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Quantifying torpor in small mammals non-invasively using infrared thermocouples

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ABSTRACT

Measurements of torpor use are pivotal for many research areas concerning the thermal biology of endotherms. Here, I used infrared thermocouples to non-invasively examine torpor patterns in the small marsupial fat-tailed dunnart (*Sminthopsis crassicaudata*). Sensors were installed inside the nesting chambers to continuously monitor fur temperature in undisturbed animals. Firstly, to verify the measurements, fur temperature was monitored simultaneously with body temperature using internal radio transmitters ($n=6$). Secondly, I conducted a food restriction study to demonstrate the reliability of the method within a physiological experiment ($n=8$). Based on the correspondence of simultaneously measured fur and body temperature during torpor bouts, I was able to confirm that infrared thermocouples provide reliable temporal information on torpor patterns. Furthermore, torpor use was successfully monitored over a 20-day food restriction study. The method can easily be adapted to suit other small mammal or bird species and presents a useful, inexpensive approach for examining torpor patterns remotely and non-invasively in the laboratory.

1. Introduction

Torpor is the most effective energy saving tool available to endotherms, and is employed by both daily heterotherms and hibernators from 15 mammalian and avian orders (Geiser, 2004). Because of its widespread use and immense impact on energy needs and survival (Geiser and Turbill, 2009), the ability to quantify torpor is of interest for many research areas. The two most commonly used methods to monitor torpor patterns in the laboratory are measurements of body temperature (T_b) and metabolic rate (MR); both of which have notable restrictions. T_b is usually monitored with thermocouple probes (eg. Fons et al., 1997), or either attached radio transmitters or data loggers (e.g. Bozinovic et al., 2007; Warnecke et al., 2007; Levesque and Tattersall, 2010). Unfortunately, thermocouple probes do not provide continuous data, radio transmitters have a limited life span, and data loggers need to be retrieved from the animal to access recorded information. For MR measurements, open-flow respirometry requires transferral of the animal into a new environment (metabolic chamber), and it is usually restricted to a time period of <24 h (e.g. Geiser and Baudinette, 1987). The combination of MR and T_b measurements is also often used to monitor torpor patterns (e.g. Lovegrove et al., 1999; Elvert and Heldmaier, 2005). However, as disturbance is known to interrupt torpor or even prevent torpor use, the best data are likely to be obtained if handling, space restrictions, or exposure to new environments are minimised. Non-invasive methods have successfully quantified torpor use; for example, Hiebert (1991) trained hummingbirds to rest on a thermocouple wire, and Willis et al. (2005) placed data loggers in the bottom of nest boxes of small marsupials. However, these methods rely on a direct contact between the measuring device and the animal. In contrast, the use of infrared radiation enables measurements of surface temperature from a distance. Infrared thermography is a commonly used method to generate thermal images (e.g. Phillips and Heath, 1995, 2004; Kondo et al., 2006; McCafferty, 2007; Munn et al., 2009) but the cameras required are quite costly. Small hand-held infrared thermometers have been used to detect whether an animal is torpid (e.g. Heldmaier et al., 1999), but they only provide single readings. Here I tested the usefulness of infrared sensors with a thermocouple ending for continuous monitoring of torpor patterns with an automated data collection system.

The infrared thermocouples (IFT) were used to continuously monitor torpor patterns in a small mammal kept in its home cage over a period of three weeks. The IFT sensors were installed inside nesting chambers to measure fur temperature (T_{fur}) of the marsupial fat-tailed dunnart (*Sminthopsis crassicaudata*). This insectivorous dasyurid employs torpor in the wild (Morton, 1978;

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Warnecke et al., 2008), and in the laboratory in response to a restricted food supply (Holloway and Geiser, 1995; Munn et al., 2010). The usefulness of IFT was tested with two studies; firstly, IFT data were verified by simultaneous measurements of T_{fur} and T_b using internal radio transmitters; secondly, torpor use was monitored in food-restricted dunnarts over 20 days. This non-invasive method is inexpensive, does not rely on the direct contact between the animal and the measuring device, and it further allows unrestricted data collection and constant access to the information recorded.

2. Methods

Dunnarts (18.6 ± 1.8 g) were kept at the University of New England, Armidale, Australia, at an ambient temperature (T_a) of 19 ± 2 °C and a light regime of 12:12 LD (lights off 18:00). The two following studies were conducted: (1) Simultaneous measurements of T_{fur} and T_b using implanted radio transmitters (n=6, November 2007) and (2) a food restriction study (n=8, July 2007).

The IFT (OS36 IR tlc series, Omega) consisted of a sensor with a thermocouple plug (Fig. 1A). The sensor was inserted into the closed end of the nesting chamber (Fig. 1B and C) and the thermocouple ending was connected to an 8-channel data logger, collecting data every 6 min (resolution 0.25 °C). The nesting chamber was a 10 cm × 4 cm cardboard tube inside a slightly larger PVC tube (see Fig. 1C) that ensured a distance between the sensor and animal of < 4 cm. No nesting material was provided. For calibration IFT were placed 4 cm above a metal hot plate (after Baghai, 2003), which was heated to 22.0 °C and 28.0 °C using a water bath (measured with a hand-held infrared thermometer, SE-100 Sein Electronics). IFT recorded every minute for 10 min at each temperature, resulting in temperatures of 22.7 ± 0.1 °C and 27.7 ± 0.8 °C (n=8), respectively.

To monitor activity, passive infrared motion detectors (LA-5017 Jaycar Electronics) were mounted on the cage lids to sum animal movements over 6 min. This enabled verification of T_{fur} measurements from IFT readings as follows: all IFT readings taken while movement was detected were omitted, for they represented the empty nesting chamber. In turn, an absence of activity indicated that the animal was resting inside the nesting chamber; thus the readings represent T_{fur} because the animal was within 4 cm of the IFT sensor whenever it was inside the chamber (see above). However, it must be noted that the exact position of the animal was unknown as I did not monitor behaviour with video cameras.

During the food restriction study, dunnarts were fed daily at 17:30 ± 30 min with a mixture of canned cat food and water-soaked cat kibble. Prior to the start of the study, 100% food consumption was quantified for each individual over an *ad libitum* feeding period of 10 days. Dunnarts were then exposed to the following feeding regime: Day 1–4: 100%, Day 5–14: 50%, Day 15–16:75%, Day 19–20: 100% (expressed as percentage of individual food consumption). Water was freely available.

To measure T_b six dunnarts were equipped with internal temperature-sensitive AM radio transmitters (XM-FH Mini Mitter, Sunriver) as part of a study on thermal energetics (Warnecke and Geiser, 2010). Prior to implantation, transmitters (1.0 g) were waxed, calibrated in a water bath from 10° to 40 °C (to the nearest 0.1 °C) and implanted intraperitoneally under general anaesthesia (for details see Warnecke and Geiser, 2010). Antennas were attached beneath the cages and multiplexed to a receiver, and a custom-written data logging programme (written by T. Ruf and B. Lovegrove, modified by G. Körtner) recorded transmitter pulses every 6 min. T_{fur} and T_b were measured simultaneously for each animal over 2–8 days (mean count of data points: 227 ± 167; n=6). Food and water were provided *ad libitum*.

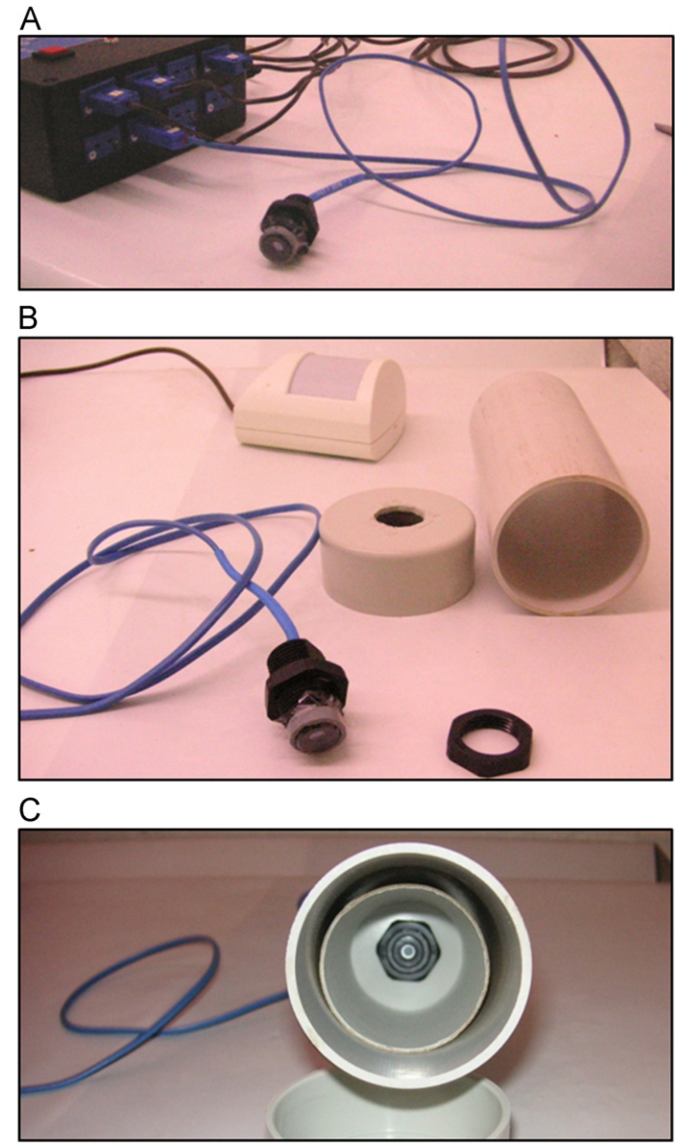

Fig. 1. The infrared thermocouple in different stages: (A) connected to the data logger; (B) with the dissembled nesting chamber and activity sensor; and (C) inserted into the closed end of the nesting chamber (see text for details).

The position of the animal in relation to the IFT sensor was not monitored, which means that T_{fur} potentially varied depending on the exact resting posture; therefore, I chose not to use a temperature threshold to define torpor bout duration. Instead, I analysed the characteristic shape of torpor bouts in daily T_{fur} traces after Willis et al. (2005), where torpor onset was measured at the start of the steep decrease in T_{fur}, and the completion of arousal at the time immediately before normothermic values were reached (see arrows in Fig. 3; Munn et al., 2010). I used *StatistiXL* 1.7 to test for differences in T_b, T_{fur} and T_a and differences in torpor bout duration (ANOVA).

3. Results and discussion

IFT provided reliable information on torpor occurrence in dunnarts, as verified by simultaneous measurements of T_b and T_{fur}. The method has many potential applications for various