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Physiology

Behavioural responses of naked mole rats to acute hypoxia and anoxia

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Naked mole rats (NMRs) are among the most hypoxia-tolerant mammals. Other species respond to hypoxia by either escaping the hypoxic environment or drastically decreasing behavioural activity and body temperature (T_b) to conserve energy. However, NMRs rarely leave their underground burrows, which are putatively hypoxic and thermally stable near the NMRs' preferred T_b. Therefore, we asked whether NMRs are able to employ behavioural and thermoregulatory strategies in response to hypoxia despite their need to remain active and the minimal thermal scope in their burrows. We exposed NMRs to progressively deeper levels of hypoxia (from 21 to 0% O_2) while measuring their behaviour and T_b. Behavioural activity decreased 40–60% in hypoxia and T_b decreased slightly in moderate hypoxia (5–9%) and then further with deeper hypoxia (3% O_2). However, even at 3% O_2 NMRs remained somewhat active and warm, and continued to explore their environment. Remarkably, NMRs were active for greater than 90 s in acute anoxia and T_b and metabolic rate decreased rapidly. We conclude that NMRs are adapted to remain awake and functional even at the extremes of their hypoxia-tolerance. This adaptation likely reflects variable and challenging levels of environmental hypoxia in the natural habitat of this species.

1. Introduction

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_a (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 1*a*,*b*, circles) and were not significantly affected by hypoxia ≥9% O_2 (figure 1*a*,*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 1*c*). 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 anapyretic

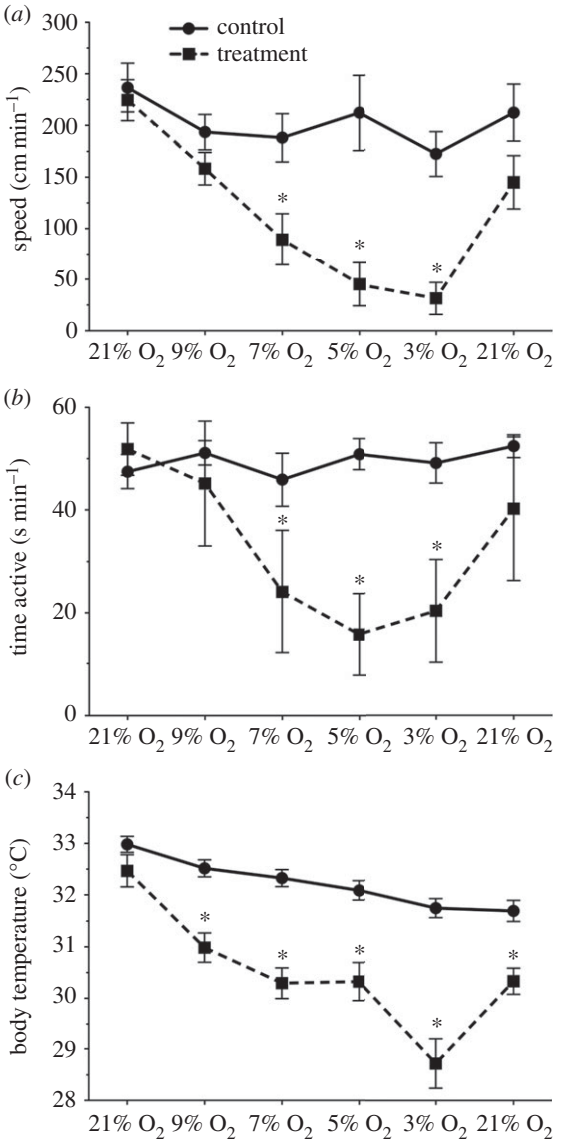

Figure 1. NMRs decrease activity and T_b in hypoxia but remain awake and active. Summaries of NMR movement speed (*a*), time active (*b*) and T_b (*c*) during normoxia ($n = 9$) or progressive hypoxia ($n = 8$). Data are mean ± s.e.m. Asterisks (*) indicate significant differences between normoxia and hypoxia ($p < 0.05$).

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

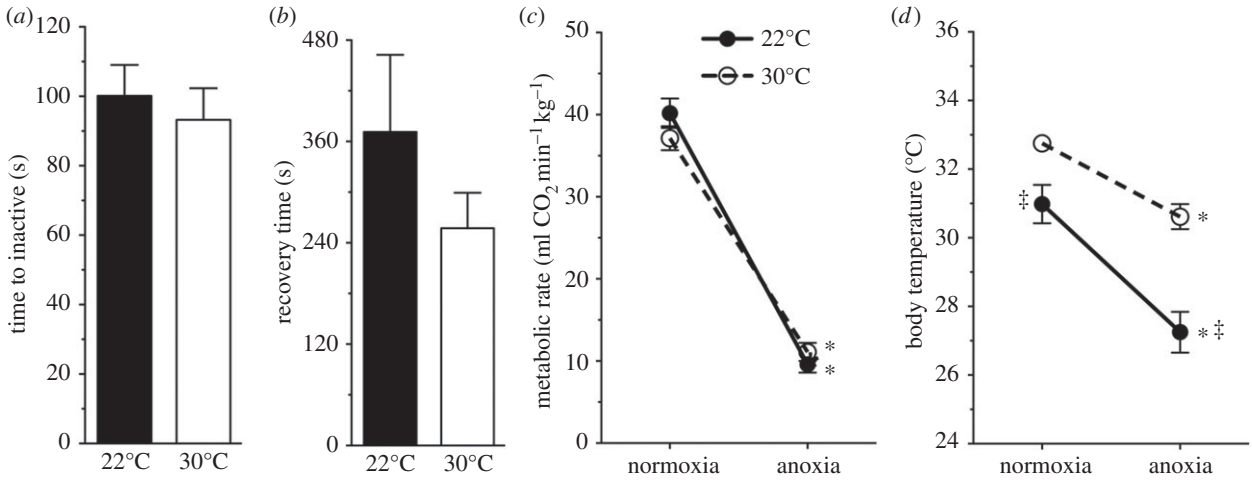

Figure 2. NMRs tolerate short-term anoxia. (*a*,*b*) Summaries of time active in anoxia until cessation of movement (*a*), time elapsed in recovery until restoration of movement (*b*), metabolic rate (*c*) and T_b (*d*). Data are mean ± s.e.m., $n = 7$ per group. Asterisks (*) indicate significant differences between normoxia and anoxia, double daggers indicate significant differences between 22 and 30°C ($p < 0.05$).

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_as 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 2*a*). Animals recovered in normoxia and regained consciousness and mobility within 4–6 min of reoxygenation (figure 2*b*). 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 2*c*). T_b also dropped rapidly in anoxia, decreasing by 2 and 4°C in T_as of 30°C and 22°C, respectively (figure 2*d*).

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.

Ethics. All procedures were conducted in accordance with the relative animal care and experimentation guidelines of the Canadian Council on Animal Care and were approved by the University of Ottawa Animal Care Committee (protocol no. 2535).

Data accessibility. Supporting data have been uploaded to Dryad and can be accessed at http://dx.doi.org/10.5061/dryad.11m62 [14].

Authors' contributions. M.E.P. conceived of and designed the study and wrote the manuscript. A.N.I. and A.M.K. performed the experiments, analysed all data and edited the manuscript. All authors gave final approval of the published version and agree to be accountable for all content therein.

Competing interests. We have no competing interests.

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