Naked mole rats (NMRs; *Heterocephalus glaber*) are among the most hypoxia-tolerant mammals and are able to survive hours at 3% O$_2$, days at 8% O$_2$ and 18 min in anoxia in a laboratory setting [1–3]. Mammals rely on continuous oxygen delivery for aerobic energy production but oxygen availability is often limited by environmental factors, such as life in densely populated burrows. Matching metabolic demand to energy supply is the key to tolerating prolonged hypoxia and vertebrates have evolved adaptive strategies that contribute to this balance. These strategies can be grouped into two categories: (i) increasing oxygen delivery to tissues and (ii) reducing energy demand via metabolic rate depression [4]. This second mechanism is successfully employed by the most anoxia-tolerant vertebrates, which typically reduce body temperature ($T_b$) and enter into a coma-like state during seasonal periods of severe hypoxia or anoxia. Conversely, it is speculated that in the wild—given their deep nests and the large number of animals within the colony—that NMRs likely encounter chronic hypoxia throughout their lives. Therefore, it is likely that NMRs not only cannot escape this environment but also must perform their daily activities with reduced O$_2$ availability. Furthermore, NMR burrows have a stable ambient temperature ($T_a$) within a few degrees Celsius of their preferred $T_b$ [5,6], which offers minimal scope for thermoregulatory energy savings in hypoxia.

Despite these apparent restrictions on the use of behavioural and thermoregulatory strategies in response to hypoxia within their burrows, we hypothesized that NMRs would nonetheless use these strategies to the maximum
degree permitted by their ecophysiology. Previous reports of behavioural and thermal responses to extreme hypoxia in NMRs are mostly observational and these parameters have not been evaluated empirically. To address this knowledge gap, we held awake and freely behaving NMRs at their typical burrow $T_b$ (approx. 30.5°C), and exposed them to progressively deeper levels of hypoxia while tracking behavioural activity and $T_b$. In addition, we exposed NMRs to anoxia to determine how long they were able to maintain activity and whether they adjusted $T_b$ and metabolism in anoxia.

2. Abridged methodology

(a) Experimental design

NMRs were individually placed, unrestrained, into a custom-designed experimental chamber maintained at 30.3 ± 0.4°C (figure S1; for complete details regarding experimental methodology, see the electronic supplementary material). Behavioural parameters including movement speed (cm min\(^{-1}\)), cumulative duration of activity (s min\(^{-1}\)) and $T_b$ were monitored throughout experiments. The experimental chamber was sealed and constantly ventilated with air (21% O\(_2\), balance N\(_2\)) at a flow rate of 350–400 ml min\(^{-1}\). For control experiments ($n = 9$), NMRs were monitored for 6 h in normoxia. For hypoxic experiments ($n = 8$), NMRs were monitored for 1 h in normoxia followed by 1 h periods of progressive hypoxia (9, 7, 5 and 3% O\(_2\)), followed by a 1 h recovery period in normoxia. For anoxia experiments, NMRs were placed in a 200 ml chamber (figure S2), and gases were supplied at 500 ml min\(^{-1}\) to rapidly remove O\(_2\). Baseline activity, metabolic rate and $T_b$ measurements were collected and then the chamber was rapidly switched to anoxia by supplying pure N\(_2\). Behaviour was monitored until the animals stopped moving and appeared to lose consciousness, at which point $T_b$ was recorded and then the animal was removed from the chamber to recover.

3. Results and discussion

(a) Naked mole rats decrease physical activity and $T_b$

in acute hypoxia but remain active

NMR movement speed and total time active were unchanged through 6 h of normoxia (figure 1a,b, circles) and were not significantly affected by hypoxia ≥9% O\(_2\) (figure 1a,b, squares). Conversely, both variables decreased in 7, 5 and 3% O\(_2\). The maximum suppression of speed occurred in 3% O\(_2\) (approx. 86% reduction), whereas time active was lowest in 5% O\(_2\) (approx. 70% reduction). Activity levels returned to baseline upon reoxygenation. $T_b$ tended to decrease throughout control experiments but this trend did not reach significance (figure 1c). Conversely, relative to normoxia, $T_b$ was significantly lowered by approximately 1.5–2°C within the range of 9–5% O\(_2\), and then dropped 1.5°C further in 3% O\(_2\). $T_b$ recovered upon reoxygenation.

This behavioural response of NMRs to acute hypoxia is similar to findings from other hypoxia- and anoxia-tolerant species. For example, the anoxia-tolerant crucian carp decreases locomotor activity but nonetheless remains active in nearly anoxic environments [7]. Most mammals use behavioural means to escape hypoxia, or if hypoxia cannot be avoided, to move to cooler regions to take advantage of anapletic energy savings in hypoxia. Indeed, there is an inverse relationship between survival time in hypoxia and $T_b$ in most small mammals [8]. Moving to colder environments facilitates a downward shift in the thermal set point, which contributes to this metabolic benefit. Conversely, hypoxia-tolerant mammals typically enter into a coma-like state when exposed to acute hypoxia, and remain inactive until normoxia is restored [8]. However, NMRs putatively live in chronic hypoxia in an environment in which $T_a$ may fluctuate by as little as 1°C per year [5], and thus cannot readily escape hypoxia or move to cooler regions. Therefore, it is not surprising that NMRs exhibit a unique behavioural strategy in acute hypoxia, which is characterized by (i) a lower O\(_2\) threshold below which behaviour is effected by hypoxia, (ii) reduced activity in general, but (iii) maintenance of some activity and responsiveness to their environment.

Relative to their unique behavioural phenotype in hypoxia, the thermal response of NMRs to acute hypoxia is more consistent with that of other mammals, although the magnitude of this...

Figure 1. NMRs decrease activity and $T_b$ in hypoxia but remain awake and active. Summaries of NMR movement speed (a), time active (b) and $T_b$ (c) during normoxia ($n = 9$) or progressive hypoxia ($n = 8$). Data are mean ± s.e.m. Asterisks (*) indicate significant differences between normoxia and hypoxia ($p < 0.05$).
response is blunted by the limited thermal scope in their natural environment. For example, previous studies reported that mouse and hamster \( T_b \) decrease by 7°C and 3.5°C, respectively, when they are exposed to 5.5% \( O_2 \) [9]. The thermoregulatory pattern we observe during hypoxia is notable within the context of the ecophysiology of this species. As NMRs likely live in chronic hypoxia in nature, 21% \( O_2 \) in a laboratory setting presumably represents a hyperoxic environment, whereas 9% \( O_2 \) may approach their natural burrow atmosphere. It is, therefore, notable that \( T_b \) plateaus at a reduced level in moderate hypoxia (9–5% \( O_2 \)) but then drops further in severe hypoxia (3% \( O_2 \)). An intriguing finding of our study is that NMR \( T_b \) dropped below \( T_a \) in 3% \( O_2 \); however, the variability around the mean for this dataset overlaps with the measurement errors associated with the instruments used to measure \( T_b \) and \( T_a \) and this finding should be interpreted cautiously; it is likely that \( T_b \) drops to very near \( T_a \), but not beyond. Nonetheless, the rapid drop in \( T_b \) in hypoxic NMRs is clear and remarkable.

Another important observation of our study is that NMR \( T_b \) remains elevated in normoxia at cold temperatures. There is disagreement in the literature as to whether or not NMRs are able to thermoregulate in normoxia at temperatures below their thermoneutral zone (TNZ: 31–34°C [6]). Specifically, one study found that NMR \( T_b \) tracks within less than 1–2°C of \( T_a \) [6], whereas others have reported that NMRs can maintain \( T_b \) at levels significantly higher (\( T_b - T_a \leq 13.2°C \)) in \( T_a \) well below their TNZ [10,11]. Our results agree well with the earlier studies. However, differences in experimental approach between these studies shed light on the likely physiological mechanisms that facilitate rapid heat loss in NMRs in hypoxia. For example, in [11], the relative humidity was 100%, which would prevent evaporative water loss. Conversely, in [6], animals were exposed to a very high airflow rate, which would facilitate convective cooling, and 0% relative humidity, which would facilitate evaporative cooling (R. Buffenstein 2017, personal communication). In these experiments, evaporative water loss accounted for greater than 300% of metabolic heat production [6], which would explain the animals’ inability to maintain \( T_b > T_a \). Conversely, in our experiments, airflow through the chamber was slow but constant and the relative humidity level was approximately 50%. NMRs exposed to 3% \( O_2 \) spent considerable periods of time (approx. 85%) lying on their backs, maximizing the exposure of abdominal skin surface to ambient air. When in this position, abdominal skin appeared very pink, indicating a high degree of blood flow, likely to facilitate heat transfer. Taken together, these observations from our laboratory and others suggest that NMRs may maximize peripheral circulation and use evaporative cooling, along with reduced behavioural activity, to rapidly reduce \( T_b \) in hypoxia.

(b) Naked mole rats maintain activity in acute anoxia and recover fully following reoxygenation

NMRs remained active in anoxia for approximately 100 s in both experimental temperatures before locomotor activity ceased (figure 2a). Animals recovered in normoxia and regained consciousness and mobility within 4–6 min of reoxygenation (figure 2b). Few animals can survive an anoxic challenge, and mammals, in particular, fare poorly under these conditions. For example, mice remain active for 26 s in anoxia at room temperature and do not recover [12]. In light of this, the ability of NMRs to maintain activity and consciousness in anoxia for up to 100 s is remarkable, as is the fact that animals recovered within a few minutes. Metabolic rate in anoxia decreased by approximately 75% in just 90 s (figure 2c). \( T_b \) also dropped rapidly in anoxia, decreasing by 2 and 4°C in \( T_a \) of 30°C and 22°C, respectively (figure 2d).

A previous study did not report any change of NMR \( T_b \) in response to anoxia [3]. Although it is not specified in that paper, rectal \( T_b \) measurements were presumably taken after the animals had lost consciousness, as taking rectal temperature from active NMRs in a sealed jar is not a trivial task. In our experiments, NMR \( T_b \) dropped within 90 s of the onset of anoxia and had decreased by the time animals lost consciousness. Therefore, it is likely that these authors missed the anoxia-related decrease in \( T_b \) due to their experimental design. In support of this interpretation, our \( T_b \) measurements from NMRs that had lost consciousness in anoxia agree well with the \( T_b \) measurements in [3]. Interestingly, in our experiments, all animals were observed urinating on themselves with the onset of anoxia, which is a strategy that supports very rapid evaporative cooling in ectotherms [13], and likely
contributed to the rapid heat loss in anoxic NMRs, along with higher rates of air flow in the chamber.

4. Conclusion
Taken together, our results indicate that NMRs use behavioural and thermoregulatory strategies that are consistent with reduced metabolic rate in acute hypoxia or anoxia. The degree to which these strategies are employed by NMRs is limited by their warm and constantly hypoxic burrow environment and appears to correlate with the severity of the hypoxic stress, such that at more extreme levels of hypoxia, NMRs enter into a coma-like state and cease all activity.

References