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Original scientific article

Housing behaviour of the naked mole rat (*Heterocephalus glaber*) under laboratory conditions

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Summary

The naked mole rat (*Heterocephalus glaber*) is a rodent that has gained importance as a biomedical research model for various conditions including hypoxic brain injury, cancer and nociception. It is captured from the wild and housed under laboratory conditions during research. Much is unknown about how to optimize housing conditions for the animals in captivity. This study was designed to establish whether the animals will replicate in the laboratory their natural behaviour of having separate resting, waste disposal and eating areas. A total of 52 naked mole rats were kept in four colonies of different sizes and housed in two types of cage design. It was found that, in all four colonies, their behavior was similar to that in the wild with regards to separating their resting, eating, defecation and urination areas. Urination and defecation commonly occurred in the outer corners of the cages while resting and eating mostly occurred in the inner parts of the cages. Average daily feed consumption was 7.6 grams per naked mole rat. Weekly weight gain averaged 0.44 grams per naked mole rat. In this study, the four colonies of naked mole rats behaved similarly in their selection of resting, waste disposal and eating area. However, additional studies are needed to investigate further whether these behaviours can be affected by colony origin, colony size or cage size. The results of our study indicate that resting, eating and waste disposal behaviours need to be taken into consideration when housing naked mole rats, to optimize the comfort of these animals in captivity.

Introduction

The naked mole rat (*Heterocephalus glaber*) is a subterranean rodent belonging to the family Bathyergidae. They are found in semi-arid regions of East Africa, mainly in Somalia, Kenya and Ethiopia. They are eusocial (Schumacher et al. 2015; Jarvis 1981), living in colonies of up to 300 animals of overlapping generations who collectively care for the young and, in addition, show division of labour (Jarvis 1981; Susan et al. 2012).

Naked mole rats are gaining in importance for biomedical research, being used as animal models for studies of neurodegenerative diseases, aging, cancer, nociception, hypoxia and bioprospecting. They are hypoxia-tolerant at the neuronal level, insensitive to acid-induced pain and acidic fumes, are cancer resistant and long lived. These characteristics make naked mole rats unique compared to laboratory mice and rats (Clarke and Faulkes 1998; Kim et al. 2011; Schumacher et al. 2015; Abiyselassie 2018). Naked mole rats housed in laboratory conditions are shown in Figure 1, and their biological characteristics and some environmental requirements are listed in Table 1.

Figure 1. Naked mole rats housed at the South Eastern Kenya University.

Despite the use of naked mole rats in laboratory experiments, there is still a lack of knowledge about optimal housing methods in captivity. To create a good laboratory environment for this species, it is necessary to study their behaviour under laboratory conditions in relation to their behaviour in the wild. This will allow optimization of their housing conditions, to assure well-being and correct handling of this species, as well as the quality of the research.

Studies indicate that, in the wild, naked mole rats have separate areas in their tunnels for resting, eating, urination and defecation (Jarvis and Sherman 2002). This study was designed to find whether these animals would replicate this behaviour under laboratory conditions and if the size of the colony or cage affects this behaviour.

Materials and Methods

Ethical statement

The experiments were conducted after licensing (KWS/BRM/5001), and obtaining a permit (KWS/904) to capture naked mole rats, by the Kenya Wildlife Service (KWS) which licenses all research on wildlife in Kenya. The study also adhered to the prevention of cruelty to animals act, chapter 360, laws of Kenya (2012), and directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes (European Union 2010).

Table 1. Biology and environmental requirements of naked mole rats.

Characteristic	Description	References
Behaviour	Eusocial rodent that lives in colonies of up to 300 animals of mixed generations	Kress et al. 2017; Schuhmacher et al. 2015
Breeding	Colony composed of a single breeding female, one to three breeding males and other hormonally suppressed colony members	Clarke and Faulkes 1998; Kim et al. 2011
Gestation	66-74 days	Abiyselassie 2018
Litter size	Mean litter size of 12 pups	Buffenstein 2005
Body	Cylindrical body measuring 8-10 cm long and a tail 3-5cm long	Jarvis and Sherman 2002; Abiyselassie 2018
Adult weight	30-50 grams	Jarvis and Sherman 2002
Body temperature	32°C and poikilothermic	Schuhmacher et al. 2015
Longevity	Up to 32 years	Kim et al. 2011
Diet	Roots and tubers	Abiyselassie 2018
Drinking	They solely obtain water requirements from succulent food they consume	Jarvis and Sherman 2002
Habitat	Subterranean in underground tunnels that extend up to 3 kilometres depending on food availability and colony size	Schuhmacher et al. 2015
Tunnel humidity	Up to 90%	Schuhmacher et al. 2015
Tunnel temperature	28-32°C	Abiyselassie 2018

Figure 2. Mole hills at Ngaindeithia village, Makueni County, Kenya. The mole hills are created by naked mole rats pushing soil onto the surface while digging tunnels underground.

Animals, housing and experimental design

The experiment was undertaken at the South Eastern Kenya University.

A total of 52 naked mole rats were used in the study. They were captured from their natural burrows in Makueni County, Kenya, while they were digging fresh mole hills (see Figure 2). Animals weighing above 18 grams were chosen for the experiment and smaller individuals were immediately returned to their burrows.

Four colonies were established for the study. The study colonies were named according to their site of origin: Ngaindeithia A, Ngaindeithia B, Kasaini and Darajani. Each colony was housed separately in one cage which was also an experimental unit. Each colony was composed of naked mole rats of both sexes in different proportions. Body weights at the beginning of the experiment ranged between 18-41 grams, but the ages of the animals were unknown. All animals were marked for identification by drawing numbers on their backs using a marker pen.

Two cage sizes were built to study the effect of cage size and the colony density on behaviour: Type 1 with 120 cm length x 40 cm width x 30 cm height (Figures 3 and 6A) and Type 2 with 70 cm length x 50 cm width x 20 cm height (Figures 4 and 6B). Cages were made of 3 mm diameter clear plastic acyclic glass (perspex) and they were covered with pieces of plywood with holes to allow for ventilation.

The cages were divided by partitions into three compartments to simulate the natural habitat and the tunnel structures of the naked mole rats, giving them opportunity to use separate areas for different behaviours. The partitions are shown in Figures

Table 2. Colony housing details

Colony	Ngaindeithia A	Darajani	Kasaini	Ngaindeithia B
Animal number in colony	20	15	10	7
Cage type	1 (4800 cm^2)	1 (4800 cm^2)	2 (3500 cm^2)	2 (3500 cm^2)
Space/animal	240 cm^2	320 cm^2	350 cm^2	500 cm^2
Total weight of the colony at the beginning	652.6 g	492.7 g	324.2 g	219.8 g
The mean weights of animal at the beginning	32.63 ± 4,4 g	32.85 ± 5,9 g	32.42 ± 6,0 g	31.40 ± 5,7 g

Figure 3. Cage Type 1: 120 cm length x 40 cm width x 30 cm height, the floor area was 4800 cm^2. The cage was divided into three compartments.

3 and 4 and indicated by lines in Figures 6A and 6B. The partitions and outer sides of the cages were opaque during the experiments; the cage in Figure 4 was covered on the outer side with opaque material during experimentation period. This was to mimic an underground burrow with solid earthen walls.

The four colonies had a different number of naked mole rats. Colonies Ngaindeithia A (n=20) and Darajani (n=15) were housed in Cage Type 1 and Kasaini (n=10) and Ngaindeithia B (n=7) in Cage Type 2. The floor area per individual animal and the total weight of the colony at the beginning of the experiment are shown in Table 2.

The cage bedding consisted of wood shavings of fine texture made from local trees; was not pre-treated with any chemicals and was changed weekly. The cage bedding was about 2.5 cm deep. No additional materials were provided in the cage.

Temperature in the animal room was maintained at 28-31°C, to simulate the temperature in the animals' natural burrows. Humidity was maintained at 50-70% to prevent drying and scaling of the mole rat skin. Both temperature and humidity in the room were measured by a thermo hygrometer (Brannan, England). Room temperature was maintained by the use of two 250 watt infrared lamps (Euro-matt) and a fan heater (1500 watts, Intertronic, UK). By blowing hot air, the fan heater assisted evaporation of water from plastic basins, thus raising humidity levels.

The light-dark cycle in the animal room was 12/12, with lights on from 06.00 to 18.00 hours. Ventilation of the cages was achieved by covering them with plywood that had 3 holes each of 2 cm diameter (each aligned per cage compartment) to allow circulation of air. The plywood cover was also not closely fitting. The animal room with cages and other equipment is shown in the Figure 5.

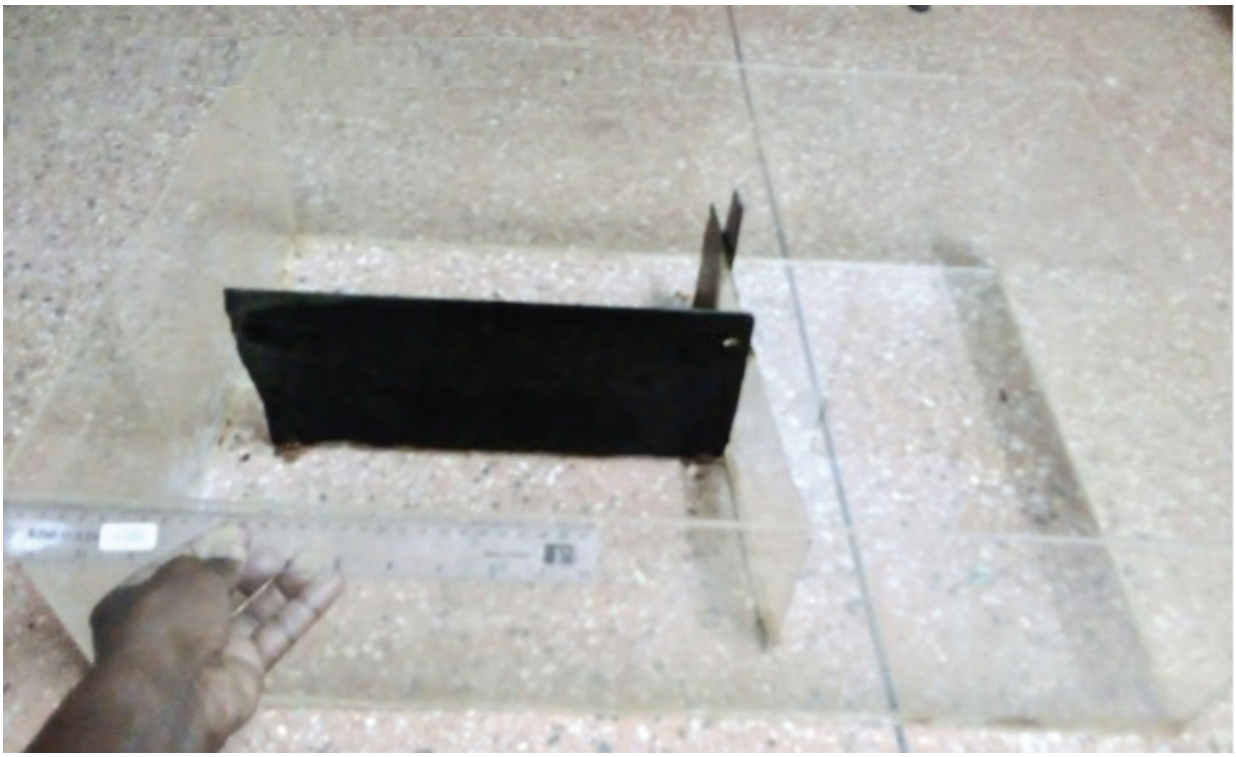


Figure 4. N Cage Type 2: 70 cm length x 50 cm width x 20 cm height, the floor area was 3500 cm^2. The cage was divided into three compartments.

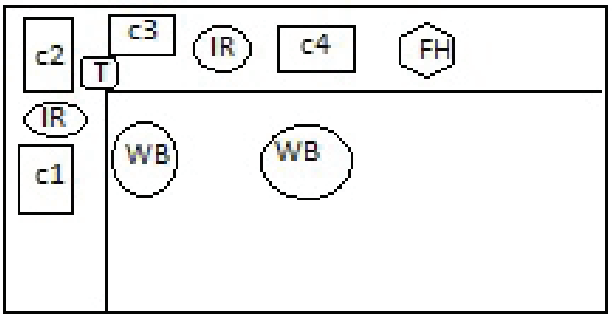


Figure 5. The animal room showing placement of various items: the four naked mole rat colonies (c1-c4), infrared lamps (IR), thermo hygrometer (T), fan heater (FH), water basins (WB).

Feeding and food consumption

Animals were provided with food chopped into pieces of about 1 cm, which made it necessary for the animals to spend time chewing the food. Enough food was provided to allow *ad libitum* consumption such that there was always uneaten food remaining the following day. Since each cage had three compartments, food was placed in the middle of each compartment sequentially during the experimental period. The diet consisted of fresh carrots, sweet potatoes and Irish potatoes. There was no pre-treatment of the food except for washing in clean water. Animals were fed daily at around 09.00 am after measuring food remaining from the previous day and observing their behaviour. No water was provided since the animals obtain their water requirements from their succulent diet (Jarvis and Sherman 2002).

To calculate the food consumed in each cage, the remaining food was subtracted from the food provided, taking a factor for environmental moisture loss into consideration, i.e. the food consumed was calculated as amount fed - remaining food - environmental moisture loss. Environmental moisture loss was averaged at 22.2% and was calculated by leaving a known amount of food undisturbed in the same room with naked mole rats and weighing it again after 24 hours. The loss in weight was attributed to moisture loss to the environment.

Food consumption was investigated for 28 days.

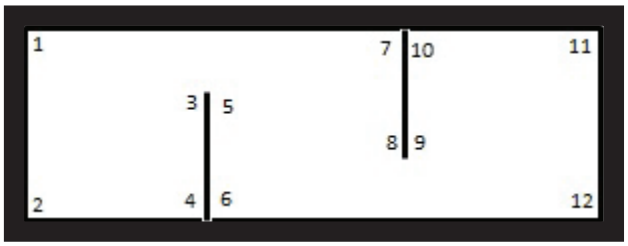
Behavioural observations and weighing of the animals

The animals were observed daily between 08.30 and 09.00 am by a single observer, before providing fresh food. Sites for resting, eating, urination and defecation in each cage (Figures 6A and 6B) were recorded. A resting site was defined as an area where naked mole rats were observed huddling together, an eating site was the area where food remains were observed, while urination and defection sites were the areas where wet bedding and feces were observed. Behavioural observations were made for 20 days.

All the naked mole rats in each colony were weighed weekly for four weeks. Their individual weights were summed to give the colony weight. Weekly weight gain per colony was calculated as (. Colony weight gain divided by number of naked mole rats in the colony gave the weight gain per naked mole rat.

After the experiments, the mole rats were maintained in the facility for use in future experiments.

A



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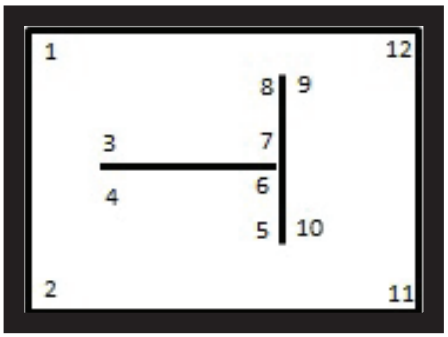

Figure 6. Cage type 1 (A) and cage type 2 (B) seen from aerial view. The lines inside both cage types are partitions dividing the cages into three compartments. Numbers 1-12 in each cage show the points where behavioural observations were made, i.e. urination, defecation, eating and resting behaviours. The points were classified as either outer corners (points 1, 2, 11 and 12) or inner areas (points 3-10).

Statistical analysis

Food consumption data were analysed using one way ANOVA with Tukey's multiple comparison tests. Weight gain among the four colonies was analysed using one way ANOVA tests while the t-test was used to compare weight gain between large and small colonies. Both ANOVA and t-test were carried out using Graph Pad Prism 5.0. Chi-square test was used to analyse behavioural observations.

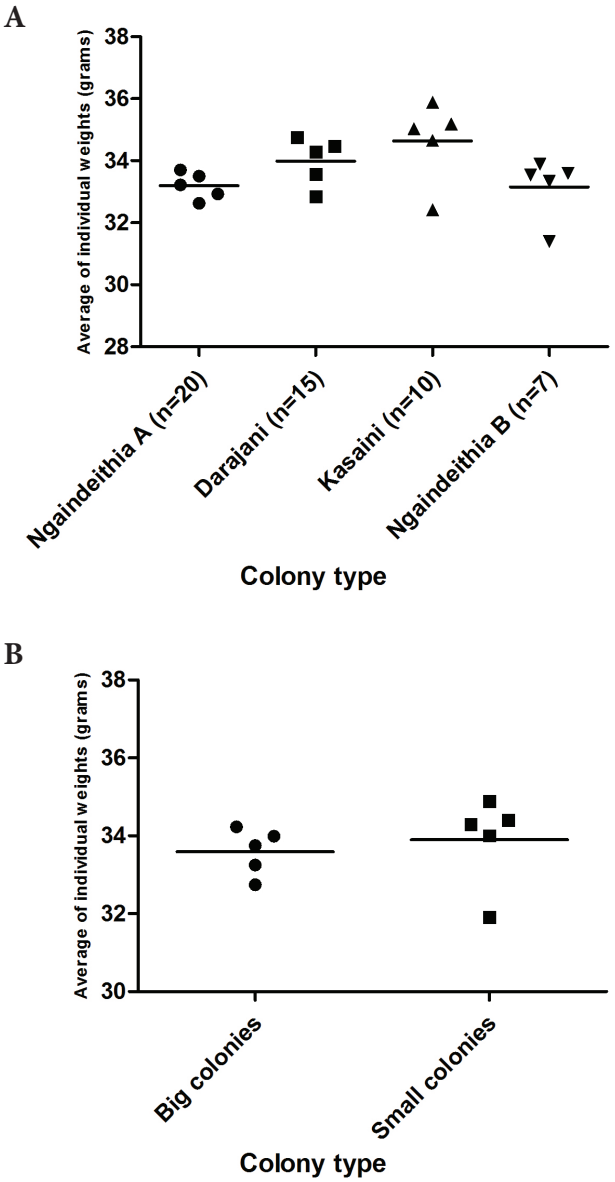

Figure 7. Scatter plots at each colony represents means of individual animal weight at days 1, 7, 14, 21 and 28. Horizontal bars on the scatter plots represent overall mean of the individual weights for the entire experimental period. Figure 7A shows scatter plots of mean individual weights for the four colonies. Figure 7B shows scatter plots of mean individual weight for the colonies grouped into either big colonies i.e. Darajani (n=15) and Ngaindeithia A (n=20) or small colonies i.e. Kasaini (n=10) and Ngaindeithia B (n=7).

Results

Naked mole rat weights

Weekly weight gain averaged 0.44 g per animal. The iincrease in total animal weight per colony from the beginning to the end of the experiment was 34.7 g (Kasaini), 28.5 g (Darajani), 21.4 g (Ngaindeithia A) and 17.4 g (Ngaindeithia B). Kasaini colony had the highest (median and mean) weight gain while Ngaindeithia A colony had the lowest (median and mean) weight gain as well as the smallest variation in weights (Figure 7A). However, there was no significant difference in weight gain between the four colonies and there was no significant difference in weight gain between small and large colonies (Figure 7B).

Food consumption

The average daily food consumption per naked mole rat was 7.6 grams after factoring environmental moisture losses of 22.2% per day (Ngaindeithia A=6.4 grams, Darajani=7.2 grams, Kasaini=7.8 grams and Ngaindeithia B=9.1 grams). Environmental moisture loss was associated with the animal room temperatures kept above 28°C. Weekly food consumption per individual naked mole rat in Ngaindeithia B colony exceeded that in other colonies. Large colonies (Ngaindeithia A and Darajani) consumed significantly less ($P < 0.05$) food per week compared to Ngaindeithia B colony (Figure 8).

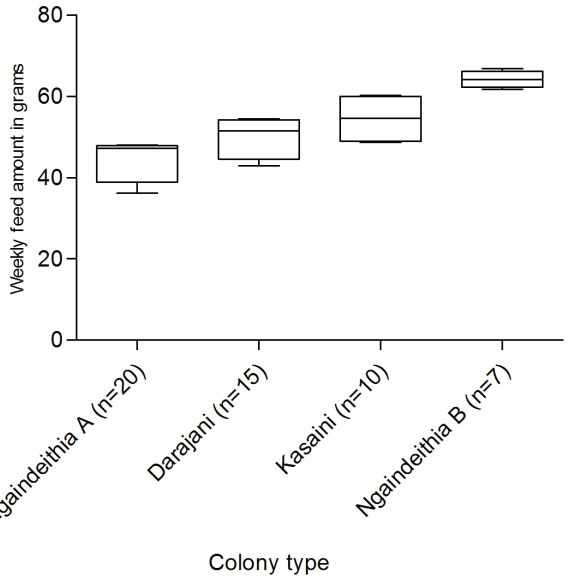

Figure 8. Box and whisker plots showing weekly food consumption (in grams) per naked mole rat in each of the four colonies. The whiskers outside the boxes represent minimum and maximum values while lines within the boxes represent median values.

Behavioural observations

The animals were consistent by undertaking a particular behaviour, for example urination, around a particular site in the cage and did not use other sites. Hence every day, each behaviour was recorded at one site which was either an outer corner or inside area of the cage. Thus on each day there was just one data point per behaviour per colony.

Urination and defecation occurred mostly at the outer corners (points 1, 2, 11 and 12) while resting and eating occurred predominantly at the inner areas (points 3-10) of the cages (See Figures 6 A and B). Although the Darajani colony had more defecation recorded at the inner areas of the cage than the other colonies ($p = 0.035$, chi square test), most defecation occurred at the outer corners. Thus, in all colonies, the pattern of separating toilet (urination and defecation) from eating and resting areas occurred irrespective of colony size or cage design (Figure 9).

Discussion and conclusion

The European Union directive 2010/63 (European Union 2010), emphasizes refinement of accommodation and care of laboratory animals to reduce animal suffering and distress. The directive also advises on accommodation and care of animals based on specific needs and characteristics of each species. Similar emphasis is expressed in the FELASA (Federation of European Laboratory Animal Science Associations) report on provision of environmental enrichments through complexity, resting and bedding facilities to allow for species specific behaviours and reduce experimental variability (FELASA 2006).

This study was designed to find out whether captive naked mole rats can replicate in the laboratory their natural behaviour in the wild of having separate resting, waste disposal and eating areas.The animals were housed at a room temperature between 28 to 31°C and humidity of 50-70%. They were kept

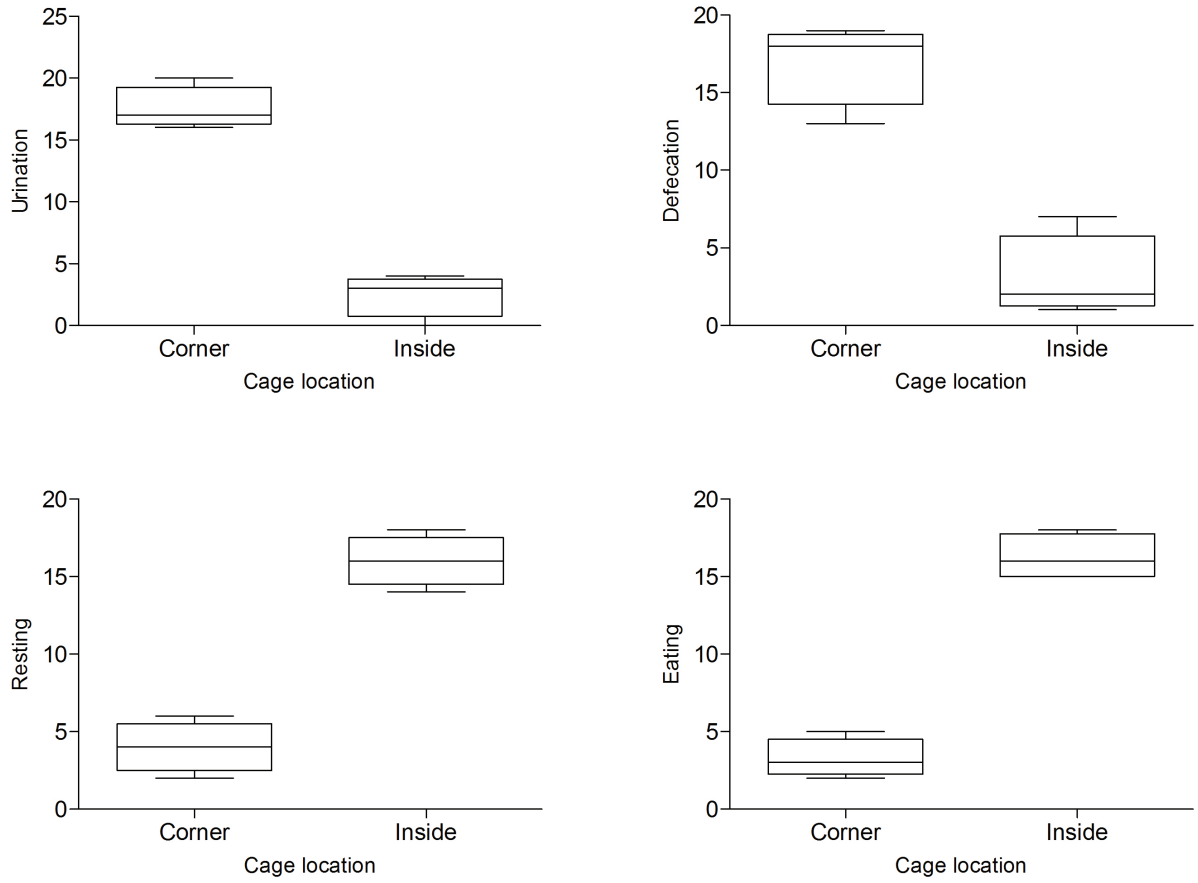

Figure 9. Box and whisker plots showing the location of the toilet, eating and resting behaviours, classified as either outer corners of the cages (points 1, 2, 11 and 12) or inside areas of the cages (points 3 to 10). For all colonies, each behaviour was counted once a day for 20 days. The number of times a behaviour was recorded on the inner or outer areas is shown on the vertical axis. The whiskers outside the boxes show minimum and maximum counts for the particular behaviour while lines within the boxes show the medians.

in ventilated cages with three compartments and provided with fine wood shavings for bedding and resting, and food consisting of fresh carrots and potatoes. These conditions were designed to simulate the environmental conditions in nature as reported by Schumacher et al. (2015) where they live in an underground network of chambers and tunnels, with temperatures ranging between 28 to 32°C depending on tunnel depth, high humidity levels of up to 90%, and feed on roots and tubers.

In the laboratory, all four naked mole rat colonies had eating and resting areas separated from waste disposal areas (defecation and urination). Resting and eating sites were found to be at the same place or adjacent to each other, but separate from urination and defecation sites, which were also at the same place or adjacent to each other. This behaviour reflects that seen in the wild, where naked mole rats are reported to urinate and defecate only in the toilet chamber to avoid contamination. In addition, the animals dig new sites for urination and defecation if the old ones become full (Jarvis and Sherman 2002; Rosamond Gifford Zoo 2006). A study by Margullis et al. (1995) observed the same behaviour in captive naked mole rats which also separated eating sites from urination and defecation sites.

The observation that naked mole rats in small colonies consumed more food but without a significant difference in weight gain compared to the naked mole rats in large colonies indicates they may have been more active and therefore expended more energy. The finding that there was no significant difference in weight gain between the four colonies, suggests that the number of naked mole rats per cage may not influence weight gain. During the experiment, the naked mole rats were fed on a diet of fresh sweet potatoes, carrots and Irish potatoes. The observed weekly weight gain of 0.44 g per naked mole rat indicates this diet is suitable for use in the laboratory. In both wild and captive conditions, they feed on roots, tubers and bulbs such as sweet potatoes, Irish potatoes, grapes, apples, bananas and other succulent plant material (Rosamond Gifford Zoo 2006; Judd and Sherman 1996; Jarvis and Sherman 2002). The animals also practice coprophagy to maximize extraction of nutrients from their food (Abiyselassie 2018).

Since there is a lack of data on standard housing of the naked mole rat under laboratory conditions, the findings of this study provide an initial recommendation of how to house the naked mole rat. These findings are open for further improvement, but already fulfil the intentions of EU directive 2010/63 (European Union 2010).

Based on this study, it is recommended that captive naked mole rats should be provided with cages designed to allow them to maintain separate locations for waste disposal (urination and defecation), resting and eating. This is an important consideration to increase comfort and well-being of these animals in the laboratory. However, additional studies are needed to investigate further the effect of colony size, colony origin and cage size on naked mole rats' behaviour in captivity.

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Conflict of interest

The authors declared no potential conflict of interest.

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