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ORIGINAL PAPER

# Ultradian oscillations in brain temperature in sheep: implications for thermoregulatory control?

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## Abstract

We compared body temperature patterns and selective brain cooling (SBC) in eight adult female sheep in an indoor (22–25 °C) and outdoor (mean ~ 21 °C) environment, by measuring brain, carotid arterial, and jugular venous blood temperatures at 5-min intervals using implanted data loggers. To investigate whether ultradian oscillations in brain temperature had thermoregulatory consequences for the sheep, we determined the cranial arterio-venous (AV) temperature difference as an indicator of respiratory evaporative heat loss (REHL). The 24-h pattern of SBC was similar in both environments, despite carotid blood temperature fluctuating 0.4 °C more outdoors compared to indoors. The sheep employed SBC more often during the night than during the day, but SBC was abolished at intervals of 1–3 h throughout the 24-h period. The suppression of SBC appeared to be associated with events that increased sympathetic nervous system activity, including shifts between stages of sleep. Short-term changes (over 5-min) in brain temperature were positively correlated with changes in the AV temperature difference 5 min later, and negatively correlated with changes in carotid temperature 10 min later. These data support the idea that increases in brain temperature modulate thermoregulation by increasing REHL, which leads to a decrease in carotid blood temperature. Ultradian oscillations in core temperature of sheep, therefore, appear to arise as a consequence of frequent brain temperature changes invoked by non-thermal inputs, in animals housed both in indoor and outdoor environments.

**Keywords** Selective brain cooling · Respiratory evaporative heat loss · Thermoregulation · Sleep

## Introduction

While in most mammals the brain is always warmer than the blood that reaches it (Mitchell et al. 2002), artiodactyls (such as sheep, pigs, and antelope) can have a brain temperature that is lower than arterial blood temperature. Those species activate a specialized vascular network in a process known as selective brain cooling [SBC; for reviews see Jessen (2001); Mitchell et al. (2002)]. Initially viewed as a mechanism to protect the brain from thermal damage during hyperthermia, SBC now is believed to have a broader role in modulating thermoregulatory and osmoregulatory demands of an animal. By cooling the hypothalamus to a temperature below the temperature of arterial blood temperature, SBC reduces the drive on heat loss effectors such as panting, so allowing an animal to conserve body water (Strauss et al. 2017). The finding that SBC is greater in dehydrated than in euhydrated animals at the same body temperature in a hot environment (Fuller et al. 2007) supports the concept that

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SBC is advantageous to an animal faced with simultaneous heat stress and water shortage.

SBC, however, also is evident in euhydrated, normothermic mammals and, in the laboratory, SBC has been observed at night, when carotid blood temperatures are decreasing. Short-term oscillations in brain temperature, that are associated with the activation and suppression of SBC, have been reported in laboratory-housed mammals throughout the 24-h cycle, independent of core body temperature (Baker and Hayward 1968; Fuller et al. 1999, 2011; Hayward and Baker 1969). Similar ultradian fluctuations have not been reported in free-living mammals, although there are few published original records of brain and arterial blood temperature available for inspection. It is also possible that such oscillations are unique to the captive, domesticated species in which they have been observed.

It is now well documented that SBC is influenced by non-thermal inputs, particularly through increased sympathetic nervous system activity, which results in the constriction of nasal mucosal blood vessels and closure of arteriovenous anastomoses, reducing venous effluent and the supply of cool blood to the cavernous sinus (Fuller et al. 2011). Thus, factors that alter the general sympathetic state of an animal influence brain temperature. Indeed, in one of the first studies of SBC, Baker and Hayward (1968) demonstrated that when sheep were startled by noises, handled, or shown food, there was a rise in cerebral arterial blood temperature which then led to an increase in hypothalamic temperature. The oscillations in brain temperature, therefore, may arise as artifacts of standard laboratory procedures. On the other hand, brain temperature oscillates during sleep states (Baker and Hayward 1968; Fuller et al. 1999; Hayward and Baker 1969) and during changes in posture (Baker and Hayward 1968); so, the oscillations may be endogenous and evident in animals outside of the artificial setting of captivity. As far as we are aware, no study has compared the brain or arterial blood temperature patterns in the same animals housed under laboratory and field conditions. Our first aim, therefore, was to compare the pattern of these temperatures, and SBC, in the same sheep when they were housed outdoors and indoors, with minimum human interference.

A further aim was to investigate if brain temperature oscillations that are driven by non-thermal inputs, whether endogenous or exogenous, have consequences for thermoregulation of the animals. Because hypothalamic temperature sensors provide about half of the input that stimulates evaporative heat loss (Jessen and Feistkorn 1984), an increase in hypothalamic temperature (with sufficient thermal input from other body sites) may increase evaporative heat loss, altering heat balance, and leading to a decrease in central arterial blood temperature. Abolishing SBC in sheep by upper respiratory bypass attenuated substantially the rise in rectal temperature that otherwise occurs during fever (Laburn et al. 1988), while a rapid rise in brain temperature on the return of drinking water (at the same temperature as the body core) to water-deprived sheep resulted in a rapid decrease in carotid arterial blood temperature (Fuller et al. 2007). If hypothalamic temperature decreases, then the opposite response should occur. However, it is also possible that the animals have a body temperature tolerance zone (Jessen and Kuhnen 1996), within which small excursions in brain temperature do not elicit changes in heat loss or generation.

If changes in hypothalamic temperature do elicit changes in the body's thermal status, and consequently carotid blood temperature, one would expect a delay in the response of blood temperature as a result of thermal inertia, when an alteration in heat balance changes heat storage and ultimately blood temperature. We, thus, assessed the temporal relationship between changes in brain and carotid blood temperature. We also attempted to determine whether oscillations in brain temperature led to changes in respiratory evaporative heat loss (REHL). In sheep, the cranial arteriovenous (AV) temperature difference, calculated as the difference between carotid arterial and jugular venous blood temperature, provides an index of REHL (Vesterdorf et al. 2011). We predicted that if rapid changes in brain temperature were followed by changes in carotid blood temperature, the changes in carotid blood temperature would be preceded by changes in the cranial AV temperature difference.

## Materials and methods

### Animals

Experiments were performed on eight adult female Dorper sheep (Dorset × Persian, *Ovis aries*). Body mass at start of the experiments was 42.0 ± 3.5 kg (mean ± SD). The Dorpers, which are a mutton breed with a light wool coat (30–40 mm), were obtained from the South African Agricultural Research Council, Irene, where they had been exposed to temperate summer heat in outdoor enclosures before being transported to our indoor animal facility in Johannesburg (Central Animal Service, University of the Witwatersrand, Medical School).

### Experimental protocol

After being housed for at least 2 weeks in the indoor animal facility, the sheep underwent surgery for the implantation of temperature probes and data loggers (see below). For at least 12 days after this surgery, the sheep remained in the indoor animal facility at an ambient temperature of 22–25 °C (with relatively dry air, vapor pressure ≈ 1.2 kPa), after which they were transported to a temperature-controlled climatic

chamber (7.5 $m^2$ floor area) where they were held for 4 days. On the first 2 days in the chamber, used for habituation to the chamber, the dry-bulb temperature was maintained at 23 °C. On the following 2 days, the sheep were exposed to heat, with dry-bulb temperature increased to 40 °C between 09:00 and 15:00. Lucerne chaff and commercial sheep pellets (Epol, Johannesburg, South Africa) were provided each morning between 08:00 and 10:00 and water was available ad libitum. After the climatic chamber experiments, the sheep were transported, in two groups (first sheep 1–4, then, 13 days later, sheep 5–8), back to the South African Agricultural Research Council, Irene, where they were housed in a 1-ha grassed enclosure. The sheep were able to seek cover under a roofed area in the enclosure, and did so at night. The sheep were undisturbed by humans, except for feeding of commercial sheep pellets at ~ 10:00 each day. Water was provided ad libitum. The sheep were housed at the outdoor facility during February and March (southern hemisphere late summer) when the average 24-h dry-bulb temperature was 20.5 ± 1.9 °C (ranging daily between an average of 15.7–28.1 °C in February, and 13.7–25.4 °C in March). Climatic data were obtained from a nearby (2 km away) meteorological station (Irene, South African Weather Services). Mean hours of sunshine on each day were 11.5 h for sheep 1–4 and 10.9 h for sheep 5–8, while vapor pressure typically varied daily between 1.6 and 2.0 kPa. Sunrise occurred at about 06:00, while sunset was at 18:40. In the indoor animal facility, lights were switched on at 06:00 and off at 18:00 each day, but animals also were exposed to natural light through windows, meaning that day-length was similar to that at the outdoor facility.

## Surgery

We implanted miniature data loggers with thermistor sensors for temperature measurement in the brain, jugular vein, and carotid artery. Anesthesia was induced by intramuscular injection of ketamine (2.5 $mg.kg^{-1}$, Anaket-V, Centaur Labs, Johannesburg, South Africa) and medetomidine hydrochloride (0.04 mg $kg^{-1}$, Domitor, Novartis, Johannesburg, South Africa), and maintained with isoflurane (2–3% in oxygen, Isofor, Safe Line Pharmaceuticals, Johannesburg, South Africa). Lignocaine (0.1 g, Bayer Animal Health, Johannesburg, South Africa) mixed with adrenaline (5 ml, Kyron Labs, Johannesburg, South Africa) was injected under the scalp at the site where the brain probe would be inserted, to anesthetize the periosteum and to reduce bleeding. Once maintenance of anesthesia was adequate, atipamezole (0.2 mg $kg^{-1}$ i.m. Antisedan, Novartis, Johannesburg, South Africa) was used to reverse the effects of medetomidine hydrochloride. The sheep received prophylactic long-acting penicillin (4–7 ml i.m., Peni LA, Phenix, South Africa), an analgesic and anti-inflammatory medication (3.0–4.5 ml s.c., Dexa-Tomanol, Centaur Labs, Johannesburg), administered according to body mass. Respiratory rate, heart rate, and percentage hemoglobin oxygen saturation were monitored throughout surgery.

A thermistor (AB0E3-BR11KA103N, Thermometrics, Edison, NJ) in a blind-ended and thin-walled polytetrafluroethylene tube (OD 0.9 mm; segment of a Straight Aortic Flush 4F Catheter, Cordis, The Netherlands) was inserted into the left common carotid artery at a position midway along the length of the neck, and advanced 80 mm towards the heart. A second thermistor was inserted into the left jugular vein at the same position on the neck, and advanced 80 mm towards the head. The bases of the thermistor probes were secured by purse-string sutures in the vessel walls. Outside the vessels, each thermistor was connected via PTFE-covered leads to a data logger (see below). The loggers were placed in a subcutaneous pouch dorsal to the vessels. For brain temperature measurement, a third data logger was positioned subcutaneously in the neck and its lead was advanced subcutaneously to the skull, where it was connected to a head plate and guide tube. The guide tube, constructed from cellulose acetate butyrate tubing (length 44 mm, OD 3.2 mm, ID 1.6 mm; World Precision Instruments, Sarasota, USA) and sealed at the tip by a stainless steel cap, was inserted via a burr hole drilled through the skull. Anatomic markers, verified in our previous study (Maloney et al. 2001), were used to guide the probe tip near to the hypothalamus. The guide tube was connected to a small polyvinyl chloride headplate (10 × 10 × 3 mm), which was secured to the skull by two bone screws. The headplate and all connecting wires were sutured over with skin so that no equipment was externalized. The wounds were treated with a topical antiseptic spray (Necrospray, Centaur Labs, Johannesburg, South Africa) and a stent bandage was sutured over the neck wound to prevent any anatomical dead space from occurring (the bandage was removed 1 week after surgery). After surgery, the sheep were returned to pens in the indoor animal facility.

At the end of experiments, using the same anesthetic and surgical procedures as before, we removed all the implanted equipment (data loggers, thermistors and headplate). The hole in the skull was sealed with bone wax (Ethicon, Johnson & Johnson, Midrand, South Africa). The wounds had healed and there were no signs of infection or scar tissue. Examination of the carotid arteries and jugular veins in all sheep revealed no occlusion, that is, the thermistor probes had measured the temperature of free-flowing blood. After recovery from surgery, the sheep were returned to stock at the Agricultural Research Council, where they were in good health and behaved normally.

## Body temperature measurement

The miniature data loggers (StowAway, Onset Computer Corporation, Pocasset, MA) had a mass of ~ 50 g when covered in inert wax (Sasol, EXP987, Johannesburg, South Africa) and dimensions of ~ 50 × 50 × 25 mm. They had a storage capacity of 32 kb, a measurement range from + 34 to + 46 °C, and a resolution of 0.05 °C. The loggers were set to record temperatures averaged over 5-min epochs (logger sampling rate every 2 s). The wax-coated loggers were calibrated against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany) in an insulated water bath, and had a calibrated accuracy equal to or better than the resolution of the loggers (0.05 °C).

## Data analyses

A comparison of temperatures between animals in the indoor and outdoor facilities was made by averaging the temperatures for each sheep over a 5-day period in each treatment (indoor or outdoor). We excluded data from at least a week following surgery, and at least 4 days after transport to the field, to remove possible confounding effects of stress. For each group of four sheep, we used the 5 consecutive days at the outdoor facility that best matched the indoor facility in terms of average dry-bulb temperature, and which were free of rainfall. Despite sheep 1–4 and 5–8 being housed outdoors over two different 5-day periods, the patterns of brain and carotid blood temperature in the two groups of sheep were remarkably similar (data not shown). We investigated the responses of sheep to heat indoors and outdoors by comparing responses on the second day of heat exposure in the climatic chamber to those on the hottest day when the sheep were housed outdoors.

SBC was calculated as carotid blood temperature minus simultaneous brain temperature, so that positive values reflected SBC. The cranial AV temperature difference was calculated as carotid blood temperature minus simultaneous jugular blood temperature. We analyzed the data using a linear mixed model with study site (indoors or outdoors; indoors was used as the reference site), time of day, and hourly air temperature as fixed effects, and animals as a random effect. Original 5-min recordings of body temperatures were used to find the daily mean, minimum, maximum, and amplitude of body temperatures for each animal. A paired *t*-test was used to compare the amplitude of body temperatures (carotid and brain) between the indoor and outdoor environments, as well as for comparisons of the use of selective brain cooling between the two environments. Comparisons of mean temperatures in the night and day were made by averaging the original 5-min temperatures between 18:40 and 06:00 (night) and 06:00 and 18:40 (day). SBC and the AV temperature difference were compared between night and day by paired *t* tests.

Pearson correlation was used to investigate the relationships between SBC (carotid blood–brain temperature difference) and either brain or carotid blood temperature, with points averaged across the sheep at every 5-min interval over the 5 days in each environment (number of data points = 1440 for each environment). Because sheep 1–4 and 5–8 were housed outdoors at different times, we show the analyses for sheep 1–4 only (Fig. 2); the pattern was similar for sheep 5–8 (within 95% CI of regression line for sheep 1–4).

To analyze short-term variability in internal body temperatures, we calculated the changes in brain, carotid blood, and the cranial AV temperature difference from one 5-min epoch (scan interval of the logger) to the next, in each animal. For each animal, the 1439 successive measurements of each temperature change were sorted into classes of 0, 0.01–0.25, 0.26–0.50, 0.51–0.75, and 0.76–1.0 °C 5 $min^{-1}$. Decrements in temperature were given positive values for this analysis; decrements and increments occurred at about the same frequency.

To investigate the temporal relationship between changes in brain temperature, and changes in carotid blood temperature or changes in the AV temperature difference, we used linear regression to correlate the change in brain temperature, from one 5-min epoch to the next, with changes in the AV temperature difference (or change in carotid blood temperature) at the same time, 5 min later, and 10 min later (based on inspection of oscillations evident in original temperature tracings, and to allow for thermal lag). We carried out the analyses for each animal on its own, in each environment (indoors and outdoors). We considered the relationship between the change in brain temperature and the change in AV temperature difference (or change in carotid temperature) to be significant if the correlation was significant in at least seven out of the eight animals (sign test, two-tailed $P = 0.07$), in both environments.

Statistical analyses were carried out using GraphPad Prism (GraphPad Software, La Jolla, CA), and R v3.6.1 (R Core Team 2019) with the lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017) and car (Fox and Weisberg, 2019) packages. Values of $P < 0.05$ were considered significant (except for the sign test, above), and for *t* tests the mean temperature difference had to be greater than or equal to 0.05 °C (the resolution of the data loggers) for us to claim a significant difference. Use of parametric tests was checked with Shapiro–Wilk tests of normality. All data are reported as mean ± SD.

## Results

### Comparison of temperature patterns in sheep housed indoors and outdoors

Comprehensive descriptions of the results of the linear mixed models for carotid temperature–brain temperature (SBC), and carotid–jugular temperature (AV temperature difference) can be found in Supplement 1. For both models, there were small, but significant, fixed effects for time of day and environmental temperature.

Figure 1 shows the mean difference between carotid blood and brain temperature (positive values indicate SBC), the mean difference between carotid blood and jugular blood temperature (AV temperature difference), brain temperature, and carotid blood temperature over 24 h, averaged over 5 days, in the eight sheep when they were housed at the indoor and outdoor facilities. The mean 24-h dry-bulb temperature was 22.1 ± 0.8 °C for sheep 1–4 housed outdoors together over the 5 days, and 20.3 ± 0.7 °C for sheep 5–8 housed outdoors together over another 5 days; the dry-bulb temperature in the indoor animal facility was maintained at 22–25 °C. The daily amplitude of carotid blood temperature was 0.4 °C greater in the field (1.39 ± 0.17 °C) than indoors (0.97 ± 0.18 °C; $t = 8.20$, $P < 0.0001$). Similarly, the daily amplitude of brain temperature also was 0.4 °C greater in the field (1.44 ± 0.18 °C) than indoors (1.01 ± 0.20 °C; $t = 4.95$, $P = 0.001$).

Despite differences in the pattern of the animals' brain and carotid blood temperatures in the indoor compared to outdoor environment, SBC followed a similar 24-h pattern in both environments. The carotid blood–brain temperature difference was different, on average, between the two environments, but the effect was small (linear mixed model, site:outdoor: estimate = − 0.07 °C, 95% CI: − 0.07 to − 0.06 °C, $P < 0.01$]) When they were indoors, the sheep exhibited SBC for 49 ± 24% of the 24-h period, similar to the 44 ± 23% when outdoors ($t = 1.09$, $P = 0.31$). Typically, SBC occurred at night and in the afternoon. The mean difference between carotid blood and brain temperature was greater at night than during the day (indoors: 0.00 ± 0.17 °C day, 0.22 ± 0.26 °C night, $t = 6.35$, $P = 0.0004$; outdoors: − 0.06 ± 0.18 °C day, 0.21 ± 0.24 °C night, $t = 9.40$, $P < 0.0001$). There was high inter-individual variability in the use of SBC, with one sheep (sheep 8) exhibiting a brain cooler than carotid blood 83% of the time and another only 20%. Sheep 8 also displayed the greatest daily maximum SBC, with an average of 1.4 °C, compared to a maximum average of 0.55 ± 0.15 °C for the other sheep. This high magnitude of SBC in sheep 8 is unlikely to reflect a measurement error, because the mean amount by which brain temperature exceeded carotid blood temperature when SBC was not employed in sheep 8 (0.31 °C) was not different to that in the other sheep (0.40 ± 0.16 °C).

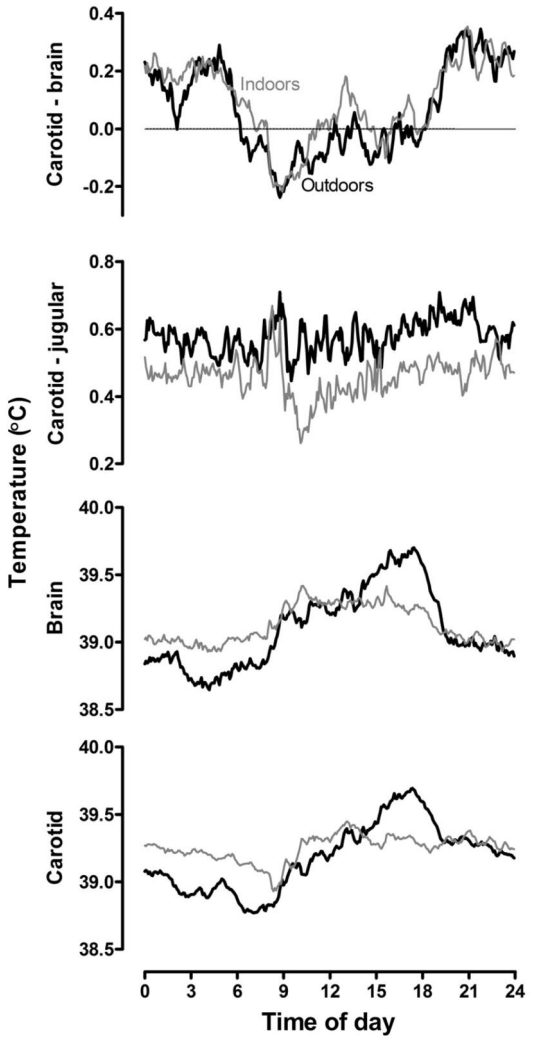


**Fig. 1** Means of carotid blood temperature, brain temperature, and the differences between carotid blood and jugular blood temperature (cranial arterio-venous difference) and carotid blood and brain temperature (selective brain cooling; shown by positive values), at 5-min intervals, determined for eight sheep over 5 days, in an outdoor and indoor environment, as a function of time day. SDs omitted for clarity

On average, the mean cranial AV temperature difference always was positive, near to 0.5 °C, with a maximum just greater than 1 °C (indoors: 1.09 ± 0.19 °C, outdoors: 1.21 ± 0.12 °C). Jugular blood temperature never exceeded carotid blood temperature in the outdoor environment. Indoors, jugular blood temperature infrequently exceeded or equalled carotid blood temperature when body temperatures were increasing between 09:00 and 10:30. The mean AV temperature difference was not different between night and day (indoors: 0.44 ± 0.10 °C day, 0.47 ± 0.11 °C night,

$t = 3.17$, $P = 0.02$ but the mean difference was less than 0.05 °C; outdoors: 0.57 ± 0.10 °C day, 0.59 ± 0.12 °C night, $t = 0.57$, $P = 0.58$). However, the mean AV temperature difference was greater in the sheep when they were housed outdoors (0.58 ± 0.10 °C) compared to when they were indoors (0.46 ± 0.10 °C; linear mixed model, site:outdoor: estimate = 0.13 °C, 95% CI: 0.12 to 0.13 °C, $P < 0.01$). A conspicuous peak in the AV temperature difference occurred in the early morning (between 07:00 and 09:00) and was associated with an abrupt decrease in SBC. Thereafter, the AV temperature difference decreased, particularly when the sheep were housed indoors, when the mean difference was only 0.26 °C at 10:00.

The smaller daily amplitude in the rhythm of carotid blood and brain temperatures when the sheep were housed indoors compared to outdoors (Fig. 1) also is evident in Fig. 2, which shows the relationships between the carotid blood–brain temperature difference and either brain or carotid blood temperature, with points showing averages across four sheep (sheep 1–4) at every 5-min sample over the 5 days in each environment. Even though the 5-min carotid blood and brain temperature were positively correlated with each other (indoors, $r = 0.50$, $P < 0.0001$; outdoors, $r = 0.81$, $P < 0.0001$), they had opposite relationships with the magnitude of SBC. The carotid blood–brain temperature difference was positively correlated with carotid blood temperature (indoors, $r = 0.34$, $P < 0.0001$; outdoors, $r = 0.33$, $P < 0.0001$), but negatively correlated with brain temperature (indoors, $r = -0.51$, $P < 0.0001$; outdoors, $r = -0.41$, $P < 0.0001$). As shown by the $r^2$ values (Fig. 2), variability in the body (brain and carotid blood) temperatures of the animals accounted for between 11 and 26% of the variability in the carotid–brain temperature difference, indicating that SBC was not strongly influenced by thermal inputs.

The relationship between thermal inputs and SBC is further explored in Fig. 3, which shows the patterns of SBC, the AV temperature difference, brain temperature and carotid blood temperature over 24 h, on the hottest day when the sheep were housed outdoors (dry-bulb temperature reached 29 °C between 14:00 and 15:00) and on a day of heat exposure in the indoor facility (40 °C between 10:00 and 15:00). Despite mean brain and carotid blood temperature reaching, or exceeding, 40 °C in the afternoon, SBC was absent or of low magnitude. In the indoor facility, the ramped increase in dry-bulb temperature at 09:00 was associated with a rapid, and transient, 0.6 °C decrease in the AV temperature difference (so that the AV temperature difference was close to zero) and a rapid rise of more than 1 °C in carotid blood and brain temperatures. SBC was absent during this period and afterwards, when brain and carotid blood temperatures

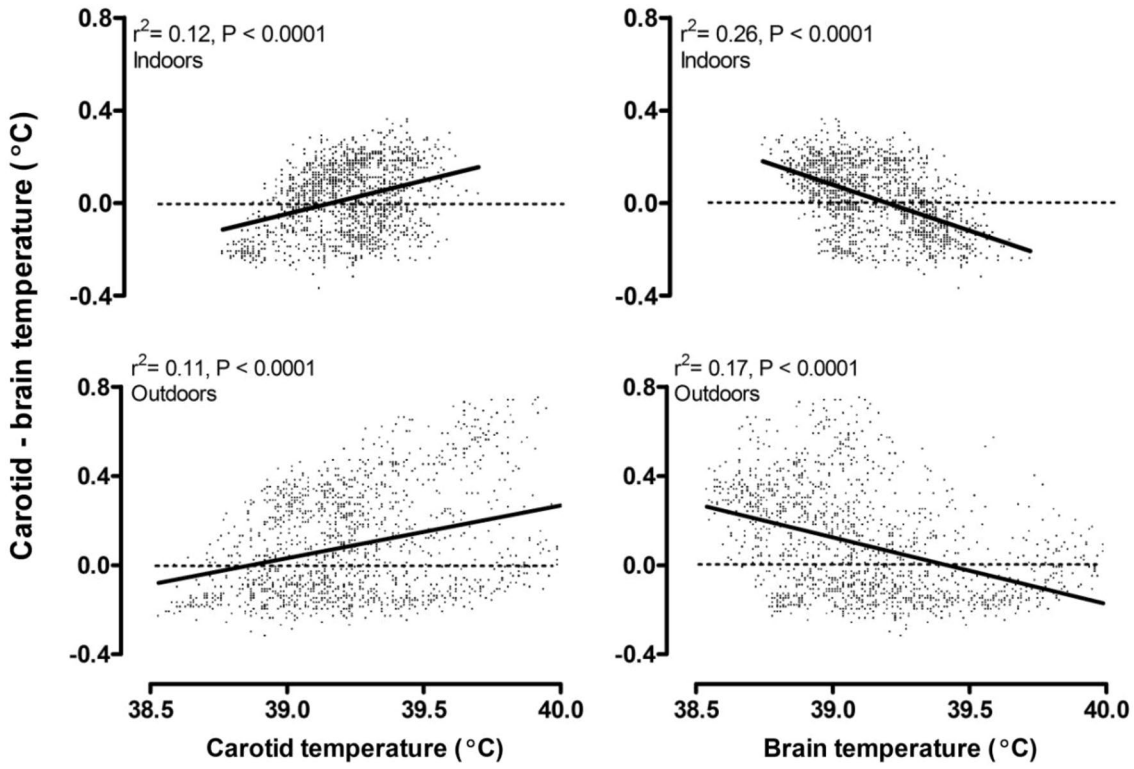


**Fig. 2** The relationship between carotid blood temperature and SBC (carotid blood–brain temperature difference) and brain temperature and SBC, in indoor (upper panels) and outdoor (lower panels) environments. Points shown are averages for four sheep (sheep 1–4) at each 5-min interval over 5 days in each environment

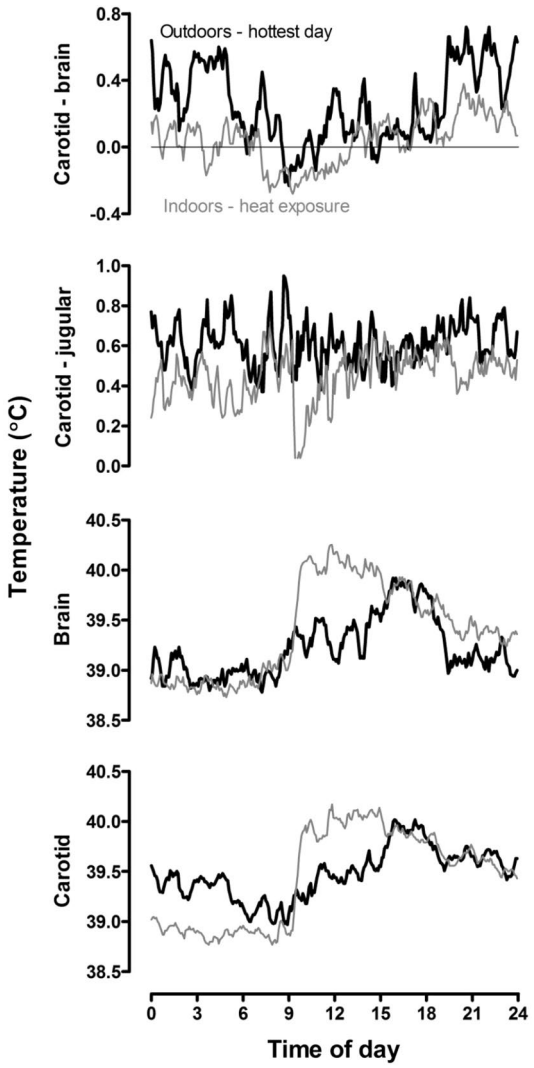


**Fig. 3** Mean carotid blood temperature, brain temperature, and the differences between carotid blood and jugular blood temperature (cranial arterio-venous difference) and carotid blood and brain temperature (selective brain cooling shown by positive values), at 5-min intervals, for eight sheep on 1 day in an outdoor and indoor environment, as a function of time day. In the indoor environment, dry-bulb temperature was 23 °C except between 09:00 and 15:00 when it was increased to 40 °C. In the outdoor environment, dry-bulb temperature averaged 23 °C, and reached a maximum of 29 °C between 14:00 and 15:00. SDs omitted for clarity

had stabilized at ~ 40 °C and the AV temperature difference was ~ 0.5 °C.

## Variability in temperatures

Although the average temperatures, of the group of eight sheep, displayed a clear pattern across the day (Fig. 1), within each individual, the brain and carotid blood temperature, and the difference between them, was highly variable over the course of the day. Figure 4 shows the typical pattern of temperatures for one sheep on 1 day housed indoors and on 1 day housed outdoors. In all of the sheep, there were oscillations in brain and carotid blood temperature, and hence SBC, at intervals of about 1–3 h. Fluctuations in the AV temperature difference also were evident at similar intervals, and often occurred in the opposite directions to those of the carotid blood–brain temperature difference.

In both the laboratory and the field setting, brain temperature was more variable over 5-min intervals than was carotid blood temperature. On average, brain temperature fluctuated 0.03 °C more over each 5-min interval than did carotid blood temperature (indoors: brain $0.07 \pm 0.01$ °C 5 $min^{-1}$, carotid $0.05 \pm 0.01$ °C 5 $min^{-1}$, $t = 3.46$, $P = 0.01$; outdoors: brain $0.08 \pm 0.02$ °C 5 $min^{-1}$, carotid $0.06 \pm 0.01$ °C 5 $min^{-1}$, $t = 4.15$, $P = 0.004$). The change in temperature from one 5-min epoch to the next was smaller than 0.25 °C in 99% of cases for carotid blood temperature (99.0% indoors, 99.3% outdoors) and in 94% of cases for brain temperature (95.5% indoors, 93.8% outdoors). As a sum for all animals in both environments (a total of 23,024 measurements), there were only 71 occasions when brain temperature changed by more than 0.5 °C in 5 min, and only 22 occasions when carotid blood temperature changed by a similar magnitude.

Correlations to assess the short-term (over minutes) temporal relationship between changes in brain temperature, and changes in carotid blood temperature and the AV temperature difference are shown in Fig. 5 for one sheep outdoors. For this typical sheep, changes in brain temperature, from one 5-min epoch to the next, were most strongly positively correlated with the changes in the AV difference 5 min later, and negatively correlated with the changes in carotid blood temperature 10 min later. Analysis of these relationships for all of the animals revealed that linear regressions were significant for the relationship between the change in brain temperature and the change in AV temperature difference 5 min later (indoors, mean slope for eight animals $= 0.20 \pm 0.16$, significant in seven animals; outdoors, mean slope $= 0.16 \pm 0.14$, significant in seven animals), but not for the change in AV difference at the same time (indoors, mean slope $= 0.05 \pm 0.21$, significant in four animals; outdoors, mean slope $= 0.03 \pm 0.24$, significant in three animals) or 10 min later (indoors, mean slope $= -0.02 \pm 0.08$, significant in one animal; outdoors, mean slope $= 0.00 \pm 0.07$, significant in two animals).

For the relationship between the change in brain temperature and the change in carotid blood temperature, there was a significant negative correlation for the change in carotid blood temperature 10 min later (indoors, mean slope $= -0.10 \pm 0.04$, significant in eight animals; outdoors, mean slope $= -0.08 \pm 0.03$, significant in seven animals). Changes in carotid blood temperature and changes in brain temperature, over the same 5-min epoch, were positively correlated (indoors, mean

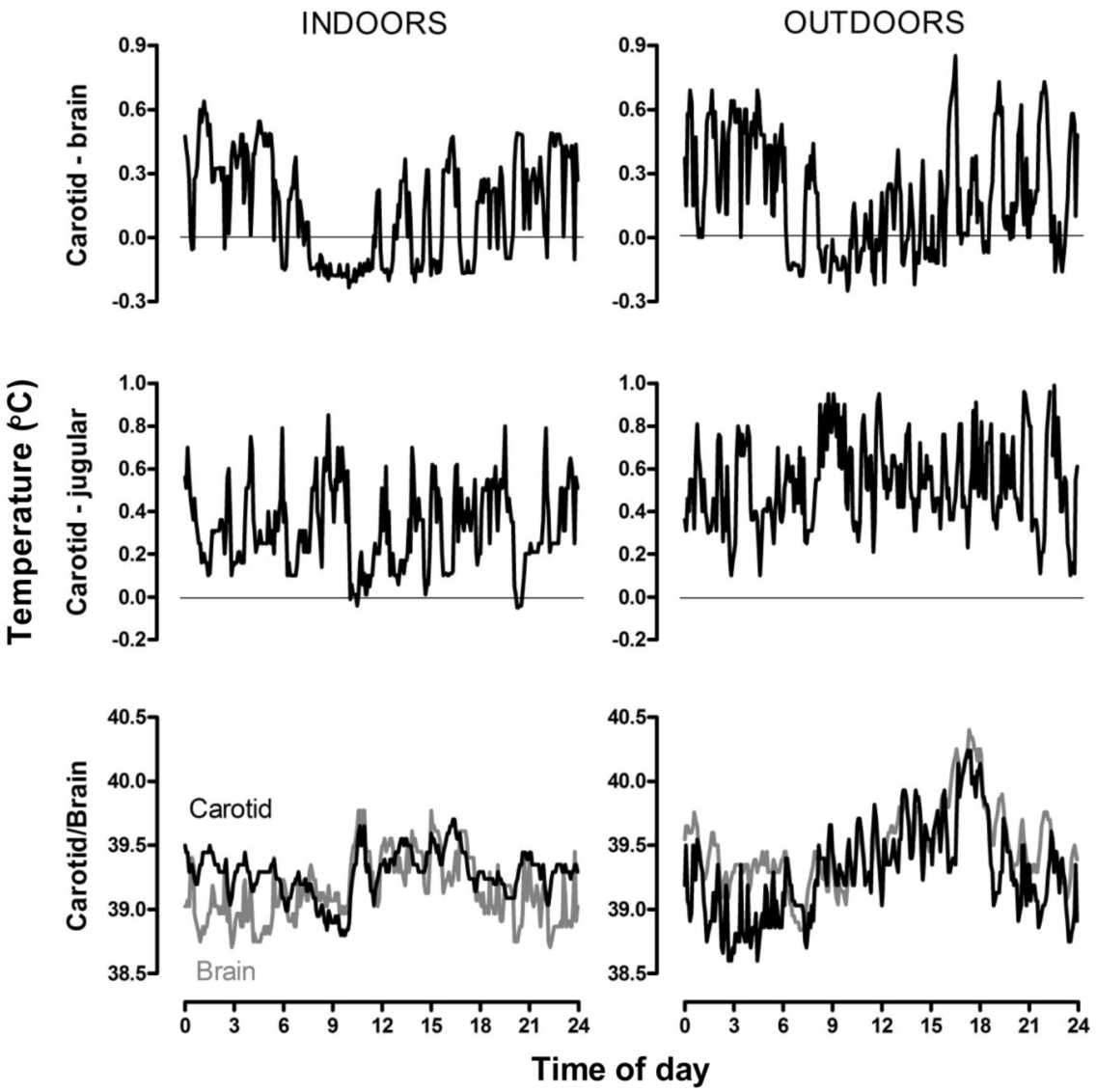


**Fig. 4** Original 5-min values of carotid blood temperature, brain temperature, and the differences between carotid blood and jugular blood temperature (cranial arterio-venous difference) and carotid blood and brain temperature (selective brain cooling; shown by positive values), in the same sheep on 1 day in an indoor and 1 day in an outdoor environment, as a function of time of day

slope = 0.24 ± 0.18, significant in seven animals; outdoors, mean slope = 0.22 ± 0.10, significant in seven animals), as shown for the sheep in Fig. 5 (top right panel). Five minutes later, the changes in carotid blood temperature were not correlated with brain temperature (indoors, mean slope = − 0.06 ± 0.07, significant in six animals; outdoors, mean slope = 0.02 ± 0.11, significant in three animals). Increases in brain temperature, therefore, appeared to increase REHL (as shown by the increased AV temperature difference 5 min later), which in turn led to a decrease in carotid blood temperature (as shown 10-min later). Decreases in brain temperature had the opposite effect.

## Events associated with alterations in SBC

On the day when we relocated the animals from the indoor unit to the farm, the handling and transport of the sheep at about 09:00 were associated with a rapid increase of about 1 °C in brain and carotid blood temperatures, at a time when these temperatures normally increased gradually by about 0.4 °C (Fig. 6, compared to Fig. 1 which shows the normal pattern at that time of day). At this time, the AV temperature difference increased and remained above the normal mean difference for a further 1–2 h. SBC occurred as usual on the night before the relocation, but was not evident (except for

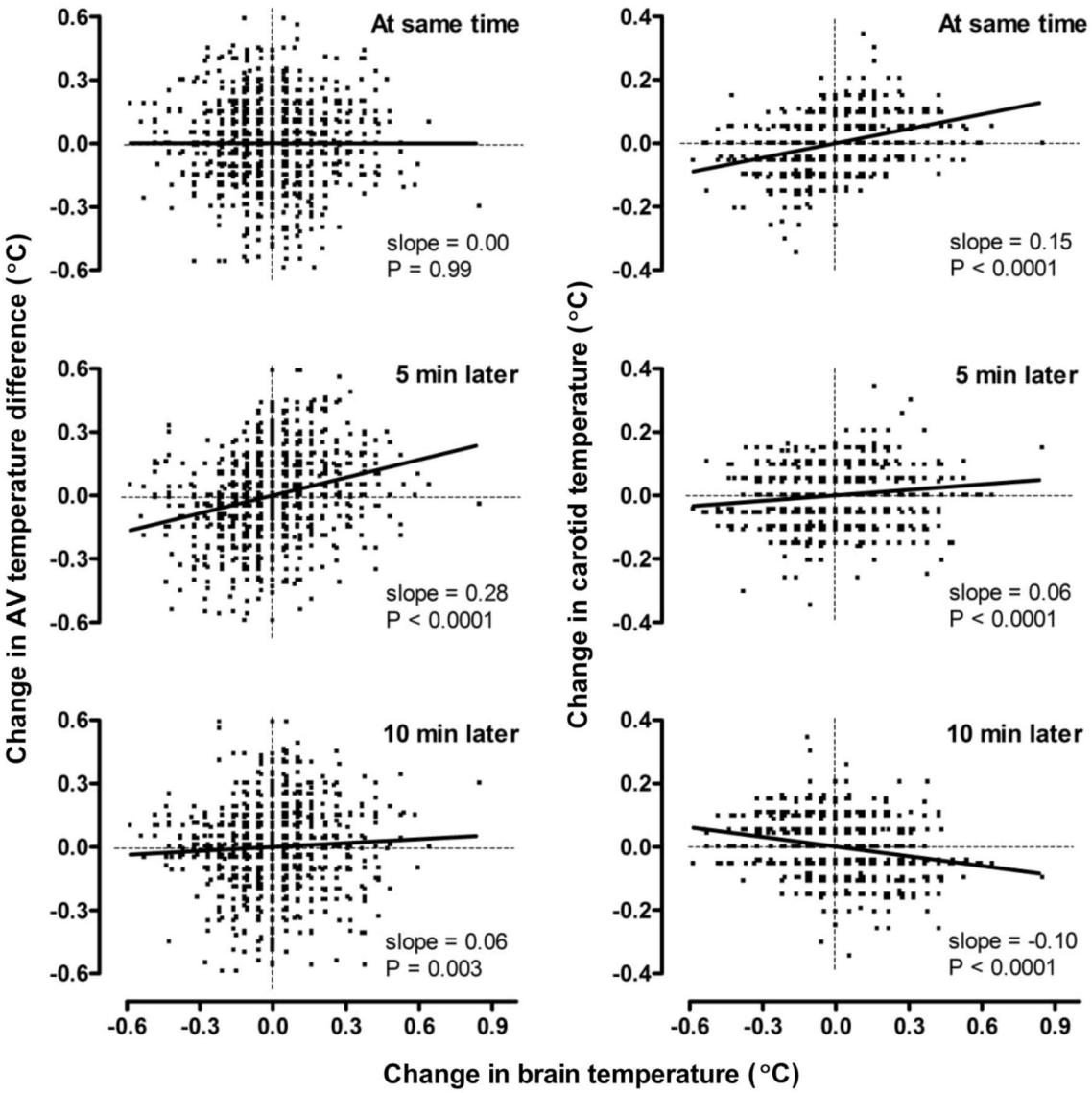


**Fig. 5** Linear regressions for the relationship between the change in brain temperature, over 5-min intervals, and the change in carotid blood temperature and the cranial arterio-venous (AV) temperature difference at the same time, and 5 and 10 min later, for one sheep outdoors. For this sheep, the change in brain temperature, from one 5-min epoch to the next, was most strongly positively correlated with the change in AV difference 5 min later, and negatively correlated with the change in carotid blood temperature 10 min later

brief periods in the evening) for the remainder of the day after relocation. After transport to the outdoor facility, SBC was attenuated for a further 3 days (data not shown).

While the abolition of SBC (as shown in Fig. 6) clearly was associated with disturbance, including the presence of humans, SBC also frequently was abolished when the sheep were not disturbed, at intervals of between 1 and 3 h. As shown in Fig. 4, oscillations in the carotid blood–brain temperature difference occurred even at night. Figure 7 shows in more detail the carotid blood–brain temperature difference, and carotid blood and brain temperatures, of the sheep of Fig. 4, over the time period of midnight to 09:00. SBC was abolished every 1–2 h during the night and attenuated following apparent waking (see also Fig. 1).

## Discussion

We have shown that short-term oscillations in brain temperature were evident in sheep in both an indoor and an outdoor environment, and that the 24-h pattern of SBC was similar whether the animals were housed indoors or outdoors. That the pattern of SBC was so similar indoors and outdoors was remarkable given that the environmental heat load which

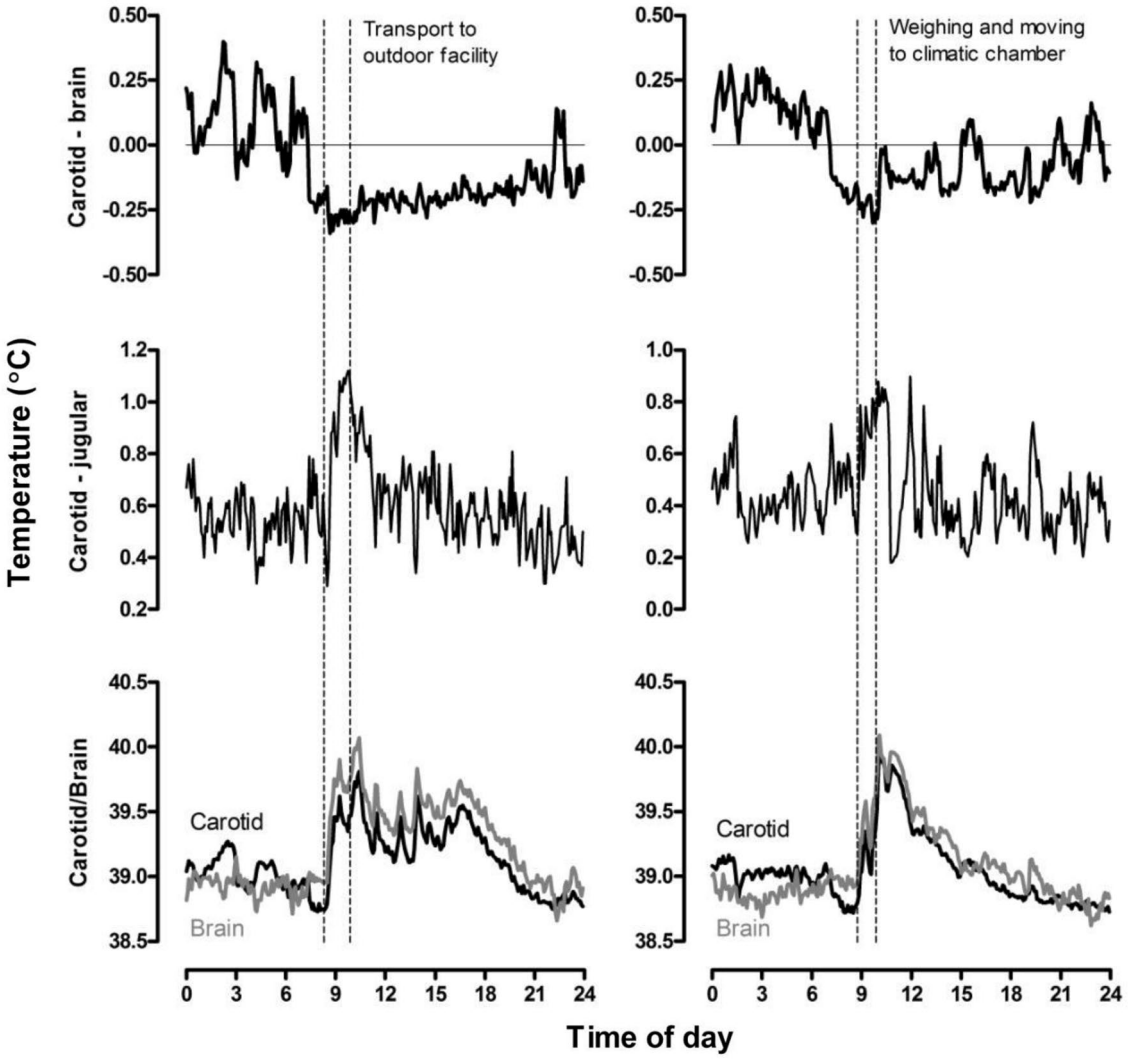


**Fig. 6** Mean carotid blood temperature, brain temperature, and the differences between carotid blood and jugular blood temperature (cranial arterio-venous difference) and carotid blood and brain temperature (selective brain cooling shown by positive values), at 5-min intervals, for four sheep (sheep 1–4) on a day when the animals were transported from the laboratory to the field (left) and on a day when the sheep were weighed and moved to the climatic chamber (right), as a function of time of day. SDs omitted for clarity

was imposed on the animals in the two environments was quite different. The indoor environment had a constant temperature for 24 h, while the outdoor environment followed the natural nychthemeral cycle, which included variations in solar radiation and wind exposure that were not experienced indoors. We also did not match standard care procedures (such as feeding or cleaning times) or control other potential stressors (such as noise) in the two environments. Not surprisingly, the thermal balance of the sheep was different in the two environments. The cranial AV temperature difference was higher, overall, in the outdoor environment (Fig. 1). Unless cranial blood flow was different, the higher AV temperature difference indicates higher REHL (Vesterdorf et al. 2011), due, presumably, to a higher heat load, in the outdoor environment. Also, as previously shown in sheep (Faurie et al. 2004), body temperature was more variable in the outdoor environment than in the indoor environment (Figs. 1 and 2). Yet, in the two environments, SBC was similar, in amplitude (Fig. 1), in circadian pattern (Fig. 1) and in episodic ultradian oscillations (Fig. 4). As reported previously (Fuller et al. 2011), the sheep exhibited SBC more often at night, and the magnitude of SBC at night was greater than that during the day, even when the sheep were exposed to heat during the day, a stimulus that led to an increase in body temperature of about 1 °C. SBC typically was switched on and off at intervals of about 1–3 h throughout the 24-h period (Fig. 4). Also, although the sheep were exposed, in groups, to the same environments, there were substantial differences in the degree to which individual sheep employed SBC.

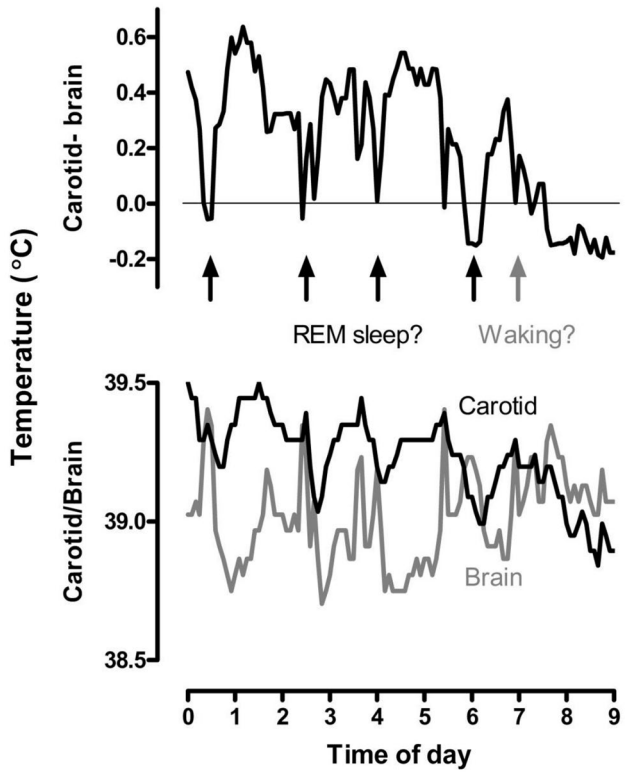


**Fig. 7** Original 5-min values of carotid blood temperature, brain temperature, and the difference between carotid blood and brain temperature (selective brain cooling; shown by positive values), in one sheep in the indoor facility over 9 h. Arrows show proposed times at which REM sleep and waking were likely to have occurred

We, therefore, reconfirm our previous contention (Fuller et al. 1999, 2007) that there are major, and sometimes dominant, non-thermal inputs in the control of SBC. Sympathetic stimulation inhibits SBC, and, in sheep, appears to do so by rapidly reducing the supply of cool blood to the cavernous sinus, most likely as a result of constriction of nasal mucosal blood vessels and arteriovenous anastomoses (Fuller et al. 2011). A reduction in the volume of cool blood delivered to the cavernous sinus will reduce or abolish SBC and cause a rise in brain temperature. As shown in Fig. 6, events associated with high sympathetic activation abolished SBC for a prolonged period. While we kept human presence to a minimum, our animals were subjected to standard care procedures, so it is likely that events such as feeding and cleaning in the morning may have been associated with some of the fluctuations in brain temperature and SBC, as reported previously in pigs (Baldwin and Ingram 1968; Fuller et al. 1999) and sheep (Baker and Hayward 1968; Fuller et al. 2007; Maloney et al. 2001). Oscillations in brain temperature and SBC, however, also were conspicuous in the afternoon and night (Fig. 4), when the sheep usually were undisturbed.

It is likely that some of the oscillations in brain temperature and SBC were associated with changes in sleep state, as has been proposed for pigs (Fuller et al. 1999). In sheep, brain temperature decreases, independent of arterial blood temperature, during slow-wave sleep (SWS), but during REM sleep it rises without a comparable rise in arterial blood temperature (Baker and Hayward 1968), so reducing or abolishing SBC. The spike in brain temperature associated with REM sleep is not confined to sheep (Parmeggiani 2007). We did not measure the EEG in our sheep, so we have no direct evidence of sleep stage, but strong circumstantial evidence leads us to conclude that the episodic spikes in brain temperature, and abolition of SBC, evident in Fig. 7, were related to the sheep entering REM sleep. Undisturbed sheep housed indoors experience alert wakefulness for about 16 h, drowsiness for about 4 h, and sleep for about 4 h; of that sleep period, about 30–45 min is REM sleep and 3–3.5 h is SWS (Ruckebusch 1972; Toutain et al. 1977). REM sleep typically occurs at night (Ruckebusch 1972), but episodes of SWS and REM sleep have been observed during the day (Ruckebusch 1975). The mean duration of REM sleep episodes is between 4 and 6 min (Baker and Hayward 1968; Hayward and Baker 1969; Toutain et al. 1977), and there are about seven or eight episodes of REM sleep, on average, per night. Those characteristics are consistent with the temperature oscillations we observed in our sheep at night (Fig. 7), where there were short episodes, every 1–2 h, when SBC was abolished. Also consistent with the interpretation that the temperature changes were associated with sleep changes is the finding that REM sleep was associated with a mean rise in brain temperature of 0.6 °C in sheep (Baker and Hayward 1968; Hayward and Baker 1969). As shown in Fig. 7 (and Fig. 4), brain temperature increments at night in our sheep were of a similar magnitude. So, it appears that SBC, along with other autonomic functions under hypothalamic control (Shapiro et al. 1974), is suppressed during REM sleep.

The suppression of SBC at night, therefore, occurred at about the same interval that is predicted for periods of REM sleep in sheep, while SBC was evident in the longer periods, between each REM episode, that are predicted for SWS. Morning waking, likely to be accompanied by an increase in sympathetic drive, also was associated with attenuated SBC. Sheep also wake and stand during the night, usually after every 2–3 sleep cycle (REM and SWS periods; 30). Baker and Hayward (1968) reported that changes in posture altered brain temperature, with standing associated with a rise in brain temperature. In their sheep, however, standing usually was observed when the sheep were startled by noise or handling; so, changes in sympathetic tone alone provide a plausible explanation for the rise in brain temperature.

While SBC in sheep, therefore, appears to have major non-thermal influences on its control, the question remains of whether short-term oscillations in SBC, driven non-thermally, have thermoregulatory outcomes. In our sheep

housed indoors at 22–25 °C, or outdoors with moderate heat at times, we had predicted that small oscillations in brain temperature might have little consequence for carotid blood temperature. However, we found that a short-term (over 5 min) change in brain temperature was associated with a change in carotid blood temperature, in the opposite direction, 10 min later (Fig. 5). Since the prime determinant of carotid blood temperature is the temperature of mixed venous blood, changes in carotid artery temperature reflect changes in body heat storage. Thus, the small, short-term changes in SBC, and brain temperature, had a demonstrable influence on thermal status of the animal.

One mechanism by which short-term changes in brain temperature could affect the thermal status of the body is by modulation of REHL. We did not measure REHL directly, but did measure the cranial AV temperature difference. We found that short-term rises in brain temperature were associated with increases in the cranial AV temperature difference. In sheep, an increase in the cranial AV temperature difference reflects an increase in REHL (Vesterdorf et al. 2011). As has been reported previously in Merino sheep (Vesterdorf et al. 2011), we observed few, and only transient, episodes when jugular blood temperature exceeded carotid blood temperature. On average, the AV temperature difference was positive (Fig. 1), indicating that the head was generally a heat sink and that the sheep were implementing REHL at all times. The AV temperature difference in the indoor environment may represent passive evaporation associated with respiration, but the elevated difference in the outdoor environment likely represents active heat dissipation by panting. We suspect that neither the indoor nor the outdoor environment was thermoneutral for our sheep, and that the sheep were constantly modulating REHL. Changes in brain temperature, from one 5-min epoch to the next, were positively correlated with changes in the cranial AV temperature difference 5 min later, and negatively correlated with changes in carotid blood temperature 10 min later (Fig. 5). As indicated by the mean slopes for the regressions between the temperature changes, a 1 °C increase in brain temperature, theoretically, could elicit a 0.2 °C increase in the AV temperature difference, followed by a 0.1 °C decrease in carotid blood temperature 10 min later. There appeared to be about a 5–10-min thermal lag period, therefore, in which REHL increased in response to an increase in brain temperature, and then the increase in REHL led to a decrease in carotid blood temperature. Because carotid blood temperature is a major determinant of brain temperature, brain temperature then decreased, as shown by the patterns in Fig. 7 from a sheep at night.

Our conclusion that ultradian oscillations in SBC, and corresponding brain temperature, influenced the thermal status of the body via modulation of REHL relies on changes in the AV temperature difference reflecting changes in REHL. Although REHL can increase without a change in the AV temperature difference if there is increased blood flow to the nasal mucosa at the same time as an increase in REHL, in sheep an increase in the AV temperature difference always was associated with an increase in REHL (Vesterdorf et al. 2011). Because sheep use both cutaneous and respiratory evaporative cooling, the AV temperature difference does not account for all evaporative heat loss, and the oscillations in brain temperature may modulate cutaneous loss too. Even within one sheep, the allocation of evaporative heat loss to respiratory and cutaneous routes depends on fleece thickness (Hofmeyr et al. 1969). Having a shorter fleece may allow maintenance of thermal balance without any evaporative heat loss, and short-term oscillations in brain temperature may not modulate non-evaporative heat loss. Baker and Hayward (1968) did not observe changes in arterial blood temperature in response to oscillations in brain temperature in sheep housed in small pens at 20–25 °C. One explanation may be that their Hampshire–Columbia ewes had a shorter fleece and maintained heat balance without using significant REHL. They also measured temperature over periods of only 1–5 h, and so may not have revealed the oscillations that we saw in response to normal sheep behaviors.

That short-term oscillations in brain temperature can modulate only evaporative heat loss is supported by our observations of such oscillations in pigs (Fuller et al. 1999). Short-term oscillations in SBC, and consequently brain temperature, occurred in pigs, as a consequence of non-thermal inputs. However, they occurred usually without a concomitant change in arterial blood temperature. Pigs do not sweat and dissipate little heat through respiratory evaporation; so, increases in brain temperature (at a particular arterial blood temperature) are unlikely to affect carotid blood temperature to the same extent as they would in sheep, which rely on panting mainly to maintain a constant core temperature in the heat (Lee 1950).

Oscillations in the abdominal temperature of sheep, with a frequency of 1–3 h, similar to those we observed in carotid blood temperature, have been demonstrated previously in Merino sheep housed at 17–20 °C (Mohr and Krzywanek 1990). The authors reasoned that changes in microbial activity and resultant heat production, following feeding, accounted for some of the oscillations. While it is possible that feeding and drinking (Beatty et al. 2008) alter core temperature by changing heat balance, the oscillations that we observed did not appear to be synchronized with feeding or drinking activity. Instead, our data support the concept that ultradian oscillations in sheep core temperature result primarily from non-thermal factors, particularly those associated with alterations in sympathetic nervous system activity. Sympathetic activation is known to inhibit SBC (Mitchell et al. 2002), and it is possible that ultradian variation in autonomic nervous system activity, demonstrated in several species and shown to be synchronized to the REM/

non-REM sleep cycle (Gordon and Lavie 1986), may underlie the temperature oscillations that we observed.

While the mechanisms underlying consistent ultradian oscillations in body temperature are unknown (Heldmaier et al. 1989; Refinetti and Menaker 1992), pharmacological blockade of both parasympathetic and sympathetic activity has been shown to abolish the normal ultradian rhythm of body temperature in Djungarian hamsters (Braulke and Heldmaier 2010). In rats, in which brown adipose tissue thermogenesis contributes to oscillations in brain and abdominal temperature, blockade of beta-3 adrenoceptors interrupted the normal ultradian temperature rhythmicity (Ootsuka et al. 2011). Although the physiological mechanisms underlying brain temperature increases are likely to differ between rodents (which do not implement SBC; Fuller et al. 1998) and sheep (which do implement SBC), an ultradian oscillation in autonomic nervous system activity may represent a common source for the ultradian body temperature rhythms observed in these animals.

Ultradian oscillations in brain temperature in our sheep induced opposite changes in carotid blood temperature. The sheep, accustomed to heat and with a wool coat, employed REHL throughout the day, with the AV temperature difference similar at night and in the day. The sheep displayed a remarkably consistent overall 24-h pattern of SBC, regardless of differences in the environment, and exhibited SBC at night within the normothermic range of body temperatures. However, as has been shown before in Merino sheep (Maloney et al. 2007), there was high variability between individual animals in terms of how they implemented SBC. Original 5-min-interval data also revealed frequent oscillations in SBC. SBC was abolished in response to non-thermal inputs likely to be associated with sympathetic stimulation, including exogenous factors like human presence and endogenous factors like sleep stage variation. The high inter-individual variability in the use of SBC may reflect differences in animal temperament that determine how an individual responds to external stimuli (Maloney et al. 2007). Sheep, like pigs in the laboratory, appear to be particularly fractious, with repeated attenuation of SBC whether housed in the field or laboratory, and when exposed to heat. In the face of a higher heat load, however, and particularly when dehydrated (Fuller et al. 2007), thermal inputs may override strong non-thermal inputs allowing sustained SBC, and water conservation, to occur.

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**Data availability** Data will be made available upon request to the corresponding author to any researcher wishing to use them for non-commercial purposes.

## Compliance with ethical standards

**Conflict of interest** No conflicts of interest, financial or otherwise, are declared by the authors.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in this study involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Animal Research Ethics Committee, University of the Witwatersrand, number 2004/94/5).

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