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Measuring subcutaneous temperature and differential rates of rewarming from hibernation and daily torpor in two species of bats

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ABSTRACT

Prolonged and remote measurement of body temperature (T_b) in undisturbed small hibernators was not possible in the past because of technological limitations. Although passive integrated transponders (PITs) have been used previously to measure subcutaneous temperature (T_{sub}) during daily torpor in a small marsupial, no study has attempted to use these devices at T_bs below 10 °C. Therefore, we investigated whether subcutaneous interscapular PITs can be used as a viable tool for measuring T_b in a small hibernating bat (*Nyctophilus gouldi*; *Ng*) and compared it with measurements of T_b during daily torpor in a heterothermic bat (*Syconycteris australis*; *Sa*). The precision of transponders was investigated as a function of ambient temperature (T_a) and remote T_{sub} readings enabled us to quantify T_{sub}–T_b differentials during steady-state torpor and arousal. Transponders functioned well outside the manufacturer's recommended range, down to ~5 °C. At rest, T_{sub} and rectal T_b (T_{rec}) were strongly correlated for both bat species (*Ng* $r^2 = 0.88$; *Sa* $r^2 = 0.95$) and this was also true for *N. gouldi* in steady-state torpor ($r^2 = 0.93$). During induced rewarming T_{sub} increased faster than T_{rec} in both species. Our results demonstrate that transponders can be used to provide accurate remote measurement of T_b in two species of bats during different physiological states, both during steady-state conditions and throughout dynamic phases such as rewarming from torpor. We show that, at least during rewarming, regional heterothermy common to larger hibernators and other hibernating bats is also present in bats capable of daily torpor.

1. Introduction

Small endothermic animals have large surface area to volume ratios and therefore heat loss over the body surface is substantial. Because of the size–heat loss relationship small endothermic species have to carefully balance energy supply and demands, and many species show pronounced daily and/or seasonal fluctuations of body temperature (T_b), especially at low ambient temperatures (T_a) to reduce the T_b–T_a differential and to minimize this heat loss and energy consumption (Geiser, 2004). Thus many endotherms are not strictly homeothermic but rather heterothermic and therefore are, from a thermal point of view, some of the most interesting.

Unfortunately, remote measurements of body temperature (T_b) in small heterothermic animals were not possible in the past because of the lack of suitable devices for such measurements. However, remote measurements of T_b in heterotherms are crucial for the provision of reliable data because study animals are easily disturbed (Speakman et al., 1991; Luo et al., 2014). Although implantable temperature sensitive devices have been used successfully in free-ranging heterothermic animals (for example; Dausmann, 2005; Bieber and Ruf, 2009; Rojas et al., 2014), they are often limited to animals >20 g and are particularly difficult to use in animals with limited body cavity space such as bats and birds. Therefore past investigations of T_b in undisturbed small animals <20 g have primarily been undertaken using external transmitters that measure skin temperature (T_{sk}). Although there is a correlation between T_{sk} and T_b, external transmitters are also affected by T_a and may not always provide precise measures of T_b; especially when the relationship between T_b and T_a changes, as is the case for heterothermic species during torpor (Barclay et al., 1996; Willis and Brigham, 2003). In addition, lightweight externally adhered transmitters have a limited battery life and are often shed by animals within a short period of time (from a few days to around 1 month) making long-term T_b measurements of small animals very difficult. The development of miniaturized, lightweight temperature-sensitive passive integrated transponders (PITs) enables investigators not only to minimize the stress associated with animal handling but record T_b continuously in unrestrained animals over a range of physiological conditions and prolonged time periods (Roark and Dorcas, 2000; Wacker et al., 2012; Langer and Fietz, 2014).

The majority of bat species are small with many weighing less than 10 g (Simmons and Conway, 2003) which is still considered by some to be prohibitively small for even the lightest available devices. Due to their small size and energetically expensive locomotion, many bats

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use torpor, primarily to minimize energy expenditure during rest, and it is highly likely that the majority of small bat species are capable of entering torpor in one form or another (Stawski et al., 2014b). Nevertheless, our understanding of the thermal biology of many of the smallest bat species remains extremely limited.

To date detailed information on the use of transponders has only been gathered for normothermic individuals or daily heterotherms during shallow torpor ($T_b > 10$ °C). The accuracy and reliability of transponders at T_b below 10 °C, as is often found in hibernators, has not been investigated in detail. Moreover, the use of transponders to measure differentials between core T_b and subcutaneous body temperature (T_{sub}) and their transient changes during torpor entry, steady-state torpor and arousal from torpor has not been undertaken—although such changes have been widely observed in heterothermic mammals (for review, see Lyman, 1982).

The aims of our study therefore were as follows: 1) to assess the accuracy and reliability of transponders at temperatures below 10 °C, 2) to quantify the relationship between core T_b and T_{sub} during normothermia, steady-state torpor and arousal from torpor in a hibernating long-eared bat (*Nyctophilus gouldi*) and 3) to compare these observations with a common blossom bat only capable of expressing shallow daily torpor (*Syconycteris australis*). Both species of bat inhabit the east coast of Australia (Churchill, 2008) and are capable of entering torpor throughout the year (Coburn and Geiser, 1998; Turbill, 2006). *N. gouldi* are insectivorous bats that weigh between 5.2 and 16.5 g, with a minimum rectal T_b during torpor of approximately 2 °C (Geiser and Brigham, 2000). *S. australis* are nectar feeding bats which weigh between 13.7 and 23.0 g with a minimum recorded core T_b around 17 °C (Geiser et al., 1996).

2. Methods

2.1. Study animals and PIT implantation

Eleven *N. gouldi* (*Ng*; 10.5 ± 1.4 g) individuals were captured in mist nets at local bushland surrounding the University of New England (UNE) or at Imbota Nature Reserve and Newholme Stations near Armidale, NSW, Australia (30°35′S, 151°44′E). Bats were transferred to UNE on the night of capture and were housed in large outdoor aviaries (3 m × 1.5 m × 2 m) fitted with hessian cloth for bats to roost and animals were provided mealworms and water *ad libitum*.

Four male *S. australis* (*Sa*; 18.7 ± 1.0 g) were trapped in mist nets at Iluka Nature Reserve on the north coast of NSW, Australia (29°24′S, 153°22′E). Bats were initially hand-fed to ensure they maintained body weight, but were also given a fruit and protein mixture *ad libitum* (for more detail regarding recipe, see Law, 1992). After transfer to UNE, bats were housed in a large indoor flight cage (2 m × 2 m × 2 m) equipped with branches and large stands of foliage for bats to roost in. The room was kept at T_a 20 ± 2 °C with relative humidity greater than 55%. Before implantation of transponders individuals were given a minimum of three days (up to 14 days) to ensure a stable weight was maintained and that animals had acclimatized to captivity.

Bats were anesthetized for PIT implantation with general isoflurane/oxygen anesthesia (0.5–4%). A small (~3 mm) incision was made in the skin between the shoulder blades for transponder insertion. The skin and transponder were sterilized with 70% ethanol prior to insertion. One or two sutures (4/0 chromic gut, Ethicon, Somerville, USA) were used to close the incision site. The entire process took <15 min. Following the minor surgery bats were placed in individual cages in a warm room (~24 °C) and given 48 h to recover before being returned to their respective holding cages.

This study was conducted under a scientific license provided by the NSW Parks and Wildlife Authority (SL100084) and with Animal Ethics approval from the University of New England (AEC11-016).

2.2. PIT calibrations

Temperature-sensitive PITs (IPTT-300, Bio Medic Data Systems, Delaware, USA) are small (14 mm × 2 mm) and lightweight (0.13 g). All transponders continued to function below the manufacturer's recommended range of use (32–43 °C) down to approximately 10.0 °C, and around 16% of 126 transponders continued to function at 5.0 °C. Forty transponders that continued to work below 10 °C were calibrated to the nearest 0.1 °C with a precision reference thermometer traceable to a national standard in a water bath at temperatures between 5.0 °C and 40.0 °C. Calibrations were taken at approximately 5.0 °C increments. To assess precision and thermal inertia of transponders, three readings were taken at 5 min intervals at each temperature. Drift over time has been shown to be minimal in these devices, with <0.5 °C change over several days (Wacker et al., 2012). Transponder signals were read with a DAS-7009S Handheld Reader (Bio Medic Data Systems, Delaware, USA). Transponders were selected for implantation into bats based on the functional temperature range, correlation coefficient, and intercept of the calibration equation.

2.3. Thermocouple calibration

To measure T_a and rectal T_b of bats a fine gauge (42 SWG) copper constantan thermocouple with digital thermometer (HH81A, OMEGA Engineering, Connecticut, USA) was used. The thermocouple and digital thermometer were calibrated in a water bath against a precision thermometer traceable to a national standard, following similar methods as for PIT calibrations above, and over the same temperature range.

2.4. Normothermia

PIT readings of T_{sub} were compared with rectal temperatures (T_{rec}) to assess accuracy of T_{sub} measurements and how this correlated to core T_b. T_{rec} as a measure of core T_b was taken using a calibrated thermocouple inserted rectally to a depth of 2 cm. For comparisons of resting T_{rec} to T_{sub} animals were placed in individual calico bags within a temperature-controlled cabinet at T_a between 5.0 and 20.0 °C (*N. gouldi*, $n = 7$) or between 12.0 and 30.0 °C (*S. australis*, $n = 4$). Bats were transferred from their holding cages to the cabinet during their active phase following sunset in the evening. A maximum of four bats were measured per night and animals were left undisturbed for at least 2 days between measurements. Animals were not disturbed for at least 45 min prior to initial measurement to ensure they were calm and had adjusted to the T_a. Following exposure to each T_a for at least 1 h, T_{rec} and T_{sub} were recorded within 30 s of one another, always in the same sequence (T_{sub} followed by T_{rec}), and animals were returned to holding cages before midnight each night of measurement.

2.5. Torpor

To assess the relationship between T_{rec} and T_{sub} during induced torpor nine *N. gouldi* and two *S. australis* were kept in individual calico bags in a temperature-controlled cabinet overnight at single constant T_a between 5.0 and 20.0 °C (*N. gouldi*) or 12.0 °C (*S. australis*) without access to food or water. Both species have been shown to enter torpor overnight or in the early morning in laboratory settings (Coburn and Geiser, 1998; Geiser and Brigham, 2000) and therefore measurements of T_{sub} and T_b were taken the following morning after lights on (natural photoperiod) to ensure animals were torpid. Again, recordings of T_{rec} and T_{sub} were taken within 30 s of each other in the sequence T_{sub} followed by T_{rec}. *S. australis* individuals did not enter torpor readily in calico bags and therefore, to demonstrate the relationship between T_{sub} and T_a, supplemental T_{sub} values presented here were taken from subsequent experiments where animals were placed in respirometry chambers at constant T_a between 12.0 and 18.0 °C (for detailed information, see Currie, 2015).