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Divergent behavioural responses to acute hypoxia between individuals and groups of naked mole rats

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ABSTRACT

Most small rodents reduce energy demand in hypoxia via behavioural strategies. For example, animals may reduce their activity, and/or move to colder environments or alter huddling strategies to take advantage of anapyretical energy savings. Naked mole rats (NMRs) are among the most hypoxia tolerant mammals and are highly social; social interactions also have a significant impact on behaviour. Therefore, this species offers a fascinating model in which to study trade-offs between social interactions and energy conservation in hypoxia. We hypothesized that the need to conserve energy in hypoxia supersedes the impetus of sociality in this species and predicted that, in hypoxia, behaviour would not differ between individuals or groups of NMRs. To test this hypothesis, we placed awake, freely behaving NMRs, alone or in groups of 2 or 4, into a temperature-controlled apparatus and measured behavioural activity during 1 h each of normoxia (21% O_2), acute hypoxia (7% O_2), and normoxic recovery. We found that in normoxia, groups of 4 NMRs were significantly more active in all temperatures than were groups of 1–2 NMRs. When exposed to hypoxia, individual NMRs were ~50% less active and their speed was reduced relative to normoxic levels. Conversely, groups of 2 or 4 NMRs exhibited minor or insignificant decreases in time spent active and speed in hypoxia and huddling behaviour was not altered. Our findings suggest that social interactions influence behavioural strategies employed by NMRs in hypoxia.

1. Introduction

Naked mole rats (NMRs, *Heterocephalus glaber*) are small (30–60 g), highly social rodents that live in colonies ranging from 50 to 300 individuals (Brett 1991). Colonies of NMRs are found in underground burrows comprised of complex tunnel systems that can span for several kilometers (Brett 1991). A recent study has demonstrated that NMR burrow systems are not particularly hypoxic (Holtze et al. 2017); however, it is speculated that, given the large number of animals within the colony, NMRs likely encounter hypoxia due to respiratory consumption of environmental O_2 in certain confined burrow spaces, such as in their nest chambers. In order to cope with these putatively hypoxic conditions, NMRs have developed a remarkable suite of physiological and behavioural adaptations that enable them to tolerate O_2 levels as low as 0% for 18 min, 3% for hours, or 8% for days to weeks in a laboratory setting (Chung et al. 2016; Pamenter 2014; Park et al. 2017). For example, their metabolic rate and ventilation are markedly reduced in acute hypoxia (Chung et al. 2016; Pamenter et al. 2015), they use alternative fuel sources in anoxia (Park et al. 2017), and, in individual animals, body temperature (T_b) and physical activity are reduced (Ilacqua et al. 2017; Kirby et al. 2018). Together, these adaptations allow NMRs to maintain some degree of activity even within very low O_2 conditions in the laboratory – and presumably in nature - by maintaining a balance between reducing O_2 consumption in hypoxia while still meeting the metabolic requirements of remaining active, albeit to a significantly reduced degree, when O_2 is limited.

In hypoxia, most small rodents reduce physical activity, seek colder environments, and reduce huddling behaviour in order to reduce T_b (i.e. anapryexia (Steiner et al. 2002)), and conserve energy (Mortola and Feher 1998; Tattersall and Milsom 2003). Consistent with the first point, we have previously demonstrated that individual NMRs decrease their time spent active and speed in acute hypoxia (7% O_2 for 1 h at 30 °C) and exhibit a mild but significant decrease in T_b (Kirby et al. 2018). However, individual NMRs do not employ the hypoxic

Abbreviations: NMR, naked mole rat; T_a, ambient temperature; T_b, body temperature; TNZ, thermoneutral zone

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behavioral anapyrexic strategy of seeking colder areas if given the option to do so during hypoxia (Kirby et al. 2018), likely because of the high metabolic cost of staying warm in colder environments with no fur and minimal insulation (Kirby et al. 2018; Tucker 1981; Withers and Jarvis 1980). Although these findings in individual animals are informative, NMRs are a highly social species and are rarely isolated in their burrows. Indeed, NMRs spend the vast majority of their time in close proximity with fellow colony members, engaged in teamwork to maintain the burrow or defend the colony from intruders, or piled together in large group huddles while resting or caring for offspring within the colony nest chamber. Therefore, in this species the relationship between huddling behaviour and thermoregulation, in both normoxia and hypoxia, is likely confounded by an unusually high degree of social interaction within the highly dynamic and interactive colony structure: animals likely huddle in large groups not only as a thermoregulatory strategy, but also as a central component of their colony social behaviour.

Therefore, we asked 1) what is the effect of hypoxia on group behaviour, 2) how does exposure to hypoxia in groups influence NMR behavioural responses to hypoxia relative to exposure in isolation, and 3) how does environmental temperature influence behavioural responses to hypoxia alone or in groups? We hypothesized that the imperative to conserve energy in hypoxia, either by reducing activity and/or by altering huddling behaviour, would supersede social interaction in groups of NMRs. We predicted that, similar to individual animals, pairs and groups of NMRs would reduce overall activity in acute hypoxia. Furthermore, given that metabolic demand is decreased in huddling NMRs (Withers and Jarvis 1980), and that decreasing metabolic rate is key to tolerating prolonged hypoxia in hypoxia-tolerant species (Buck and Pamenter 2006), we predicted that, unlike in other small mammals, huddling behaviour would increase during hypoxia. Finally, given the higher metabolic activity of both normoxic and hypoxic NMRs in the cold (Kirby et al. 2018), we predicted that the incidence of huddling behaviour would increase with decreasing environmental temperature in both normoxia and hypoxia.

2. Materials and methods

2.1. Animals

Forty-six subordinate adult male and female NMRs, weighing 47.4 ± 1.2 g were used in our experiments. NMR behaviour is primarily determined by the social status of animals within their colony and particularly their reproductive status as a breeder or non-breeder (Mooney et al. 2014b). Sex-based differences in behaviour have not been reported. Therefore, we assumed that both sexes would behave similarly and, thus, did not attempt to evaluate the impact of sex on behaviour. Animals were group-housed in established colonies in commercial plastic rodent caging connected by tubing to simulate their natural underground burrow environment. Animals were fed a diet of fresh fruit, vegetables and Pronutro cereal supplements ad libitum, and were housed at the University of Ottawa at 30 °C and in 70% humidity. The animal care protocol (#2535) was in compliance with the requirements proposed by the Canadian Council on Animal Care and was approved by the University of Ottawa Animal Care Committee.

2.2. Experiment design

Animals (in groups of 1, 2, or 4) were placed in the experimental apparatus, which consisted of two circular chambers (25 cm diameter, 8 cm height) connected by a tunnel (15 cm long, 5 cm diameter). This apparatus is similar to the cage and tunnel system used to house the animals in our facility. The experimental chambers were placed on top of two Peltier plates, which provided a constant ambient temperature (T_a) within the experimental apparatus. The uniformity of the T_a within the experimental apparatus was evaluated using iButtons placed throughout the two experimental chambers and connecting tunnel. There were no significant differences in T_a throughout the chamber (data not shown). Naked mole rats were divided into three different temperature treatment groups, with chambers set at 20 °C, 30 °C, and 38 °C. The lower temperature was chosen to facilitate comparisons to previous experiments conducted at room temperature in NMRs and other rodents. The upper ambient temperature was chosen based on an ethical maximum for this species (Mcnab 1966), and falls well within the range of T_as recorded from NMR burrows in the wild (24.6–48.8 C; Holtze et al. 2017). Animals were allowed to habituate within the experimental chamber and at their experimental exposure temperature for a minimum of 1 h prior to experimentation. Animals did not exhibit behavioral signs of stress or atypical behaviors in any of the experimental temperatures.

At the commencement of each experiment, the chambers were sealed and perfused with 21% O_2 balanced with N_2 (normoxia) at a rate of approximately 500 ml/min. In pilot experiments we confirmed that inflowing and outflowing O_2 content differed by < 0.1% O_2 in all experimental conditions, when flow rate was set to 500 ml/min (data not shown). Thus, this flow rate was sufficient to eliminate any variability in environmental O_2 availability due to group-mediated respiratory depletion of chamber O_2 between groups of 1, 2 or 4 animals. After 60 mins, the chambers were perfused with 7% O_2 for 60 mins. Following hypoxic exposure, the chambers were flushed with air to return O_2 levels to 21% O_2, the recovery period. 1 h was chosen as the exposure duration based on pilot studies indicating that < 30 min is sufficient for NMRs to reach a steady state metabolic rate in hypoxia (data not shown), therefore providing a 30 min window of steady-state in hypoxia in which to evaluate physiological responses to this challenge. Each individual was exposed to 1 h of normoxia, hypoxia, and recovery, at either 20 °C, 30 °C, or 38 °C, alone or in groups of 2 or 4. During the transition between normoxia to hypoxia and from hypoxia to normoxia, gas flow was increased to 1 L/min for 10 min to rapidly bring the O_2 content of the experimental chamber to the desired level (< 10 min). Gases were mixed using calibrated rotameters (The Linde Group, Ottawa, Canada), and in all trials the inflowing air was passed through a bubbler to achieve ~50% humidity. Outflowing air from the bubbler was then passing through a flow analyzer (Q-G266 Flow Monitor, Qubit Systems, Kingston, ON, Canada) prior to entering the experimental chamber. Gas flowing out of the chamber was passed through a desiccant column and then to an O_2 analyzer (Q-S102; Qubit Systems) to measure the O_2 content of gas entering the animal chamber. The O_2 analyzer was calibrated before each experimental trial using 100% N_2 and compressed air (21% O_2, balance N_2). Flow rate (ml/min) and % O_2 were tracked over the duration of the 3 h trials using Logger Pro software (Vernier Software & Technology, Portland, Oregon, USA).

2.3. Data collection

Animal activity was monitored with an overhead camera (Basler AG, Ahrensburg, Germany) and behavioural variables were tracked using activity detection software (Ethovision XT; Noldus Information Technology, Wageningen, Netherlands). The behavioural variables tracked for all animal trials were distance moved and speed. For the multiple animal trials, distance between subjects was also be tracked. Behaviour was recorded at a rate of 25 frames/s and an animal was considered active if > 2% of the pixels recording the animal indicated movement. Behavioural recordings were also manually reviewed to confirm that the active/inactive indicator accurately determined the activity state of the recorded animals.

2.4. Statistical analysis

Average values were calculated for each dependent variable in 10 min bins and statistical analysis was conducted on the final 10 min of