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RESEARCH ARTICLE

Exploratory behaviour, memory and neurogenesis in the social Damaraland mole-rat (*Fukomys damarensis*)

Maria K. Oosthuizen[1,2,*]

ABSTRACT

Both exploratory behaviour and spatial memory are important for survival in dispersing animals. Exploratory behaviour is triggered by novel environments and having a better spatial memory of the surroundings provides an adaptive advantage to the animals. Spatial challenges can also affect neurogenesis in the hippocampus by increasing cell proliferation and enhancing survival of young neurons. In social Damaraland mole-rat colonies, the social hierarchy is largely based on body size. Individuals with different social statuses in these colonies display different dispersal behaviours and as behavioural differences have been linked to dispersal behaviour, I investigated exploratory behaviour, memory and hippocampal neurogenesis in wild-captured Damaraland mole-rats. Dispersal behaviour gives rise to differential exploratory behaviour in Damaraland mole-rats; they readily explored in a novel environment but resident, worker mole-rats explored more slowly. In the Y-maze, animals entered the escape hole significantly faster by the second day; however, they did not make fewer wrong turns with successive days of the experiment. Female dispersers did not show any improvement in time to reach the escape hole or the number of wrong turns over the 4 day experimental period. Damaraland male and female dispersers employ different dispersal strategies, and this is evident in their approach to the learning task. Females are less motivated to complete the task, leading to a difference in behaviour, and this has important survival implications for the different sexes. Finally, in the context of memory, adult neurogenesis does not seem to be a good marker in mole-rats as it is generally low and has not been investigated thoroughly enough to determine which and how other factors can influence it in these animals.

KEY WORDS: Learning, Dispersal, Y-maze, Social group differences, Memory, Wild

INTRODUCTION

Exploratory behaviours in animals are triggered by novelty and serve to gather information about their surroundings and to decrease uncertainty in novel environments (Vanden Broecke et al., 2018). Exploration is a fundamental interaction of animals with their environment and is linked to their survival (Page et al., 2018). Exploratory behaviour can be influenced by both the external and internal environments of animals. These include environmental factors such as the physical attributes of the environment and how it is perceived by the animal, social organisation of the species and individual factors such as motivation, cognition and memory (Mehlhorn et al., 2015).

Exploratory behaviour has been linked to a number of other behaviours such as foraging (Patrick et al., 2017), risk-taking behaviour (Martins et al., 2007) and anti-predator behaviour (Jones and Godin, 2010). Social dominance appears to play a role in the speed of exploration, it has been proposed that individuals with a high reproductive future would be slower explorers and low risk takers (Quinn et al., 2012), but there seems to be limited evidence for this. The relationship seems to rather be context and perhaps age dependent. In great tits, dominant territorial males explore faster while fast-exploring, non-territorial juvenile males have a lower dominance rank (Dingemanse and De Goede, 2004).

Exploration is also important for spatial movements such as dispersal (Cote et al., 2010). Dispersal is a fundamental component of ecology with important consequences for survival and reproduction (Debeffe et al., 2014). Behavioural differences between dispersers and non-dispersers or residents have been reported in a number of species and have been attributed to reducing dispersal costs and facilitation of settlement (Hoset et al., 2011). An association between dispersal and spatial memory has been shown in African striped mice, where male survival is positively correlated with greater spatial memory, potentially because they remember the environmental configuration during dispersal (Maille and Schradin, 2016). Several factors may influence the spatial cognition ability of a species. Habitat complexity has long been implicated in cognitive ability, whether in natural habitats or in the laboratory (Costanzo et al., 2009; Daniel et al., 1999). Habitat complexity has been shown to influence both the behaviour and the brains of animals (Shumway, 2008).

Mammalian studies demonstrated correlations between the complexity of spatial challenges and relative size of the brain, as well as specific brain structures (Bernard and Nurton, 1993; Safi and Dechmann, 2005). The size of the hippocampus in both mammals and birds correlates positively with the increased spatial activity that accompanies larger home ranges and specific foraging behaviours (Jacobs and Spencer, 1994; Yaskin, 2011). Adult neurogenesis is a well-known phenomenon in discrete areas of mammalian brains (Gage, 2000). In most mammals, cell proliferation takes place throughout life in the subventricular zone of the lateral ventricle (associated with olfaction) and in the dentate gyrus of the hippocampus (Zhao et al., 2008). Spatial learning and memory, which is hippocampus dependent, has been shown to enhance cell proliferation and survival of young neurons in the hippocampus of rodents and is thought to modulate an animal's reaction to novelty by contributing to behavioural flexibility (Cavegn et al., 2013; van Dijk et al., 2015).

African mole-rats (family Bathyergidae) are subterranean rodents. Of the six African mole-rat genera that comprise the family Bathyergidae, three are social (Kock et al., 2006). All social

[1]Department of Zoology & Entomology, University of Pretoria, Private Bag X20, Pretoria 0028, South Africa. [2]Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa.

*Author for correspondence (moosthuizen@zoology.up.ac.za)

M.K.O., 0000-0001-6305-8283

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mole-rats breed cooperatively, displaying a marked reproductive skew such that a single female in a colony is responsible for the production of offspring (Bennett and Faulkes, 2000). In Damaraland mole-rats (*Fukomys damarensis*), socially subordinate animals in the colony are reproductively suppressed by the breeding animals and their reproductive success while they are in the natal colony is typically very low or non-existent (Cooney and Bennett, 2000). Thus, in order to reproduce, they need to disperse.

Disperser Damaraland mole-rats of both sexes are characteristically significantly larger (but not necessarily older) than other non-reproductive animals in the colony (Bennett and Faulkes, 2000; Hazell et al., 2000). However, dispersal events may be delayed as dispersion is primarily dictated by sporadic and unpredictable rainfalls (Hazell et al., 2000). Hence, dispersers remain in their natal colony until environmental conditions are favourable (Voigt, 2014).

While the burrow systems of social mole-rats are relatively complex compared with those of the solitary species (Bennett and Faulkes, 2000; Thomas et al., 2012), tunnel systems offer much less environmental stimulation in terms of both surface area and habitat complexity compared with the above-ground environment. Mole-rats in their natural habitat thus inhabit a rather featureless and impoverished environment. Compared with that in other rodents, the hippocampal formation in mole-rats is small and the levels of adult hippocampal neurogenesis are relatively low (Oosthuizen and Amrein, 2016).

Because they live in a naturally uniform habitat, social mole-rats provide a unique opportunity to study both behavioural and neurological differences in animals with different social statuses. Animals within a single colony differ in dispersal behaviour, with some individuals having a high propensity to disperse while others will remain in the natal colony for life. The objectives of this study were to investigate the exploratory behaviour, spatial reference memory and neurogenesis of wild-trapped Damaraland mole-rats with different social statuses. Because of their dispersal behaviour, disperser animals were predicted to explore more readily and be faster in learning tasks. I was also interested in any potential sex differences in exploratory behaviour and spatial memory tasks that may arise, as dispersal behaviour frequently differs between males and females (Clutton-Brock, 2016). Previously, differences were shown in hippocampal neurogenesis of Damaraland mole-rats (Oosthuizen and Amrein, 2016), and the distribution and presence of proliferating and young neurons are expected to reflect social status.

MATERIALS AND METHODS

Twenty animals from eight colonies of Damaraland mole-rats, *Fukomys damarensis* (Ogilby 1838), were captured near Blackrock in the Northern Cape, South Africa (27°7′S, 22°52′E) during the summer, using Hickman live traps (Hickman, 1979). Once captured, the mole-rats were transported to the University of Pretoria, where behavioural experiments were conducted. Animals were subjected to experimental testing immediately after arrival in the laboratory (within 2 weeks of capture). These 20 animals were then used for immunohistochemical experiments (1 week after the commencement of the behavioural experiments; see below) to detect markers for neurogenesis in the hippocampus. Subsequent to the analysis of behavioural data, one group showed unusual results, and as this group had the smallest sample size, 16 animals from six additional colonies from Tswalu Kalahari Reserve (27°31′S, 22°19′E) were used during the summer of the following year to supplement the sample sizes for the behavioural component of the study. The experimental mazes were transported to the field laboratory, where experiments were conducted (also within 2 weeks of capture of the animals), and the animals were subsequently released.

Damaraland mole-rats were categorised into groups with different social status; namely, queens, dispersers and workers. Queens were identified by the presence of nipples and a perforate vagina; none of the animals showed signs of lactation at the time of experimentation, and animals were not visibly pregnant. Dispersers and workers were classified by physical size and body mass according to the methodology of Oosthuizen and Amrein (2016). The total sample size for the behavioural analysis was 36 mole-rats (7 queens, 8 male and 6 female dispersers, 6 male and 9 female workers), whereas the sample size for the neurogenesis experiments was 18 mole-rats (3 queens, 6 male and 2 female dispersers, 4 male and 3 female workers). Table S1 provides the body mass of all animals used in this study.

Experimental procedures were approved by the Animal Use and Care Committee at the University of Pretoria (EC013-09). Collection permits were obtained from the Northern Cape nature conservation authority (ODB 2023/2010).

Behavioural experiments

Apparatus

For the first experiment, a modified open-field box was used (Fig. S1). The apparatus consisted of an open-topped, white wooden box (dimensions 20×40×20 cm). Three black plastic tunnels (20 cm long, 7 cm diameter) projected externally from one of the long sides of the box. The tunnels were positioned 1 cm above the floor and equidistantly spaced along the side of the box. The floor of the box was covered with a thin layer of wood shavings. Between animals, the box and tunnels were wiped with 95% alcohol and distilled water (Deacon et al., 2012).

The apparatus for the second experiment was a Perspex Y-maze (Fig. S2), with the arms measuring 50×10×20 cm. At the distal end of each arm was an escape hole, 2 cm above the floor with a tunnel attached. Both ends of each arm could be blocked off with a guillotine door (Deacon, 2013).

Experimental design

Experiment 1: exploration in tunnel maze

The aim of this experiment was to investigate the exploratory behaviour and general activity levels of the Damaraland mole-rats. Animals were placed in the centre of the box and spontaneous behaviour was observed for 3 min. Five measures were recorded: the latency to enter the first tunnel, total number of entries into the tunnels, total and mean duration in tunnels and number of tunnels visited. All four feet of the animal were required to be inside a tunnel to be considered an entry (Deacon et al., 2012). Animals were considered to explore more readily if they showed a longer latency to enter the first tunnel, made more tunnel entries, spent a shorter time in the tunnels and spent more time in the open.

Experiment 2: spatial memory in the water Y-maze

To motivate animals to move, the bottom of the Y-maze was filled with about 1 cm of water (at room temperature). The arms were labelled A, B and C. For the first trial, arm A was the start arm, arm B was the wrong arm and arm C was the correct arm. On the distal end of the correct arm, an escape hole was connected to a Perspex tunnel and a dry nest box. At the ends of the other two arms, blocked tunnels were placed at the closed exits so that the arms would look identical from the centre of the maze. Animals were released into the starting arm, facing towards the blocked tunnel, and allowed 1 min

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to find the escape hole. The time to locate and enter the escape hole was recorded; again, all four feet needed to be inside the tunnel. In addition, the number of wrong entries into the arms was recorded (Deacon, 2013). The starting arm was alternated randomly between arm A and arm B; thus, half of the trials per day started in each arm. The escape hole was always to the right from the starting position; thus, when the starting position was arm A, the nest box was connected to arm C, and when the starting position was arm B, the nest box was connected to arm A. The spatial memory test trials were conducted on four consecutive days for 10 trials per day. The period that separated the trials varied between 45 and 60 min per animal. A mean of the time to complete the maze was calculated for each animal per day, and the number of errors per day per animal was calculated by dividing the total number of errors over the 10 trials per day by 10.

The mass of the animals remained stable during the experimental procedures (data not shown). All behavioural procedures were recorded with an overhead video recorder and were analysed manually upon completion of the experiments.

Experiment 3: neurogenesis

For analysis of neurogenesis markers, animals were deeply anaesthetised with fluorothane gas (Zeneca) before they were perfused intracardially with 0.9% phosphate-buffered saline (PBS; pH 7.4, Sigma) and 4% paraformaldehyde (PFA; Saarchem) in 0.1 mol l^{-1} PBS (pH 7.4). The brain was removed from the skull and post-fixed overnight in 4% PFA, then stored in 2% PFA. The left hemisphere of the brain was used to count the granule cell numbers, while the right hemisphere was used for immunohistochemistry to count proliferating and young neurons (Amrein et al., 2014).

Ki67, a nuclear protein associated with cellular proliferation, was used to mark proliferating cells. It is expressed in the nucleus during all active phases of the cell cycle, and absent in non-proliferating cells (Scholzen and Gerdes, 2000). A study on hepatic tissue showed that Ki67 mRNA increased after 12 h and expression remained elevated for the duration of their 8 day (192 h) study (Gerlach et al., 1997).

Developing and migrating neurons express a cell surface molecule, a polysialylated (PSA) form of the neural cell adhesion molecule (NCAM) called PSA-NCAM (Quartu et al., 2008). Newly generated neurons begin to migrate almost immediately, and synaptic integration is achieved between 2 and 4 weeks, during which period PSA-NCAM is expressed (Ming and Song, 2005) and can therefore be used to mark young or immature neurons.

Histoarchitecture

The left hemispheres of the mole-rat brains were rinsed in PBS, after which they were dehydrated in graded ethanol solutions. The brains were subsequently incubated in a 1:1 solution of 100% alcohol and hydroxyethylmethacrylate (HEMA; Technovit 7100, Heraeus Kulzer GmbH, Wehrheim/Ts, Germany) overnight, followed by three changes of HEMA only, for a total period of 1 month. Brains were cut horizontally in 20 µm sections and stained with a Giemsa staining solution (Giemsa stock solution 1.09204.0500, Merck, Darmstadt, Germany, diluted 1:10 in 67 mmol KH_2PO_4 buffer, pH 7.2) for 40 min at room temperature. Subsequently they were differentiated in 1% acetic acid for 15 s, dehydrated in alcohol and cover-slipped.

Immunohistochemistry

Right hemispheres of the mole-rat brains were cryoprotected in 30% sucrose and 40 µm sagittal sections were collected in series. Every 6th section was used for immunohistochemical analysis. Treatment of the sections followed the protocol previously described in Oosthuizen and Amrein (2016). In brief, free-floating sections were washed in Tris-buffered saline (TBS). For NCL-Ki67 immunohistochemistry, epitope retrieval was required; these sections were incubated in citrate buffer (pH 6.4; Target Retrieval Solution, Dako; 1:10) for 40 min at 94°C and rinsed in TBS. Sections were incubated in hydrogen peroxidase for 30 min to block endogenous peroxidase activity, and then in TBS containing Triton and normal serum and bovine serum albumin (BSA) for 1 h. Sections were incubated in primary antibodies in the same diluent (for proliferating cells: polyclonal rabbit anti-NCL-Ki-67, Dianova, 1:2500; for young differentiating cells: monoclonal mouse anti-PSA-NCAM, Chemicon/Millipore, 1:5000) overnight at 4°C. After a rinse in TBS, sections were incubated in secondary antibodies (goat anti-rabbit and donkey anti-mouse, respectively; both Vectastain, 1:300) for an hour, and then incubated in an avidin–biotin complex (Vectastain Elite ABC kit). Sections were stained with 3,3′-diaminobenzidine (DAB), mounted, cover-slipped and investigated on an Olympus BX 40 microscope.

Quantification

Design-based stereology was used to estimate granule cell numbers in the HEMA embedded sections. An optical fractionator (West et al., 1991) with a 100× oil immersion lens (NA=1.3) was used together with StereoInvestigator software (MicroBrightField, Inc., Williston, ND, USA). Granule cells were sampled in every 12th section in 10 µm high and 15×15 µm wide dissector and 2 µm top guard zones at 240 µm intervals along the *x*- and *y*-axis. A mean (±s.e.m.) of 16.3±1.5 sections per animal were counted (queens 15.75±1.9, dispersers 16.1±0.8, workers 16.8±1.7). Ki67-positive, proliferating cells and PSA-NCAM-positive young neurons located in the granular and subgranular cell layers were counted manually in every 6th section; positively stained cells in the top focal plane were discarded.

Data acquisition and statistics

For the behavioural data, statistical analysis was performed with IBM SPSS Statistics version 22 (SPSS Inc., Chicago, IL, USA). Data were not normally distributed; thus, parameters for exploratory behaviour were assessed with generalized linear models using a gamma distribution with a log link function. Generalized linear mixed models with a gamma distribution and a log link function were employed for analysis of experiment two. The consecutive trials were used as a repeated measure and days, sex and caste were chosen as fixed effects. Significance level was set at 0.05 and the main effects were tested with sequential Bonferroni tests (Oosthuizen et al., 2013).

For the immunohistochemistry, the Gundersen–Jensen coefficient of error (CE) was determined to assess experimentally introduced variance to granule cell number estimates, using the conservative approach of m=0 (Gundersen et al., 1999). The integrity of the cell counts was assessed by the contribution of the estimation procedure to group variances, conveyed by the ratio CE^2/CV^2 (CV=coefficient of variation=s.d./mean cell count).

The relationships between body mass, total granule cell number and positively stained cells for neurogenesis markers were compared using a two-tailed Spearman's r correlation analysis. In addition, a generalized linear model (GLM) with a gamma distribution and a log link function was used to analyse neurogenesis in the dentate gyrus. Here, cell numbers were employed as dependent variables to reveal caste and sex differences in the Damaraland mole-rat hippocampus and the interaction between them. Least significant difference (LSD) *post*

hoc tests were applied, and statistical significance was maintained at $P<0.05$ (Oosthuizen and Amrein, 2016).

RESULTS

Experiment 1: exploratory behaviour

The latency to enter the first tunnel was not influenced by the sex of the animals ($\chi^2=3.72$, d.f.=1, $P=0.54$) but workers were found to enter the first tunnel faster than queens and dispersers ($\chi^2=11.98$, d.f.=2, $P=0.003$; Fig. 1A). The mean time Damaraland mole-rats spent in the tunnels was not influenced by sex ($\chi^2=3.69$, d.f.=1, $P=0.055$); however, it was affected by the social status of the animals ($\chi^2=6.67$, d.f.=2, $P=0.036$). Workers spent a lot more time per visit in the tunnels compared with queens ($P=0.011$) and dispersers ($P=0.043$; Fig. 1A; Table S2).

No differences were found between sexes ($\chi^2=1.83$, d.f.=1, $P=0.176$) or castes ($\chi^2=3.6$, d.f.=2, $P=0.165$) in the total time spent in the tunnels (Fig. 1A). Similarly, the number of tunnel entries did not vary between sexes ($\chi^2=0.09$, d.f.=1, $P=0.861$) or social groups ($\chi^2=0.3$, d.f.=2, $P=0.861$); the number of tunnels visited also did not differ between the sexes ($\chi^2=0.79$, d.f.=1, $P=0.374$) or social groups ($\chi^2=0.91$, d.f.=2, $P=0.636$; Fig. 1B).

Experiment 2: memory and learning

Learning over time

Time to complete the maze was highly dependent on the consecutive days ($F_{3,1420}=4.62$, $P=0.003$). The latency to find the escape hole was significantly faster on the second day than on the first day ($P=0.008$), after which the time to enter the escape hole remained stable (day 2–3: $P=1.0$, day 3–4: $P=1.0$). *Post hoc* tests revealed that this was the case for queens ($F_{3,1420}=4.45$, $P=0.004$) and workers ($F_{3,1420}=6.45$, $P<0.001$), but not disperser mole-rats ($F_{3,1420}=1.42$, $P=0.235$). However, this difference was apparent only in disperser females ($F_{3,1420}=0.68$, $P=0.567$), not males ($F_{3,1420}=4.89$, $P=0.002$; Fig. 2A; Table S3).

The amount of wrong turns the mole-rats made also changed significantly over the consecutive days, with fewer wrong turns on the first day compared with the other days ($F_{3,1420}=4.73$, $P=0.003$). Wrong turns were not significantly different between day 1 and 2 ($P=0.064$), but animals made significantly more wrong turns on the third ($P=0.009$) and fourth ($P=0.008$) day compared with day 1, while the number of mistakes remained similar between day 2 and 4 (day 2–3: $P=1.0$, day 3–4: $P=1.0$; Fig. 2B). None of the groups showed an improvement in the number of mistakes made (Table S3).

Social group differences

Social status grouping had a significant effect on the time to find the escape hole ($F_{2,1420}=14.54$, $P<0.001$). Queens were significantly faster than dispersers ($P<0.001$) and workers ($P=0.012$), and workers were also faster than dispersers ($P=0.001$). Disperser and worker males did not differ in time to reach the escape hole ($F_{1,1420}=0.89$, $P=0.344$), whereas the females differed in speed ($F_{2,1420}=26.07$, $P<0.001$): queens were faster than disperser females ($P<0.001$) and worker females ($P=0.011$), and worker females were faster than disperser females ($P<0.001$, Fig. 2A). Significant differences in the number of wrong turns were evident between the social groups ($F_{2,1420}=12.26$, $P<0.001$). Disperser mole-rats made more wrong turns than queens ($P=0.001$) and workers ($P<0.001$). This difference was again a result of female dispersers; disperser and worker males made similar amounts of wrong turns ($F_{2,1420}=1.21$, $P=0.272$), and disperser females made more errors than both the queens and worker females ($F_{2,1420}=16.57$, $P<0.001$; Table S3).

Sex differences

Males and females showed a significant difference in time to reach the escape hole ($F_{1,1420}=10.99$, $P=0.001$). However, this difference was only in the disperser group, where disperser females were slower to find the escape hole than the males ($F_{2,1420}=43.8$, $P<0.001$); there was no difference between worker males and females in the time to reach the escape hole ($F_{2,1420}=0.27$, $P=0.605$; Fig. 2A). Worker males and females made equal numbers of errors ($F_{2,1420}=0.169$, $P=0.681$), while female dispersers made more wrong turns than male dispersers ($F_{2,1420}=11.42$, $P=0.001$; Table S3).

Experiment 3: immunochemistry

Body mass and hippocampal granule cells according to social status

Body mass was significantly different for the three social groups ($\chi^2=19.18$, d.f.=2, $P<0.001$). Worker mole-rats had significantly lower body mass compared with the queens ($P=0.004$) and

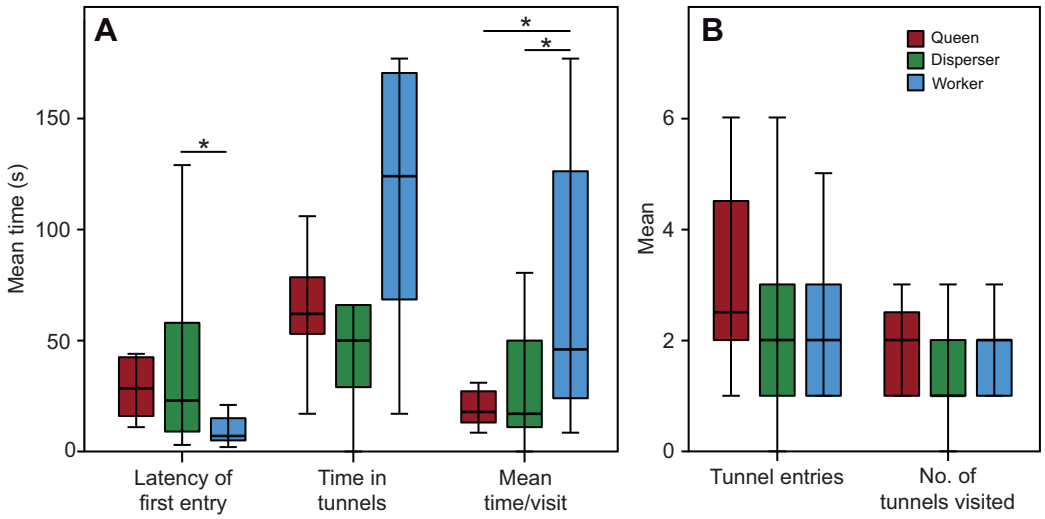

Fig. 1. Exploratory behaviour. (A) Time-related exploration measurements in the tunnel maze for the different castes of Damaraland mole-rats. (B) Activity measurements for the different castes of Damaraland mole-rats in the tunnel maze. Values are presented as median, 1st and 3rd quartile, minimum and maximum. Sample size: 7 queens, 14 dispersers, 15 workers. Asterisks indicate significance.

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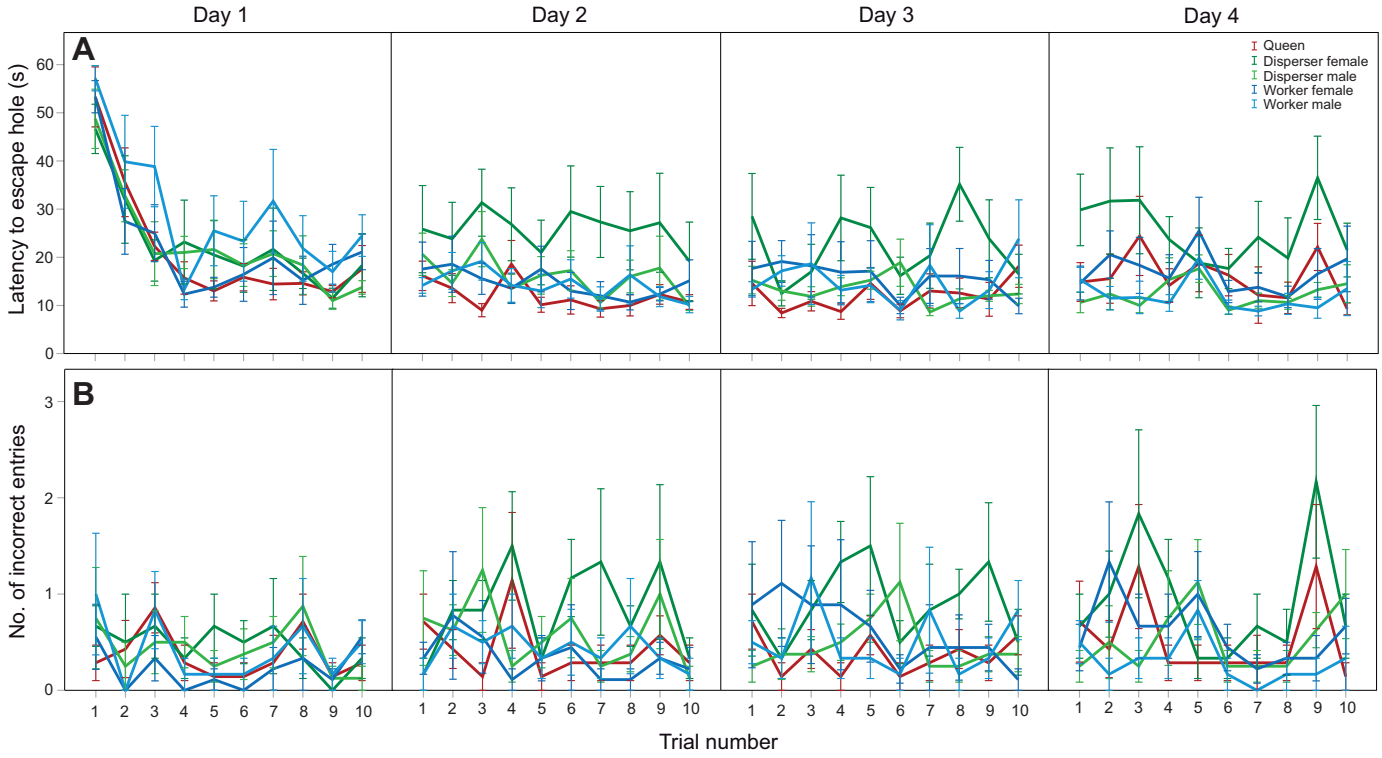

Fig. 2. Spatial memory in the Y-maze. (A) Mean latency to enter the escape hole over the four experimental days for the three social groups. (B) Mean number of incorrect entries into the different arms by the three social groups. Sample sizes: 7 queens, 8 disperser males, 6 disperser females, 6 worker males, 9 worker females. Data are means±s.e.m.

dispersers (P<0.001), while the queens and dispersers had similar body mass (P=0.181). Estimates of absolute granule cell numbers in the dentate gyrus were low compared with numbers in other rodents. Granule cell numbers were not different between the three social groups of Damaraland mole-rats (χ^2=2.71, d.f.=2, P=0.259) or between males and females (χ^2=0.175, d.f.=1, P=0.676) (Fig. 3A). The number of granule cells was not dependent on body mass (r_{16}=0.068, P=0.788; Table 1).

Effect of social status and sex on hippocampal neurogenesis

Social status had a significant effect on the number of Ki67-positive proliferating cells (χ^2=7.24, d.f.=2, P=0.027; Figs 3B and 4, Table 1), and *post hoc* analysis revealed that worker mole-rats have higher numbers of proliferating cells than both disperser mole-rats (P=0.007) and queens (P=0.041). Queens and disperser mole-rats had similar numbers of proliferating cells (P=0.566; Fig. 3B). The number of proliferating cells was not different between males and females when queens were included (χ^2=0.16, d.f.=1, P=0.687) or excluded from the analysis (χ^2=0.48, d.f.=1, P=0.490). The interaction between sex and social group influenced the number of proliferating cells (χ^2=10.47, d.f.=4, P=0.033). *Post hoc* tests showed that disperser males have significantly fewer proliferating cells than male (P=0.018) and female workers (P=0.04).

Social status was also identified as a contributing factor for the presence of PSA-NCAM-positive young neurons (χ^2=7.49, d.f.=2, P=0.024; Figs 3C and 4, Table 1). Disperser mole-rats displayed a lower number of PSA-NCAM-positive young neurons than worker mole-rats, but no difference was observed between queens and dispersers (P=0.421) or worker mole-rats (P=0.131; Fig. 3). No difference was observed between males and females when the queens were included (χ^2=0.02, d.f.=1, P=0.895) or excluded (χ^2=0.08, d.f.=1, P=0.776). The sex and social group interaction was identified as a contributing factor in the young neuron numbers (χ^2=20.15, d.f.=4, P<0.001). Queens had fewer young neurons than male workers (P=0.029) and male dispersers had fewer neurons than male (P<0.001) and female (P=0.033) workers.

DISCUSSION

Exploratory behaviour

Animals use exploratory behaviour to familiarise themselves with novel environments (Russell et al., 2010). In the laboratory, open-field tests are used to provide information regarding the level of activity as well as exploratory behaviour and anxiety experienced by an animal (Walsh and Cummins, 1976). Anxious animals tend to move less or freeze and engage in stereotypic behaviours. As mole-rats live in enclosed burrow systems slightly wider than their bodies, they may be less comfortable in an open arena, especially recently collected animals. Therefore, a modified open-field box was used to obtain a base level of activity for the animals and to establish whether there are social group differences in exploratory behaviour. All wild Damaraland mole-rats showed no hesitation to explore, and displayed results very similar to those found for another eusocial mole-rat species, the naked mole-rat, in terms of latency to enter the first tunnel, time in the tunnels and the number of tunnel entries (Deacon et al., 2012). Although mole-rats have been reported to explore slower with longer risk assessment times than surface-dwelling rodents (mice) (Deacon et al., 2012), the social Damaraland mole-rats are still faster than solitary Cape mole-rats. Cape mole-rats that have been tested in the same paradigm explore slower and are more reluctant to enter the tunnels for the first time compared with the social species (Oosthuizen et al., 2013).

No overall sex differences were noticed, but the social groups showed differences in some of the parameters measured in the exploratory experiment in this study; in particular, the ones related

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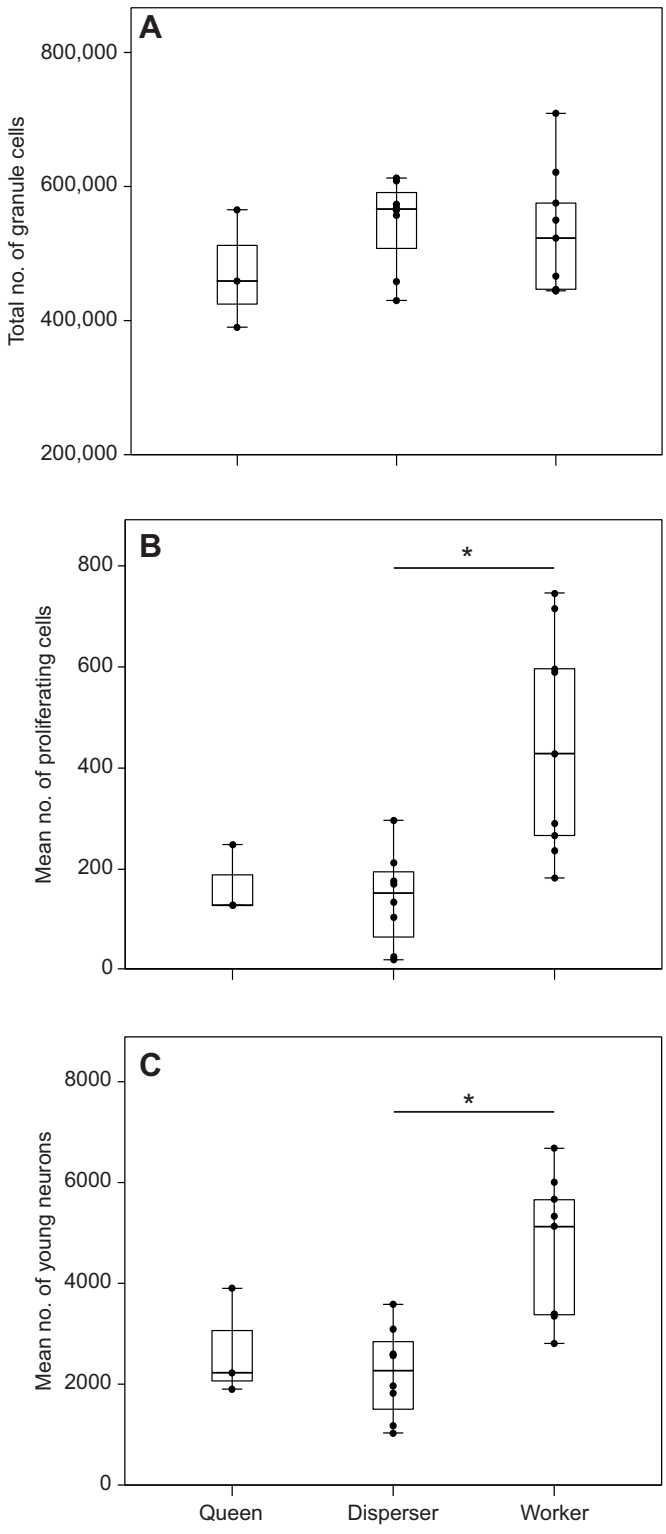

Fig. 3. Granule cell number and cellular proliferation. (A) Mean granule cell number in the three social groups. (B) Mean number of proliferating cells in the three social groups. (C) Mean number of young neurons in the three social groups. Values are presented as median, 1st and 3rd quartile, minimum and maximum, with individual data points overlaid. Sample sizes: 3 queens, 8 dispersers, 7 workers. Asterisks indicate significance.

to anxiety. The resident, worker mole-rats had a shorter latency to enter the first tunnel and they spent more time in the tunnels. The difference in exploratory behaviour of the social groups suggests that the non-dispersing worker animals are more cautious to enter open areas. This could be related to differences in both reproductive and dispersal behaviour of the animals. A study on cooperative birds suggests that exploration is dependent on the reproductive state rather than the social state of the birds, such that birds that reproduce early explore faster (Edwards et al., 2016). In social mole-rats, the reproductive status of an animal is linked to its dispersal behaviour, with primarily animals that have dispersed attaining reproductive status. Thus, dispersal behaviour seems to influence exploration, with the queens (established breeders) and potential dispersers (future breeders) exploring faster and more confidently.

Learning and memory

Damaraland mole-rats showed a significant improvement in time to reach the escape hole on the second day compared with the first day, which then remained stable for the remaining experimental days. A previous study on the solitary Cape mole-rat showed that they were slower to become familiar with the maze, and only exhibited an improvement in time to enter the escape hole by the third day (Oosthuizen et al., 2013). The difference between the two species may be related to the disposition of the animals: solitary Cape mole-rats are xenophobic and much more aggressive than the social Damaraland mole-rats (personal observation). The aggressive behaviour of the Cape mole-rat could stem from anxiety, which can influence cognitive function (Darcet et al., 2014).

Interestingly, Damaraland mole-rats made fewer wrong turns to the escape hole on the first day of trials compared with the consecutive days. Similarly, wild-trapped solitary Cape mole-rats did not show a reduction in number of errors with progressive days (Oosthuizen et al., 2013). The number of errors provides a proxy for spatial learning: fewer errors to reach the escape hole with consecutive trials would indicate that the animals have learned the spatial location of the escape hole, as opposed to just learning that an escape hole exists to get out of the water. Hence, although learning is apparent, the results of this study do not provide conclusive evidence that spatial learning took place. The lack of improvement in the number of errors may also be anxiety related in the wild-trapped animals in the maze. Wild-trapped solitary mole-rats show similar variable behaviour to the social species, whereas solitary mole-rats that have been in captivity for some time display more consistent behaviour (Oosthuizen et al., 2013). In this experiment, global cues were changed between trials by changing the orientation of the maze, thereby excluding spatial information outside the maze, such as geomagnetism, from being used as a cue. As mole-rats have very poor vision (Němec et al., 2008), they probably do use magnetic stimuli to orientate; some mole-rat species have been shown to respond to magnetic stimuli (Malewski et al., 2018). By removing magnetic cues as a stimulus, the difficulty of the learning task might have been increased and the spatial learning ability of the animals underestimated. Nevertheless, the number of wrong turns was not high, having a mean of <1 in all the groups for all days. The solitary Cape mole-rats, tested in the same paradigm, made more errors than the social Damaraland mole-rats (Oosthuizen et al., 2013), which again could be related to the differences in their burrow systems. Fish inhabiting complex habitats have been shown to complete tasks quicker, reached landmarks faster and make fewer mistakes than fish living in less complex habitats (Shumway et al., 2007).

Sex and social status differences

The existence of sex differences in spatial behaviour and cognition has been reported in several mammalian species; however, the

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Table 1. Hippocampal neurogenesis in the three mole-rat social groups

	Sex	*N*	Body mass (g)	Relative age	Granule cells	Young neurons	Proliferating cells
All animals		18	117.94±7.3	5.3±0.5	532,000±21,000	3240±380	270±50
CE					0.12±0.01	0.07±0.01	0.18±0.02
Queens	Female	3	123.25±12.19	5.7±2	471,000±62,000	2680±760	170±50
Dispersers	Male	6	150.67±7.63	5±0.9	562,000±25,000	1900±350	120±40
	Female	2	109.5±4.5	5.2±1.9	500,000±70,000	3090±490	200±100
Workers	Male	4	93.5±8.56	4.7±1	500,000±40,000	5260±790	450±140
	Female	3	85.33±9.55	6.2±1	592,000±95,000	3770±610	500±210

Body mass and age data are means±s.e.m. Estimated cell numbers (±s.e.) are provided separately for social groups and sexes: granule cell numbers are rounded to the nearest 1000, and young neurons and proliferating cell numbers are rounded to the nearest 10. CE, coefficient of error *m*=0 (Slomianka and West, 2005).

reliability and magnitude thereof is debatable (Jonasson, 2005). Sex differences have been attributed to factors such as hormones (Daniel et al., 1999; Spritzer et al., 2011) and ranging behaviour exhibited by males (Gaulin and Fitzgerald, 1986). Sex differences in dispersal behaviour are pertinent in cooperatively breeding species, where males seem to move between groups more readily (Clutton-Brock, 2016; Torrents-Ticó et al., 2018), and it has been suggested that dispersal in Damaraland mole-rats is male biased (Hazell et al., 2000; Torrents-Ticó et al., 2018). In Damaraland mole-rats in this study, sex differences in spatial behaviour were only obvious in the disperser group, where disperser females that did not show any improvement in the latency to reach the escape hole made more mistakes to reach the exit hole than all the other groups. Similarly, social group differences resulted primarily from the performance of the disperser females. During the experiment, the side of the reward arm was not controlled for; therefore, it cannot conclusively be said whether the observed differences result from poor memory or a turning bias. This would require further investigation. Nevertheless, this group difference is an intriguing finding. Initially, the sample size for disperser females was small; however, this effect remained even after the addition of several more animals in this category. Sex differences in learning performance could be related to different dispersal strategies. After dispersal, male Damaraland mole-rats are more likely to join other established groups, whereas female dispersers face a higher risk of fatality on their own once they leave their natal colony (Torrents-Ticó et al., 2018). As no sex difference in general exploratory behaviour was apparent in disperser animals, the poor performance of disperser females may indicate a lack of motivation rather than impaired cognitive function, because they appeared to be more motivated to explore the maze than to find an escape route. It has been suggested that exploration may be of more importance for potential reproductive females from an evolutionary point of view as they have to secure a safe environment for their young (Dubovický et al., 1999).

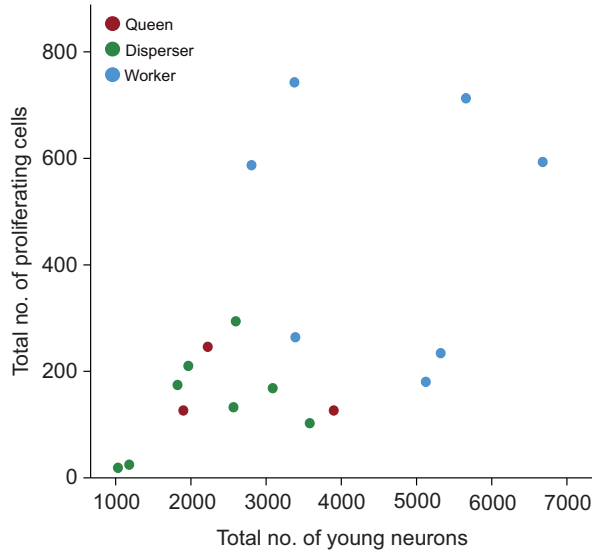

Fig. 4. Hippocampal neurogenesis. Correlation between the number of proliferating cells and the number of young neurons in Damaraland mole-rat hippocampus for the three social groups.

Neurogenesis

Overall, mole-rats have a relatively low level of neurogenesis (Amrein et al., 2014); however, social group differences have been reported previously. Oosthuizen and Amrein (2016) found queens to have the lowest number of both proliferating cells and young neurons, with disperser animals having an intermediate number and worker mole-rats having the highest number of proliferating and young neurons, with a clear separation between the social groups (Oosthuizen and Amrein, 2016). In the present study, absolute cell numbers were in the same range as in the previous study and, in addition, there was no distinction between queens and dispersers in terms of proliferating cells and young neurons, and while worker animals still had higher numbers of proliferating cells and young neurons, the number of immature neurons was not significantly more than that of the queens. Hippocampus-dependent learning has been shown to enhance survival of young neurons in rodents (Gould et al., 1999; Shors, 2009), but in this study, neurogenesis in the mole-rats appeared to be downregulated rather than upregulated. Neurogenesis can be upregulated or downregulated by many factors that may be specific to species and situations (for an overview see Oosthuizen, 2017). Stress is a powerful inhibitor of neurogenesis (Mirescu and Gould, 2006). As animals in this study were freshly captured and were handled for the behavioural experiment, stress could be a plausible explanation for reduced neurogenesis. The sample size for this part of the study was relatively small; therefore, group differences (or the lack thereof) may not have been as obvious, but given the overall low level of neurogenesis in these animals, and the large number of factors that can influence it that are not properly understood or investigated in mole-rats, neurogenesis may not be the most appropriate method to assess learning in mole-rats.

Conclusions

Dispersal can affect behaviour differentially depending on whether animals disperse or not. All Damaraland mole-rats explored readily when exposed to a novel environment; however, dispersers and non-dispersers showed different exploratory behaviour. Worker mole-rats, which remain resident in the colonies, are more reluctant to explore compared with animals that have dispersed or will disperse given the opportunity. Different dispersal strategies can also render different behaviour. Although the latency to reach the escape hole

was significantly faster by the second day, the number of wrong turns did not change over the experimental period. Female dispersers did not show an improvement in time to reach the escape hole. Damaraland male and female dispersers employ different dispersal strategies (Torrents-Ticó et al., 2018), resulting in a difference in motivation, and ultimately behaviour, which has important survival implications for the animals. Finally, in the context of memory, adult neurogenesis does not seem to be a good marker in mole-rats as it is generally low and has not been investigated thoroughly enough to determine which and how other factors can influence it in these animals.

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Competing interests
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References

Amrein, I., Becker, A. S., Engler, S., Huang, S.-H., Müller, J., Slomianka, L. and Oosthuizen, M. K. (2014). Adult neurogenesis and its anatomical context in the hippocampus of three mole-rat species. *Front. Neuroanat.* **8**, 39. doi:10.3389/fnana.2014.00039

Bennett, N. C. and Faulkes, C. G. (2000). *African Mole-rats: Ecology and Eusociality*. Cambridge, UK: Cambridge University Press.

Bernard, R. T. F. and Nurton, J. (1993). Ecological correlates of relative brain size in some South African rodents. *South Afr. J. Zool.* **28**, 95-98. doi:10.1080/02541858.1993.11448300

Cavegn, N., van Dijk, R. M., Menges, D., Brettschneider, H., Phalanndwa, M., Chimimba, C. T., Isler, K., Lipp, H. P., Slomianka, L. and Amrein, I. (2013). Habitat-specific shaping of proliferation and neuronal differentiation in adult hippocampal neurogenesis of wild rodents. *Front. Neurosci.* **7**, 59. doi:10.3389/fnins.2013.00059

Clutton-Brock, T. H. (ed.) (2016). Chapter 12: Male dispersal and its consequences. In *Mammal Societies*, pp. 401-420. Chichester, UK: John Wiley & Sons, Ltd.

Cooney, R. and Bennett, N. C. (2000). Inbreeding avoidance and reproductive skew in a cooperative mammal. *Proc. Biol. Sci.* **267**, 801-806. doi:10.1098/rspb.2000.1074

Costanzo, M. S., Bennett, N. C. and Lutermann, H. (2009). Spatial learning and memory in African mole-rats: the role of sociality and sex. *Physiol. Behav.* **96**, 128-134. doi:10.1016/j.physbeh.2008.09.008

Cote, J., Clobert, J., Brodin, T., Fogarty, S. and Sih, A. (2010). Personality-dependent dispersal: Characterization, ontogeny and consequences for spatially structured populations. *Philos. Trans. R. Soc. B* **365**, 4065-4076. doi:10.1098/rstb.2010.0176

Daniel, J. M., Roberts, S. L. and Dohanich, G. P. (1999). Effects of ovarian hormones and environment on radial maze and water maze performance of female rats. *Physiol. Behav.* **66**, 11-20. doi:10.1016/S0031-9384(98)00272-8

Darcet, F., Mendez-David, I., Tritschler, L., Gardier, A. M., Guilloux, J.-P. and David, D. J. (2014). Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. *Front. Behav. Neurosci.* **8**, 136. doi:10.3389/fnbeh.2014.00136

Deacon, R. M. J. (2013). Shallow water (Paddling) variants of water maze tests in mice. *J. Vis. Exp.* **76**, e2608. doi:10.3791/2608

Deacon, R. M. J., Dulu, T. D. and Patel, N. B. (2012). Naked mole-rats: behavioural phenotyping and comparison with C57BL/6 mice. *Behav. Brain Res.* **231**, 193-200. doi:10.1016/j.bbr.2012.03.003

Debeffe, L., Morellet, N., Bonnot, N., Gaillard, J. M., Cargnelutti, B., Verheyden-Tixier, H., Vanpé, C., Coulon, A., Clobert, J., Bon, R. et al. (2014). The link between behavioural type and natal dispersal propensity reveals a dispersal syndrome in a large herbivore. *Proc. Biol. Sci.* **281**. doi:10.1098/rspb.2014.0873

Dingemanse, N. J. De Goede, P. (2004). The relation between dominance and exploratory behaviour is context-dependent in wild great tits. *Behav. Ecol.* **15**, 1023-1030. doi:10.1093/beheco/arh115

Dubovický, M., Škultétyová, I. and Ježová, D. (1999). Neonatal stress alters habituation of exploratory behavior in adult male but not female rats. *Pharmacol. Biochem. Behav.* **64**, 681-686. doi:10.1016/S0091-3057(99)00166-5

Edwards, H. A., Dugdale, H. L., Richardson, D. S., Komdeur, J. and Burke, T. (2016). Exploration is dependent on reproductive state, not social state, in a cooperatively breeding bird. *Behav. Ecol.* **27**, 1889-1896. doi:10.1093/beheco/arw119

Gage, F. H. (2000). Mammalian neural stem cells. *Science* **287**, 1433. doi:10.1126/science.287.5457.1433

Gaulin, S. J. C. and Fitzgerald, R. W. (1986). Sex differences in spatial ability: an evolutionary hypothesis and test. *Am. Nat.* **127**, 74-88. doi:10.1086/284468

Gerlach, C., Sakkab, D. Y., Scholzen, T., Daßler, R., Alison, M. R. and Gerdes, J. (1997). Ki-67 expression during rat liver regeneration after partial hepatectomy. *Hepatology* **26**, 573-578. doi:10.1002/hep.510260307

Gould, E., Beylin, A., Tanapat, P., Reeves, A. and Shors, T. J. (1999). Learning enhances adult neurogenesis in the hippocampal formation. *Nat. Neurosci.* **2**, 260-265. doi:10.1038/6365

Gundersen, H. J. G., Jensen, E. B. V., Kiêu, K. and Nielsen, J. (1999). The efficiency of systematic sampling in stereology-reconsidered. *J. Microsc.* **193**, 199-211. doi:10.1046/j.1365-2818.1999.00457.x

Hazell, R. W. A., Bennett, N. C., Jarvis, J. U. M. and Griffin, M. (2000). Adult dispersal in the co-operatively breeding Damaraland mole-rat (Cryptomys damarensis): a case study from the Waterberg region of Namibia. *J. Zool. Lond,* **252**, 19-25. doi:10.1111/j.1469-7998.2000.tb00816.x

Hickman, G. C. (1979). A live-trap and technique for fossorial animals. *South Afr. J. Zool.* **14**, 9-14. doi:10.1080/02541858.1979.11447641

Hoset, K. S., Ferchaud, A.-L., Dufour, F., Mersch, D., Cote, J. and Le Galliard, J.-F. (2011). Natal dispersal correlates with behavioral traits that are not consistent across early life stages. *Behav. Ecol.* **22**, 176-183. doi:10.1093/beheco/arq188

Jacobs, L. F. and Spencer, W. D. (1994). Natural space-use patterns and hippocampal size in kangaroo rats. *Brain Behav. Evol.* **44**, 125-132. doi:10.1159/000113584

Jonasson, Z. (2005). Meta-analysis of sex differences in rodent models of learning and memory: a review of behavioral and biological data. *Neurosci. Biobehav. Rev.* **28**, 811-825. doi:10.1016/j.neubiorev.2004.10.006

Jones, K. A. and Godin, J.-G. Y. (2010). Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. *Proc. R. Soc. B* **277**, 625e632. doi:10.1098/rspb.2009.1607

Kock, D., Ingram, C. M., Frabotta, L. J., Honeycutt, R. L. and Burda, H. (2006). On the nomenclature of Bathyergidae and Fukomys n.ge. *(Mammalia: Rodentia). Zootaxa* **1142**, 51-55. doi:10.11646/zootaxa.1142.1.4

Maille, A. and Schradin, C. (2016). Survival is linked with reaction time and spatial memory in African striped mice. *Biol. Lett.* **12**, 20160346. doi:10.1098/rsbl.2016.0346

Malewski, S., Malkemper, E. P., Sedláček, F., Šumbera, R., Caspar, K. R., Burda, H. and Begall, S. (2018). Attracted by a magnet: Exploration behaviour of rodents in the presence of magnetic objects. *Behav. Processes* **151**, 11-15. doi:10.1016/j.beproc.2018.02.023

Martins, T. L. F., Roberts, M. L., Giblin, I., Huxam, R. and Evans, M. R. (2007). Speed of exploration and risk-taking behaviour are linked to corticosterone titres in zebra finches. *Horm. Behav.* **52**, 445-453. doi:10.1016/j.yhbeh.2007.06.007

Mehlhorn, K., Newell, B. R., Todd, P. M., Lee, M. D., Morgan, K., Braithwaite, V. A., Hausmann, D., Fiedler, K. and Gonzalez, C. (2015). Unpacking the exploration-exploitation tradeoff: a synthesis of human and animal literatures. *Decision* **2**, 191-215. doi:10.1037/dec0000033

Ming, G. and Song, H. (2005). Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223-250. doi:10.1146/annurev.neuro.28.051804.101459

Mirescu, C. and Gould, E. (2006). Stress and adult neurogenesis. *Hippocampus* **16**, 233-238. doi:10.1002/hipo.20155

Němec, P., Cveková, P., Benada, O., Wielkopolska, E., Olkowicz, S., Turlejski, K., Burda, H., Bennett, N. C. and Peichl, L. (2008). The visual system in subterranean African mole-rats (Rodentia, Bathyergidae): retina, subcortical visual nuclei and primary visual cortex. *Brain Res. Bull.* **75**, 356-364. doi:10.1016/j.brainresbull.2007.10.055

Oosthuizen, M. K. (2017). From mice to mole-rats: species-specific modulation of adult hippocampal neurogenesis. *Front. Neurosci.* **11**, 602. doi:10.3389/fnins.2017.00602

Oosthuizen, M. K. and Amrein, I. (2016). Trading new neurons for status: adult hippocampal neurogenesis in eusocial Damaraland mole-rats. *Neuroscience* **324**, 227-237. doi:10.1016/j.neuroscience.2016.03.020

Oosthuizen, M. K., Scheibler, A.-G., Bennett, N. C. and Amrein, I. (2013). Effects of laboratory housing on exploratory behaviour, novelty discrimination and spatial reference memory in a subterranean, solitary rodent, the Cape mole-rat (Georychus capensis). *PLoS ONE* **8**, e75863. doi:10.1371/journal.pone.0075863

Page, H., Sweeney, A., Pilko, A. and Pinter-Wollman, N. (2018). Underlying mechanisms and ecological context of variation in exploratory behaviour of the Argentine ant, *Linepithema humile*. *J. Exp. Biol.* **221**, jeb188722. doi:10.1242/jeb.188722

Patrick, S. C., Pinaud, D. and Weimerskirch, H. (2017). Boldness predicts an individual's position along an exploration-exploitation foraging trade-off. *J. Anim. Ecol.* **86**, 1257-1268. doi:10.1111/1365-2656.12724

Quartu, M., Pina Serra, M., Boi, M., Ibba, V., Melis, T. and Del Fiacco, M. (2008). Polysialylated-neural cell adnesion molecule (PSA-NCAM) in the human trigeminal ganglion and brainstem at prenatal and adult ages. *BMC Neurosci.* **9**, 108. doi:10.1186/1471-2202-9-108

Quinn, J. L., Cole, E. F., Bates, J., Payne, R. W. and Cresswell, W. (2012). Personality predicts individual responsiveness to the risks of starvation and predation. *Proc. R. Soc. Lond. B* **279**, 1919-1926. doi:10.1098/rspb.2011.2227

Russell, J. C., McMorland, A. J. C. and MacKay, J. W. B. (2010). Exploratory behaviour of colonizing rats in novel environments. *Anim. Behav.* **79**, 159-164. doi:10.1016/j.anbehav.2009.10.020

Safi, K. and Dechmann, D. K. N. (2005). Adaptation of brain regions to habitat complexity: a comparative analysis in bats (Chiroptera). *Proc. R. Soc. Lond.* **272**, 179-186. doi:10.1098/rspb.2004.2924

Scholzen, T. and Gerdes, J. (2000). The Ki-67 protein: from the known and the unknown. *J. Cell. Physiol.* **182**, 311-322. doi:10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9

Shors, T. J. (2009). Saving new brain cells. *Sci. Am.* **300**, 46-55. doi:10.1038/scientificamerican0309-46

Shumway, C. A. (2008). Habitat complexity, brain, and behavior. *Brain Behav. Evol.* **72**, 123-134. doi:10.1159/000151472

Shumway, C. A., Scace, J. G. and Bell, M. (2007). Cichlid fish differing in habitat complexity show spatial ability differences in a maze. *Soc. Neurosci. Abstr.* 817, 10.

Slomianka, L. and West, M. J. (2005). Estimators of the precision of stereological estimates: an example based on the CA1 pyramidal cell layer of rats. *Neuroscience* **136**, 757-767. doi:10.1016/j.neuroscience.2005.06.086

Spritzer, M. D., Daviau, E. D., Coneeny, M. K., Engelman, S. M., Prince, W. T. and Rodriguez-Wisdom, K. N. (2011). Effects of testosterone on spatial learning and memory in adult male rats. *Horm. Behav.* **59**, 484-496. doi:10.1016/j.yhbeh.2011.01.009

Thomas, H. G., Bateman, P. W., Scantlebury, M., Bennett, N. C. and Hayssen, V. (2012). Season but not sex influences burrow length and complexity in the non-sexually dimorphic solitary Cape mole-rat (Rodentia: Bathyergidae). *J. Zool.* **288**, 214-221. doi:10.1111/j.1469-7998.2012.00944.x

Torrents-Ticó, M., Bennett, N. C., Jarvis, J. U. M. and Zöttl, M. (2018). Sex differences in timing and context of dispersal in Damaraland mole-rats (*Fukomys damarensis*). *J. Zool.* **306**, 252-257. doi:10.1111/jzo.12602

van Dijk, R. M., Lazic, S. E., Slomianka, L., Wolfer, D. P. and Amrein, I. (2015). Large scale phenotyping links adult neurogenesis to the reaction to novelty. *Hippocampus* **26**, 646-657. doi:10.1002/hipo.22548

Vanden Broecke, B., Borremans, B., Mariën, J., Makundi, R. H., Massawe, A. W., Leirs, H. and Hughes, N. K. (2018). Does exploratory behaviour or activity in a wild mouse explain susceptibility to virus infection? *Curr. Zool.* **65**, 585-592. doi:10.1093/cz/zox053

Voigt, C. (2014). An example of burrow system architecture of dispersing Damaraland mole-rats. *Afr. Zool.* **49**, 148-152. doi:10.1080/15627020.2014.11407627

Walsh, R. N. and Cummins, R. A. (1976). The open-field test: a critical review. *Psychol. Bull.* **83**, 482-504. doi:10.1037/0033-2909.83.3.482

West, M. J., Slomianka, L. and Gundersen, H. J. G. (1991). Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat. Rec.* **231**, 482-497. doi:10.1002/ar.1092310411

Yaskin, V. A. (2011). Seasonal changes in hippocampus size and spatial behavior in mammals and birds. *Biol. Bull. Rev.* **1**, 279. doi:10.1134/S2079086411030108

Zhao, C., Deng, W. and Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell* **132**, 645-660. doi:10.1016/j.cell.2008.01.033