have been measured in another member of the same genus, the gemsbok [*Oryx gazella gazella* (Maloney et al., 2002)]. Gemsbok inhabit hot dry environments in southern Africa, and indeed were studied at ambient temperatures similar to those experienced by our Arabian oryx, but the rainfall experienced by our Arabian oryx was one-third of that experienced by the gemsbok.

## MATERIALS AND METHODS

### Animals and habitat

The study took place between March and July 2006 within the 2200 km<sup>2</sup> Mahazat as-Sayd Protected Area (22°25'N, 41°48'E) in the open steppe desert in Saudi Arabia, which is both the historical and the current habitat for Arabian oryx (*Oryx leucorox* Pallas 1777). Adult, wild-born oryx (two male and two female) were captured in the Protected Area in mid-March 2006. The oryx were habituated in outdoor pens for two weeks to reduce potential peri-operative stress. An additional male oryx was obtained from the breeding herd at the National Wildlife Research Center in Taif (21°15'N, 40°42'E). This male oryx remained in Taif in a partially shaded pen throughout the study period and had lucerne and water available *ad libitum*. All experimental procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (protocol no. 2005/87/5).

### Surgery

The oryx, at both locations, were darted and anaesthetized in their holding pens with etorphine hydrochloride [2.5 mg intramuscularly (i.m.), M99; C-Vet, Leyland, UK] and, once recumbent, were transported to a temporary operating theatre within 200 m of the pens. At this theatre, the animals were placed in sternal recumbency, with their heads elevated. The animals were intubated, and anaesthesia was maintained with 2–6% isoflurane (Aerrane; Astra Zeneca, Johannesburg, South Africa) administered in 100% oxygen. Respiratory rate, heart rate, arterial oxygen saturation and rectal temperature were monitored throughout the surgery, which lasted about two hours.

Under sterile surgical conditions, we implanted temperature-sensitive data loggers (see below) with sensors in the brain and carotid artery. The loggers were dry sterilized in formaldehyde vapour before implantation. After we had administered a local anaesthetic [3 ml 2% lignocaine hydrochloride; Bayer Animal Health (Pty) Ltd, Isando, South Africa] subcutaneously (s.c.) at the incision sites, the sites were shaved and sterilized with povidone

iodine antiseptic (Vetidine; Vetoquinol Veterinary Pharmaceuticals, Lure, France). For the measurement of carotid arterial blood temperature, a thermistor inserted in a blind-ended and thin-walled polytetrafluoroethylene (PTFE) catheter (o.d. 1.35 mm, i.d. 0.97 mm; Straight Aortic Flush 4F Catheter; Cordis, the Netherlands) was advanced 60 mm into the left common carotid artery towards the heart, at a position midway along the length of the neck, and secured with a purse-string suture in the arterial wall. Outside the artery, the PTFE tube was sealed on a Teflon<sup>®</sup>-coated co-axial cable (150 mm long, o.d. 3 mm; Belden, Richmond, IN, USA) connecting the thermistor to the temperature-sensitive data logger (see temperature measurements). The data logger was positioned subcutaneously, dorsal to the artery. For measurement of brain temperature, a second data logger was positioned subcutaneously, caudal to the base of the left ear. Its Teflon<sup>®</sup>-coated cable was advanced subcutaneously over the skull, where it was connected to a thermistor housed in a head plate and guide tube. The guide tube, constructed from cellulose acetate butyrate tubing (40 mm long, o.d. 1.95 mm, i.d. 1 mm; World Precision Instruments, Sarasota, FL, USA) sealed at the tip by a steel cap, was inserted through a 2 mm-diameter burr hole, which was drilled through the cranium, at coordinates pre-determined from head sections of dead oryx of similar size, so that the probe tip would be positioned near the hypothalamus. The polyvinyl chloride head plate (20×10×5 mm) was secured to the skull by two bone screws and covered by skin.

The wounds were treated with a topical antiseptic spray (Necrospray; Centaur Labs, Johannesburg, South Africa). Each oryx received a long-acting antibiotic (450 mg i.m. penicillin, Norocillin La; Norbrook Laboratories Ltd, Newry, Northern Ireland), a non-steroidal anti-inflammatory analgesic (100 mg i.m. phenylbutazone, dexaphenylarthritis injectable solution; Vetoquinol Veterinary Pharmaceuticals), a long-acting parasiticide (2 ml s.c. Ivermectin, Noromectin; Norbrook Laboratories Ltd) and a multivitamin injection (9 ml i.m. Multivit injectable solution; Univet Ltd, County Cavan, Ireland). Before anaesthesia was terminated, we fitted a neck collar containing a tracking radio transmitter (MOD-500; Telonics, Inc., Mesa, AZ, USA).

Following surgery, the oryx were transported back to their pens, where they became ambulatory within ~10 min after the effects of etorphine were reversed with diprenorphine hydrochloride [7.5 mg intravenously (i.v.) M5050; C-Vet]. The oryx at Taif remained in its pen for the duration of the study. Two weeks after surgery, the four oryx in the Mahazat as-Sayd Protected Area were released into a 2 km<sup>2</sup> fenced enclosure with natural forage and water available *ad libitum*. Ten days later, they were allowed to enter and range freely within the Mahazat as-Sayd Protected Area, where they were left undisturbed, apart from the occasional serendipitous visual contact by rangers. The four oryx separated from each other shortly after their release, with some joining other oryx in the reserve.

A year after surgery, the four oryx were radio tracked, captured and transported back to the holding pens. Those oryx, and the one at Taif, were anaesthetized again and the data loggers were removed under a surgical procedure similar to that used for the original implantation. The surgical wounds had healed and there were no signs of infection. After two weeks of recovery in pens, the four free-living oryx were re-released into the Mahazat as-Sayd Protected Area.

### Temperature measurements

The miniature thermometric data loggers (StowAway XTI; Onset Computer, Pocasset, MA, USA) had outside dimensions of ~50×45×20 mm and a mass of ~40 g when covered in inert wax (Sasol, Johannesburg, South Africa). Temperature sensors used to

measure brain and carotid blood temperatures were constructed from ruggedized glass-coated bead thermistors with insulated extension leads (bead diameter 0.3 mm; AB0E3-BR11KA103N; Thermometrics, Edison, NJ, USA). The loggers had a resolution of 0.04°C and a measurement range from +34°C to +46°C. The scan interval of the loggers was set at 5 min. Each sensor assembly was calibrated against a high-accuracy thermometer (Quat 100; Heraeus, Hanau, Germany) in an insulated water bath. After calibration, the loggers and their sensors measured blood and brain temperatures to an accuracy better than 0.05°C.

#### Climatic data measurements

We collected climatic data from a portable weather station erected near the Mahazat as-Sayd Protected Area, at the Saja/Umm ar-Rimth Protected Area (23°22'N, 42°45'E), and also at the National Wildlife Research Center in Taif. We recorded wind speed ( $\text{m s}^{-1}$ ), solar radiation ( $\text{W m}^{-2}$ ), dry-bulb temperature (°C) and relative humidity (%). We also recorded black globe temperature (°C) and rainfall on site at the Mahazat as-Sayd Protected Area for the duration of the study period. Black globe temperature provides an index of the thermal load on an animal as it integrates the effects of radiant and convective heat exchange.

#### Data analysis

Although we recovered the data a year after surgery, we analysed responses for a period when we had the most complete data set. The data loggers that measured carotid blood temperature in three of the oryx (identified as Oryx 1, Oryx 2 and Oryx 3) malfunctioned within a month after surgery (after a minimum of 4400 simultaneous measurements of brain and carotid blood temperature). The data logger that measured brain temperature in the captive male oryx in Taif (identified as Taif oryx) malfunctioned within a month after surgery (after ~7900 simultaneous measurements of brain and carotid blood temperatures). We obtained brain temperature and carotid blood temperature data from the other free-living male oryx (identified as Oryx 4) for four months after surgery (~16,500 simultaneous measurements of brain and carotid blood temperatures), but his brain temperature probe exhibited an intermittent fault that restricted data analysis to two months. Despite the fault, the data from this animal allowed us to compare selective brain cooling over two seasonal periods. Furthermore, the opportunity presented itself to compare a dominant male with a subordinate male exposed to similar environmental conditions.

We compared variables of the nycthemeral rhythm of brain and carotid blood temperatures with paired *t*-tests. We analyzed the relationship between brain temperature and carotid blood temperature by sorting all five-minute measurements of carotid blood temperature into 0.1°C classes and determining the mean, standard deviation, maximum and minimum brain temperature at each class of carotid blood temperature. We determined the threshold for selective brain cooling as the point at which the regression line of brain temperature against carotid blood temperature intercepted the line of identity ( $y=x$ ). Selective brain cooling was calculated as the difference between carotid blood and brain temperature, with positive values indicating selective brain cooling. The maximum positive difference was defined as maximum selective brain cooling capacity. Mean selective brain cooling was calculated as the average positive difference, and the frequency of selective brain cooling was calculated as the proportion of positive values.

We used mixed effects linear models (function 'lme' using the statistical package 'R') (R Development Core Team, 2008) to investigate the effect of air temperature, water vapour pressure, water

availability and carotid blood temperature on the average 24-h selective brain cooling (difference between carotid blood and brain temperatures). Water availability was a categorical predictor and divided our data into four periods: (1) water was freely available and the oryx drank daily, (2) water was freely available and the oryx drank weekly, (3) limited free-standing water (7 mm rain in May) and (4) no free-standing water (0 mm rain in June). We controlled for repeated measurements by including 'individual' and 'day of the year' as random factors. To account for potential autocorrelation between our variables, we included a first-order autoregressive process (AR1) in our model structure. We generated 16 models (1 full model, 1 null model and 14 reduced models) based on all additive term combinations and determined the best-fit model based on the Akaike's Information Criterion (AIC). Models presenting with  $\Delta\text{AIC}$  greater than two were not considered plausible models (Burnham and Anderson, 2002).

Values are expressed as means  $\pm$  s.d., and  $P < 0.05$  was considered significant.

## RESULTS

### Climate

Air and black globe temperatures varied as a function of the time of day, peaking just after solar noon (12:00) and reaching a minimum just before sunrise (Fig. 1). Air and black globe temperatures increased progressively from April (when all rain fell) to June (when no rain fell) (Table 1). Despite a difference in the number of days over which selective brain cooling was recorded for each oryx in April, there was no difference in the 24-h air temperatures experienced by the four oryx ( $F_{3,84}=2.0$ ,  $P=0.12$ ). Solar radiation showed the expected bell-shaped distribution and peaked at  $1034 \pm 64 \text{ W m}^{-2}$  around solar noon in the month in which we had the most complete selective brain cooling data set (April). Wind speed increased to a maximum of  $9.4 \pm 2.1 \text{ m s}^{-1}$  in the late afternoon and averaged  $4.6 \pm 1.5 \text{ m s}^{-1}$  over the 24-h periods during April. Mean 24-h vapour pressure and relative humidity decreased progressively from April to June (Table 1). Rainfall totalled 31 mm during 2006, substantially lower than the 10-year average of  $100 \pm 60 \text{ mm}$ . April was the wettest month (17 mm) but some rain fell during March (7 mm) and May (7 mm). Because surgery was completed in late March, we have excluded data for March from our analysis to eliminate any possible post-surgical abnormalities in responses.

### Selective brain cooling

Brain and carotid blood temperatures exhibited a nycthemeral rhythm with a trough between 06:00 and 07:00 (following sunrise)

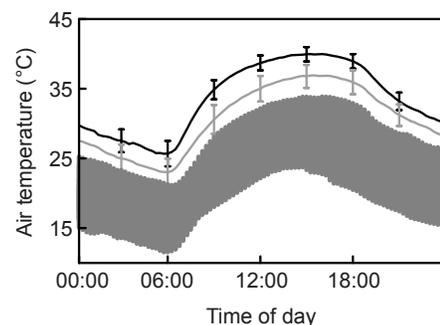


Fig. 1. Average 24 h pattern of air temperature (mean  $\pm$  s.d.) during May (light grey line) and June (black line). The shaded grey area represents the range of air temperature to which each of the four oryx were exposed for the period during which we collected selective brain cooling data in April.

Table 1. Environmental conditions (mean  $\pm$  s.d.) during the three months in which the oryx were living free in the Mahazat as-Sayd Protected Area

	April	May	June
Air temperature ( $^{\circ}\text{C}$ )			
24 h mean	25.0 $\pm$ 3.6	30.5 $\pm$ 1.7	33.7 $\pm$ 0.9
24 h minimum	17.2 $\pm$ 4.1	22.7 $\pm$ 1.9	25.3 $\pm$ 1.7
24 h maximum	31.4 $\pm$ 3.5	36.9 $\pm$ 1.9	40.2 $\pm$ 1.0
Black globe temperature ( $^{\circ}\text{C}$ )			
24 h mean	31.7 $\pm$ 3.0	35.4 $\pm$ 2.9	38.1 $\pm$ 1.4
24 h minimum	16.4 $\pm$ 3.2	20.0 $\pm$ 2.3	21.5 $\pm$ 2.8
24 h maximum	51.0 $\pm$ 5.6	55.0 $\pm$ 4.3	57.9 $\pm$ 3.3
Mean 24 h vapour pressure (kPa)	0.81 $\pm$ 0.26	0.79 $\pm$ 0.28	0.44 $\pm$ 0.05
Relative humidity (%)	25 $\pm$ 10	20 $\pm$ 9	9 $\pm$ 1
Mean 24 h wind speed ( $\text{m s}^{-1}$ )	4.6 $\pm$ 1.5	4.2 $\pm$ 1.3	4.1 $\pm$ 1.0
Mean 24 h radiation ( $\text{W m}^{-2}$ )	286 $\pm$ 50	289 $\pm$ 46	312 $\pm$ 12
Total rainfall (mm)	17	7	0

and a peak between 17:00 and 18:00 (shortly before sunset) (Fig. 2). During April, in four animals (i.e. excluding Oryx 4), the mean 24-h brain temperature was lower than carotid blood temperature ( $38.45\pm 0.06^{\circ}\text{C}$  vs  $38.70\pm 0.10^{\circ}\text{C}$ ;  $t_3=11.5$ ,  $P=0.0014$ ). Minimum 24-h brain and carotid blood temperatures were similar ( $37.36\pm 0.28^{\circ}\text{C}$  vs  $37.53\pm 0.34^{\circ}\text{C}$ ;  $t_3=0.99$ ,  $P=0.39$ ), while the maximum 24-h brain temperature was significantly lower than carotid blood temperature ( $39.34\pm 0.28^{\circ}\text{C}$  vs  $39.61\pm 0.20^{\circ}\text{C}$ ;  $t_3=4.2$ ,  $P=0.024$ ). On average, the oryx implemented selective brain cooling throughout the day, except for the period after sunrise (Fig. 2). For the four oryx, brain temperature was lower than carotid blood temperature, measured simultaneously, 87 $\pm$ 6% of the time. On average, brain temperature was  $0.24\pm 0.05^{\circ}\text{C}$  lower than carotid blood temperature for these four oryx in April. Mean selective brain cooling, the average difference between carotid blood and brain temperature when the oryx were implementing selective brain cooling, was  $0.32\pm 0.03^{\circ}\text{C}$ , and the maximum magnitude of selective brain cooling was  $1.15\pm 0.17^{\circ}\text{C}$ . The threshold for selective brain cooling, defined as the point at which carotid blood and mean brain temperatures were equal, was  $37.8\pm 0.1^{\circ}\text{C}$  (mean  $\pm$  s.d.,  $N=4$ , Table 2).

Fig. 3 shows brain temperature as a function of carotid blood temperature (upper panels in A–D) and the frequency distribution of carotid blood temperature (lower panels in A–D) in two male oryx while both had access to food and water; the male in Taif drank water daily (Fig. 3B) whereas the male (Oryx 3) in the Mahazat as-Sayd Protected Area drank less frequently (Fig. 3A). The captive male oryx (Taif oryx) employed selective brain cooling less frequently (81% vs 94%) than did the free-living male oryx (Oryx 3), despite the two oryx having the same carotid blood temperature and threshold of selective brain cooling (Table 2).

Oryx 4, a male that was frequently observed pacing the fence while in captivity, initiating fights with other males and squat-defecating, behaviours typical of a dominant male (Stanley Price, 1989), displayed less selective brain cooling than did any of the other oryx (Table 2). Compared with Oryx 3, a male that appeared to us to be a subordinate, the dominant male (Oryx 4) showed a higher threshold of selective brain cooling ( $39.6^{\circ}\text{C}$  vs  $37.8^{\circ}\text{C}$ ), a lower maximum magnitude of selective brain cooling ( $0.93^{\circ}\text{C}$  vs  $1.35^{\circ}\text{C}$ ) and implemented selective brain cooling less frequently (11% vs 94%) (Table 2). Unlike that of the other oryx (Fig. 2),

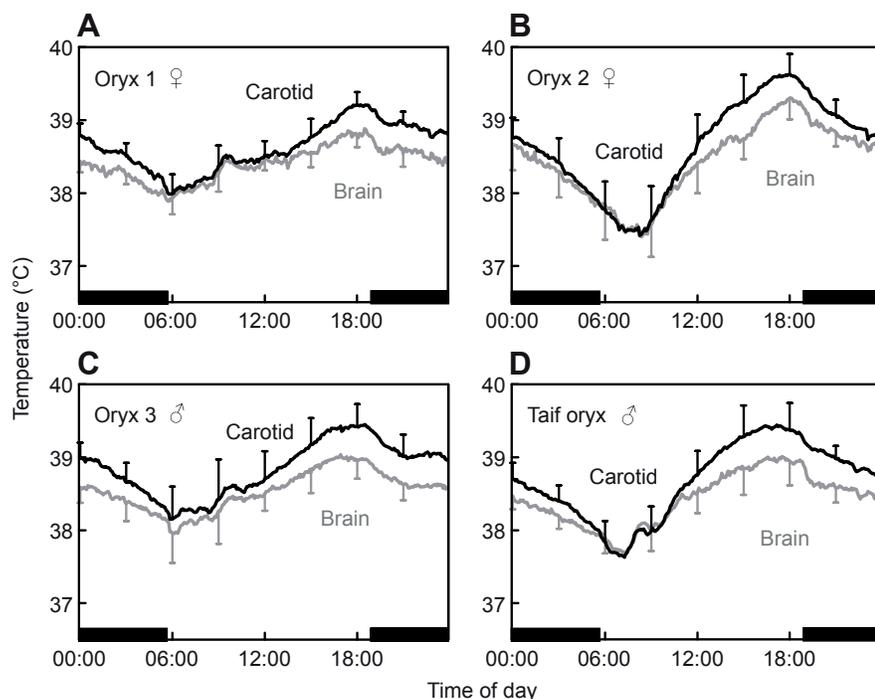


Fig. 2. Average 24 h rhythm of brain (grey line) and carotid blood (black line) temperatures for two female (A,B) and two male (C,D) oryx in April. All oryx had access to food and water *ad libitum*. Black horizontal bars indicate night-time.

Table 2. Mean brain and carotid blood temperatures recorded every 5 min and the mean difference between carotid blood ( $T_{\text{carotid}}$ ) and brain ( $T_{\text{brain}}$ ) temperatures, as well as the mean, maximum, frequency and threshold for selective brain cooling in three male and two female oryx

Oryx	Month	Mean brain temperature (°C)	Mean carotid temperature (°C)	Mean $T_{\text{carotid}} - T_{\text{brain}}$ (°C)	Selective brain cooling				$N$ (days)
					Mean (°C)	Max (°C)	Frequency (%)	Threshold (°C)	
Oryx 1 ♀	April	38.42±0.31	38.63±0.39	+0.21±0.19	0.29±0.16	0.95	90	37.9	15
Oryx 2 ♀	April	38.44±0.62	38.64±0.74	+0.20±0.26	0.30±0.19	1.10	82	37.6	24
Oryx 3 ♂	April	38.53±0.38	38.83±0.50	+0.30±0.20	0.34±0.17	1.35	94	37.8	22
Oryx 4 ♂	May	38.99±0.45	38.71±0.60	-0.28±0.25	0.18±0.14	0.93	11	39.8	28
	June	38.87±0.88	38.86±1.11	-0.03±0.32	0.32±0.19	1.03	38	39.0	29
Taif oryx ♂	April	38.41±0.45	38.66±0.60	+0.24±0.26	0.35±0.20	1.19	81	37.9	27

Data are means ± s.d. and encompass at least 4400 simultaneous measurements of brain and carotid blood temperature in each animal.  $N$  = number of days of data.

the brain temperature of the dominant male oryx (Oryx 4) was generally above that of his carotid blood temperature, particularly at low body temperatures in May (Fig. 4A), when climatic conditions were relatively mild and drinking water was available through ephemeral pools of rainwater. But selective brain cooling was enhanced in Oryx 4 when conditions were hotter and drier in June (Fig. 4B). Although Oryx 4 spent only 11% of the time with his hypothalamus cooler than his carotid blood during May (Fig. 3C), this frequency increased to 38% of the time in June

(Fig. 3D). His brain temperature was an average of  $0.28 \pm 0.25^\circ\text{C}$  higher than his carotid blood temperature during May, and only  $0.03 \pm 0.32^\circ\text{C}$  higher during June.

These differences in selective brain cooling between May and June were not simply the result of a higher carotid blood temperature during June. When analysed over the same carotid blood temperature range (37–40°C), both the frequency of selective brain cooling (30%) and the average difference between carotid blood and brain temperatures ( $0.06 \pm 0.30^\circ\text{C}$ ) indicated enhanced selective brain

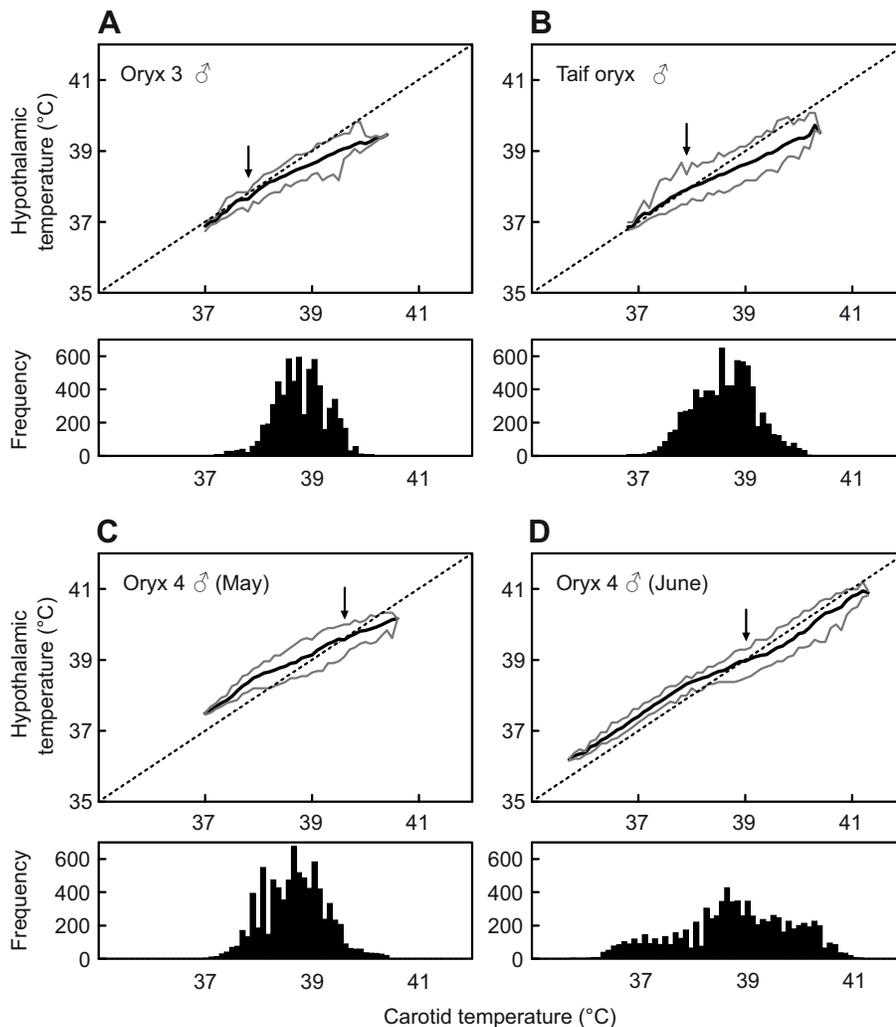


Fig. 3. Brain temperature as a function of carotid blood temperature (top panels in A–D) and the frequency distribution of carotid blood temperature (bottom panels in A–D) in two male oryx (Oryx 3 and the Taif oryx), when both animals had access to food and water *ad libitum*, and a free-living male oryx (Oryx 4), while free ranging in the desert in May and in June. Each 5-min recording of carotid blood temperature was sorted into  $0.1^\circ\text{C}$  classes, and the mean (solid black line), minimum (lower grey line) and maximum (upper grey line) brain temperatures were calculated for each  $0.1^\circ\text{C}$  class of carotid blood temperature. The broken line represents the line of identity; points below this line reflect selective brain cooling. Lower panels in A–D show the absolute frequencies with which each  $0.1^\circ\text{C}$  class of blood temperature occurred; 6491 data points were obtained for the free-living male oryx (Oryx 3), 7968 data points for the captive male oryx in Taif (Taif oryx), 8067 data points for the free-living male oryx (Oryx 4) in May and 8355 data points in June. Arrows indicate the threshold of selective brain cooling.

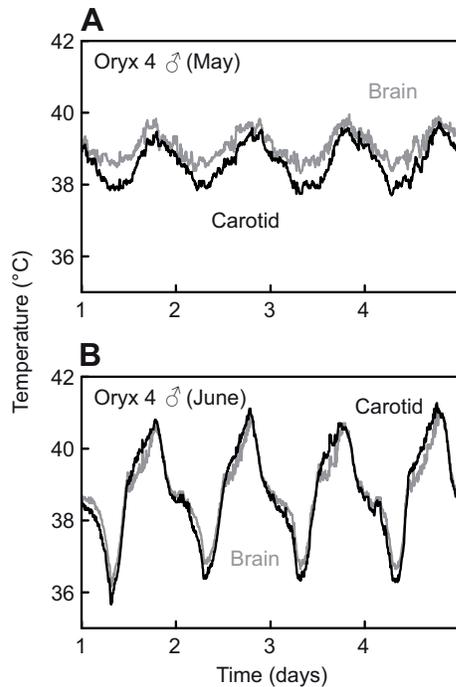


Fig. 4. Brain (grey line) and carotid blood (black line) temperatures of the dominant free-living male oryx (Oryx 4) over a four-day period in May when mean ambient temperature was  $27.9 \pm 2.0^\circ\text{C}$  (A) and a four-day period in June when mean ambient temperature was  $34.7 \pm 0.5^\circ\text{C}$  (B). No rain fell in June.

cooling during the dry June period compared with May. The threshold for selective brain cooling in Oryx 4 decreased with increasing ambient temperature and aridity, from  $39.6^\circ\text{C}$  in May to  $39.0^\circ\text{C}$  in June. Above the threshold, the difference between carotid blood and brain temperatures was higher in June ( $0.28 \pm 0.24^\circ\text{C}$ ) than during May ( $0.16 \pm 0.20^\circ\text{C}$ ). Nevertheless, the maximum magnitude of selective brain cooling was similar in May ( $0.93^\circ\text{C}$ ) and June ( $1.03^\circ\text{C}$ ), and selective brain cooling seldom exceeded  $1^\circ\text{C}$  at any time.

The mixed effects linear models allowed us to differentiate the effects of air temperature and aridity and confirmed that water availability (drinking frequency) was the best predictor for mean 24-h selective brain cooling, with the top five models all incorporating water availability (Table 3). The best model incorporated the effects of water availability, carotid temperature and water vapour pressure. Among tested explanatory variables, air temperature had no measurable effect on mean 24-h selective brain cooling; the addition of air temperature to the model changed the AIC value by more than two (Burnham and Anderson, 2002).

## DISCUSSION

Our study provides the first remote and continuous measurement of brain and carotid blood temperatures of Arabian oryx (*Oryx leucoryx*) in their natural habitat, the deserts of Saudi Arabia. Brain temperature was generally below carotid blood temperature near the peak of the endogenous nycthemeral rhythm of body temperature. However, selective brain cooling was not obligatory at high body temperatures; on occasion, brain temperature reached  $41.2^\circ\text{C}$  when the corresponding carotid blood temperature was also  $41.2^\circ\text{C}$ . Despite the amplitude of the nycthemeral rhythm of brain and carotid blood temperature of our oryx being similar to that measured in other species ( $\sim 2^\circ\text{C}$ ), selective brain cooling appeared to be enhanced in our Arabian oryx. The degree of selective brain cooling can be measured in three ways, namely the magnitude of the difference between carotid blood and brain temperature, the threshold at which selective brain cooling is implemented, and the proportion of time spent using selective brain cooling. Our Arabian oryx, which were exposed to drier natural conditions than any previous animals in which selective brain cooling has been measured, displayed a high maximum magnitude of selective brain cooling ( $1.15^\circ\text{C}$ ), a low threshold for selective brain cooling ( $37.8^\circ\text{C}$ ) and a high frequency of selective brain cooling (87%).

The view that selective brain cooling would be enhanced under dry conditions is supported by our finding that selective brain cooling was observed more frequently in the free-living male oryx (Oryx 3), which drank infrequently, than in the captive male oryx (Taif oryx), which drank daily, despite the two oryx being exposed to similar ambient temperatures and having the same carotid blood temperature. Furthermore, under hot and dry conditions (in June), male Oryx 4 decreased the threshold at which selective brain cooling was implemented compared with that in May, thereby increasing the range of body temperatures over which selective brain cooling was implemented and thus increasing the frequency of selective brain cooling (Fig. 3D). The enhanced selective brain cooling was driven primarily by water availability (drinking frequency), but carotid blood temperature and water vapour pressure also influenced 24-h selective brain cooling (Table 3).

Our conclusions about the enhancement of selective brain cooling by aridity seem robust, but our conclusions about seasonal differences in selective brain cooling in the arid-adapted oryx are tentative, because we obtained data from only a single male oryx (Oryx 4) under hot and dry conditions (June). Since non-thermal factors affecting sympathetic tone may override the thermal regulation of brain temperature (Kuhnen and Jessen, 1994; Maloney et al., 2001), particularly in free-living animals (Jessen et al., 1994; Maloney et al., 2002; Caputa, 2004), high vigilance, and consequent high sympathetic tone, may have acted to decrease the degree of selective brain cooling in the dominant male oryx. His frequency of selective brain cooling was lower, and his threshold of selective brain cooling was higher, than that of all the other oryx measured

Table 3. Akaike's Information Criterion (AIC) for the five best-fit mixed effects linear models incorporating the main factors that accounted for the variability in average 24-h selective brain cooling

Formula (fixed effects)	<i>d</i>	AIC	$\Delta_{\text{AIC}}$
Water availability + water vapour pressure + carotid blood temperature	5	-364.00	0.00
Water availability + water vapour pressure + carotid blood temperature + air temperature	6	-356.66	7.62
Water availability + carotid blood temperature	4	-351.34	12.95
Water availability + carotid blood temperature + air temperature	5	-349.22	15.07
Water availability + water vapour pressure	4	-296.80	67.49

'Individual' and 'day of the year' were included as random factors. *d*, number of parameters including random factors;  $\Delta_{\text{AIC}}$ , difference between AIC with the best model.

(Fig. 3; Table 2). It remains to be seen whether subordinate oryx have the capacity to further increase the frequency of selective brain cooling above 87% or whether they are capable of increasing the magnitude of selective brain cooling as conditions get hotter and drier seasonally. Other species capable of selective brain cooling increase the magnitude of selective brain cooling during conditions of water deprivation and heat stress, as shown in both laboratory-housed sheep (Fuller et al., 2007) and Bedouin goats (Jessen et al., 1997; Jessen et al., 1998). Compared with euhydration, dehydrated goats and sheep increased selective brain cooling with no alteration in the threshold body temperature at which selective brain cooling was implemented. When animals were not heat stressed and were able to lose heat through non-evaporative avenues, two restrained camels at rest showed no obvious difference in brain and blood temperatures between hydrated and dehydrated states (Schroter et al., 1989).

We conclude that the Arabian oryx uses selective brain cooling to facilitate homeostasis in hot and dry environments. Arid-zone mammals possessing a carotid rete may employ selective brain cooling to attenuate thermal drive by reducing brain temperature, which reduces evaporative heat loss and ultimately conserves water by transferring cooling to non-evaporative means (Kuhnen, 1997; Jessen, 1998; Jessen, 2001; Mitchell et al., 2002). Our oryx, like free-living eland [*Tragelaphus oryx* (Fuller et al., 1999)], gemsbok (Maloney et al., 2002), black wildebeest [*Connochaetes gnou* (Jessen et al., 1994)], kudu [*Tragelaphus strepsiceros* (Hetem et al., 2008)] and springbok [*Antidorcas marsupialis* (Mitchell et al., 1997)], displayed the greatest magnitude of selective brain cooling when at rest, while body temperature was rising, in the late afternoon (Fig. 2). Selective brain cooling appeared to be enhanced in our Arabian oryx compared with that exhibited by gemsbok, another species from the same genus (Maloney et al., 2002), exposed to similar ambient temperatures but less aridity (rainfall was three times higher than that experienced by our oryx). Our oryx displayed a lower threshold of selective brain cooling ( $37.8 \pm 0.1^\circ\text{C}$  vs  $39.8 \pm 0.4^\circ\text{C}$ ), a higher frequency of selective brain cooling ( $87 \pm 6\%$  vs  $15 \pm 15\%$ ) and a higher magnitude of maximum selective brain cooling ( $1.2 \pm 0.2^\circ\text{C}$  vs  $0.5 \pm 0.3^\circ\text{C}$ ) than did the gemsbok. Indeed, the threshold for selective brain cooling in Arabian oryx was lower than that observed in any of the African antelope species mentioned above (Jessen et al., 1994; Mitchell et al., 1997; Fuller et al., 1999; Hetem et al., 2008), implying that they spent a higher proportion of time selectively cooling the brain. Whether the apparent enhanced capacity for selective brain cooling in the Arabian oryx reflects differences in the physiology or the anatomy of the carotid rete heat exchanger remains to be elucidated. Even though the magnitude of selective brain cooling in our oryx was much less than the  $2.7^\circ\text{C}$  originally reported for the exercising captive Thomson's gazelle (Taylor and Lyman, 1972), it was double the magnitude that substantially reduced both the respiratory water loss (Kuhnen, 1997) and the metabolic cost of thermoregulation (Kuhnen and Jessen, 1991) in goats.

### CONCLUSION

We have shown that Arabian oryx use enhanced selective brain cooling in the extremely hot and hyper-arid conditions of the Arabian desert. The enhancement took the form of increased frequency of, and lower threshold for, selective brain cooling; the magnitude of selective brain cooling in the arid-adapted Arabian oryx was similar to that observed in other free-living artiodactyls (Jessen et al., 1994; Jessen and Kuhnen, 1996; Mitchell et al., 1997; Fuller et al., 1999; Maloney et al., 2002), implying that there may be a physiological

limit of about  $1^\circ\text{C}$  to selective brain cooling in free-living artiodactyls. Because the hypothalamus is sensitive to changes in temperature of a few tenths of a degree (Kuhnen, 1997), such selective brain cooling would be sufficient to suppress evaporative cooling and therefore reduce water loss. Since selective brain cooling does not disrupt other homeostatic systems, it may provide an economical form of autonomic thermoregulation. The carotid rete may ultimately provide artiodactyls with greater acclimatization potential to the hot and dry conditions predicted to occur in the future.

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