CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Laterality in the Upper Galilee Mountains blind mole rat *Nannospalax galili*

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled “Laterality in the Upper Galilee Mountains blind mole rat *Nannospalax galili*” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 16.8.2023.

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Maja Rendulić

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Abstract

Lateralization is the differential use of one side of the body, occurring as a result of asymmetries in the brain. Environmental factors, as well as various aspects of animals' biology, ecology and behaviour can have an effect on laterality, such as sociality, predation risk, sex, personality, genetics, hormones, stress and captivity, which can be detected through turning biases. In the current study, individual and population-level laterality is examined in the Upper Galilee Mountains blind mole rat *Nannospalax galili*, a species presumed to be undergoing ecological speciation with the diverging populations inhabiting abutting rendzina and basaltic soil. Animals' turning biases were evaluated either in a Y- or a T-maze, and their reaction time was measured, i.e. the time necessary to make the choice. Individual laterality was evaluated, and comparisons were made between groups based on various characteristics (e.g. sex, captivity, locality, soil type of origin). A significant right-side preference was detected in all the individuals together. The wild-caught group showed an overall stronger lateralization bias regardless of the side, which may be due to an effect of captivity. The basaltic-soil mole rats showed a right-side preference while rendzina ones showed no preference. Reaction time was significantly higher in basaltic-soil animals, which may reflect their vigilance and caution due to living under higher population densities. These results provide further insight into the distinctions between the basaltic and rendzina-soil blind mole rats.

Key words: Basaltic and rendzina soil, behavioural asymmetry, ecological speciation, laterality, *Nannospalax galili*, subterranean rodent, turning biases

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List of the abbreviations used in the thesis

L_I	relative laterality index
L_A	absolute laterality index

1. Introduction and Literature Review

1.1. Introduction

Lateralization is the differential processing of information by the left and right brain hemispheres, resulting in a function being localized to one side of the body (Rogers 2002). It can be detected through biases in responses, for examples turning biases (García-Muñoz et al. 2013; Jacobs & Oosthuizen 2021), handedness and paw preferences (Hopkins et al. 2006; Manns et al. 2021), asymmetrical use of sensory organs such as eyes, nostrils, ears and antennae (Brown et al. 2004; McGreevy & Rogers 2005; Frasnelli et al. 2012). Based on their occurrence at the population-level, these asymmetries can be divided into the following categories: 1) no asymmetry, resulting from the individuals not showing a side preference, 2) antisymmetry, when half of the individuals show a bias for the left side, while the other half shows a bias for the right, and 3) population-level asymmetry, when the majority of individuals are lateralized in the same direction (Rogers 2002; Ströckens et al. 2013). An individual benefits from being lateralized since the duplication of information processing on both sides of the brain is avoided by specializing each hemisphere for separate functions, enabling the animal to simultaneously preform more tasks (Vallortigara 2000). A summary of the general pattern of lateralized functions is given in Table I. On the population-level however, being lateralized may be a vulnerability if a predator learns and takes advantage of a directional bias (Vallortigara & Rogers 2005); the opposite is true as well – the prey may predict the behaviour and the hunting strategy of a predator (Hori 1993). It is hypothesised by the Evolutionary Stable Strategy (Maynard Smith 1982) that the benefits of being lateralized on the population-level for social species outweigh the costs – by having their biases aligned in the same direction, the individuals within a group can coordinate better (Frasnelli & Vallortigara 2018). The dilution effect is also an important factor, as an individual in a large group has a lower chance of being captured by a predator (Foster & Treherne 1981).

Table I. A summary of the general pattern of lateralized functions between the two brain hemispheres.

Left brain hemisphere	Right brain hemisphere
Behaviour under familiar settings	Novel, unexpected stimuli
Considered responses	Rapid responses
Positive emotions	Negative emotions
Foraging, food reward, prey capture	Aggression, agonistic behaviour
Inhibition of aggression and intense emotions	Predator detection and escape
	Fear, withdrawal, avoidance
	Spatial information

Not many studies can be found on laterality in subterranean rodents (Schleich 2016; Jacobs & Oosthuizen 2021; Jacobs & Oosthuizen 2023); one of the reasons may be difficulties in studying these animals in the wild and keeping them in captivity (Burda et al. 1990). However, they could be excellent models for studying this phenomenon – living in complex underground burrow systems, they require a highly efficient and developed sense of spatial orientation and navigation (Hildebrand 1985). They exhibit a wide spectrum of social systems, ranging from solitary to social and eusocial species (Lacey 2000). Some species are extremely aggressive, territorial and xenophobic (Guttman et al. 1975; Begall et al. 1996; Bennett et al. 2006). Those aspects may influence behavioural asymmetries as they are mainly dominated by the right-brain hemisphere (Rogers 2002; Rogers et al. 2013). In the highly social species, there are differences between members of the same colony – morphological between the reproductive and non-reproductive individuals, but also behavioural among the non-breeders based on their size and age (e.g. Faulkes et al. 1991; Jarvis 1991; Lacey & Sherman 1991; O'Riain et al. 1996; Lövy et al. 2013, Šklíba et al. 2016a, Zöttl et al. 2016), which consequently may lead to differences in laterality. In the wild, they are exposed to predators such as reptiles, small mammals and birds of prey (e.g. Busch et al. 2000; Németh et al. 2016 and references therein), but in captivity, that effect is absent, which may result in changes in cognitive performance and thus in changes in laterality (Bibost & Brown 2014; van Schaik et al. 2016). Subterranean rodents are being kept in captivity in many institutions worldwide, including universities, research centres and zoological gardens (Šumbera, personal communication; ZIMS; Zootierliste), so laterality can also be explored in regard to captivity. By examining laterality of different species of subterranean rodents and among different individuals of the same species, comparisons can be made based on different aspects of their biology, ecology and behaviour to better understand this phenomenon in mammals.

Figure 1. *Nannospalax galili* (taken by Lövy, 2015).

The Upper Galilee Mountains blind mole rat *Nannospalax galili* (Figure 1) is an ideal model to study laterality for multiple reasons. It is solitary, highly aggressive, territorial and xenophobic (Nevo et al. 1986) – all of those qualities could be manifested as behavioural asymmetries with a left-side bias, considering they are primarily processed by the right-brain hemisphere (Rogers 2002; Rogers et al. 2013). Importantly, this species is presumed to be currently undergoing ecological speciation. The pattern of two separate populations inhabiting two different types of soil that adjoin each other without a physical barrier is consistent across two recently studied localities (Hadid et al. 2013; Lövy et al. 2015; Lövy et al. 2017; Lövy et al. 2019). Many differences driven by adaptations to life in two ecologically different soil types (for details see next chapter) have been detected between the two populations, including behavioural, genetic and physiological (Polyakov et al. 2004; Hadid et al. 2013; Li et al. 2015; Šklíba et al.

2016b); all of which might lead to differences in laterality. Considering all this information, it is surprising that laterality has not yet been studied in any species of blind mole rats.

1.2. Literature review

1.2.1. Subfamily Spalacinae

The blind mole rats, Spalacinae, are Old World fossorial herbivorous rodents, highly specialized for life underground (Nevo 1999; Musser & Carleton 2005). They feed on subterranean storage organs of plants, such as tubers, bulbs, onion and roots (e.g. Nevo 1999). The present study will follow the approach of authors recognizing two genera within the Spalacinae subfamily – *Nannospalax* and *Spalax* (Savić 1982, Savić & Soldatović 1984, Németh et al. 2009). The former genus includes smaller species with quite variable karyotypes (2n = 36-62) living in south-eastern Europe, eastern Mediterranean and Middle East, while species belonging to the latter genus are larger, found only in continental Eurasia, and have higher and less diverse diploid chromosome numbers (2n = 60 or 62) (Savić & Soldatović 1984, Hadid et al. 2012; Arslan et al. 2016).

The superspecies *Nannospalax ehrenbergi* is widely distributed primarily throughout the eastern Mediterranean, ranging from Israel in the south to Turkey in the north, with isolated populations in coastal northern Africa. It is listed as Data Deficient by the IUCN Red List of Threatened Species, due to uncertainties with the taxonomy of the species – it represents an actively speciating taxon (Schlitter et al. 2017). The *N. ehrenbergi* complex has split into four chromosomal forms during the last 2-2.3 million years (Nevo 1999). In Israel, four chromosomal forms are recognised as separate species (Nevo et al. 2001), occurring in ecologically different regions along the aridity gradient which is increasing eastwards and southwards. Each of those proposed species has adapted to their local ecological conditions and a different combination of temperature and humidity – *N. golani* (2n = 54) in cool and dry Golan Heights, *N. galili* (2n = 52) in cool and humid Upper Galilee Mountains, *N. carmeli* (2n = 58) in warm and humid Lower Galilee Mountains and central part of the country, and *N. judaei* (2n =

60) in warm and dry Samaria Mountains, Judea and northern Negev desert (Nevo 1973, Nevo 1991, Nevo 1999, Nevo et al. 2001) (Figure 2).

Figure 2. Distribution of the chromosomal forms of *Nannospalax ehrenbergi* in Israel (taken from Singaravelan et al. 2013).

1.2.2. Speciation in Spalacinae

Speciation in Spalacinae has generally been assumed to be allopatric and/or peripatric (Nevo 1991; Nevo et al. 2001), meaning a geographic barrier is involved in isolation of the forms and gradual formation of separate species. Surprisingly, a case of ongoing ecological speciation with an ongoing gene flow, i.e. speciation without a physical barrier within populations, has been proposed in one of the four species, *N. galili* (Polyakov et al. 2003; Hadid et al. 2013). The species originates from the Upper Galilee Mountains in the northern part of Israel, where two basalt plateaus exist – Dalton and Alma. Reddish-brown basaltic soil is covering the basaltic bedrocks. The basaltic islands are surrounded by much older chalk and marl sedimentary rocks and covered by pale rendzina soil (Levitte 2001; Weinstein et al. 2016). The two populations of blind mole rats live in the two ecologically different types of soil, which abut each other but with no topographic barrier in between at two localities near the villages of Gush Halav and Rihaniya (Figure 3). Basaltic soil is more difficult to dig through than rendzina due to a higher proportion of water and clay making the soil plastic and sticky, but it offers a higher diversity and availability of underground plant storage organs the staple diet of blind mole rats (Lövy et al. 2015; Lövy et al. 2017). Owing to a larger food supply, the population density of mole rats is several times higher in basaltic soil than in rendzina, despite it being a more challenging environment in terms of digging costs (Hadid et al. 2013; Lövy et al. 2015). The differences in physical parameters, characteristics of food resources and population structure of the blind mole rats seem to be consistent across the area where those two soils meet, which is an important prerequisite for a potential ecological speciation (e.g. Hadid et al. 2013; Lövy et al. 2015; Lövy et al. 2017; Lövy et al. 2019).

Figure 3. Typical vegetation on basaltic (left) and rendzina soil (right) (taken from Lövy et al. 2015).

The two blind mole rat populations have also been found to exhibit different behaviours. Their burrowing patterns reflect the food availability, soil conditions and population density – basaltic-soil mole rats construct shorter, but more complex and branched tunnels, while rendzina mole rats dig longer and more linear tunnels (Lövy et al. 2015). Due to an around five-times higher population density, mole rats in basaltic soil are spatially limited by each other’s burrow systems and can only increase the complexity of burrows within their own territories, while the ones in rendzina have a much lower chance of meeting a conspecific (Lövy et al. 2015). Basaltic-soil mole rats have also been found to push more soil into the aboveground mounds, dig tunnels at a higher rate and overall have a higher level of outside-nest activity than rendzina ones, which is explained by a stronger territoriality due to a higher population density (Šklíba et al. 2016b).

2. Aims of the Thesis

The objective of this study was to examine and compare individual and population-level laterality of *Nannospalax galili* from rendzina and basaltic soils that are likely undergoing ecological speciation. The main aims were 1) to determine whether there is a laterality preference in turning biases on a species, population and individual level, 2) to test whether sex, captivity, locality and soil type of origin have an effect on the direction or strength of laterality, and 3) to test whether the reaction time of the animals differs in regard to the beforementioned parameters. For these purposes, captive and wild-caught *N. galili* originating from two localities, in both of which rendzina and basalt soil types are present, were tested for their turning preferences in either a Y- or a T- maze. I expected no significant overall directional bias among the blind mole rats (Kimchi & Terkel 2003; Frasnelli & Vallortigara 2018). I presumed the wild-caught group would show a somewhat stronger lateralization bias, regardless of the side (Jacobs & Oosthuizen 2021; Jacobs & Oosthuizen 2023). Considering all the parameters in which the rendzina and basaltic-soil populations differ (Hadid et al. 2013; Lövy et al. 2015; Lövy et al. 2017), I assumed there would be a difference regarding laterality as well, either in the degree of direction of bias – I anticipated a left-turning bias among the basaltic-soil, but not rendzina animals. The left-side preference would indicate a higher usage of the right brain hemisphere which is responsible of aggressive and agonistic behaviours (Rogers 2002; Rogers et al. 2013; Jacobs & Oosthuizen 2021), and the basaltic-soil mole rats are said to be the more active, aggressive and territorial of the two populations (Šklíba et al. 2016b). For the same reason, I would expect the basaltic-soil mole rats to show a faster passage through the maze.

3. Methods

3.1. Study microsite

All of the experimental animals were captured in the eastern Upper Galilee mountains in the north of Israel. The local climate is Mediterranean, characterized by a rainy winter (October to March) followed by a hot and dry summer (April to Septermber). Local mean annual rainfall is 714 ± 163 mm, majority of which falls from December to March (Fick & Hijmans 2017).

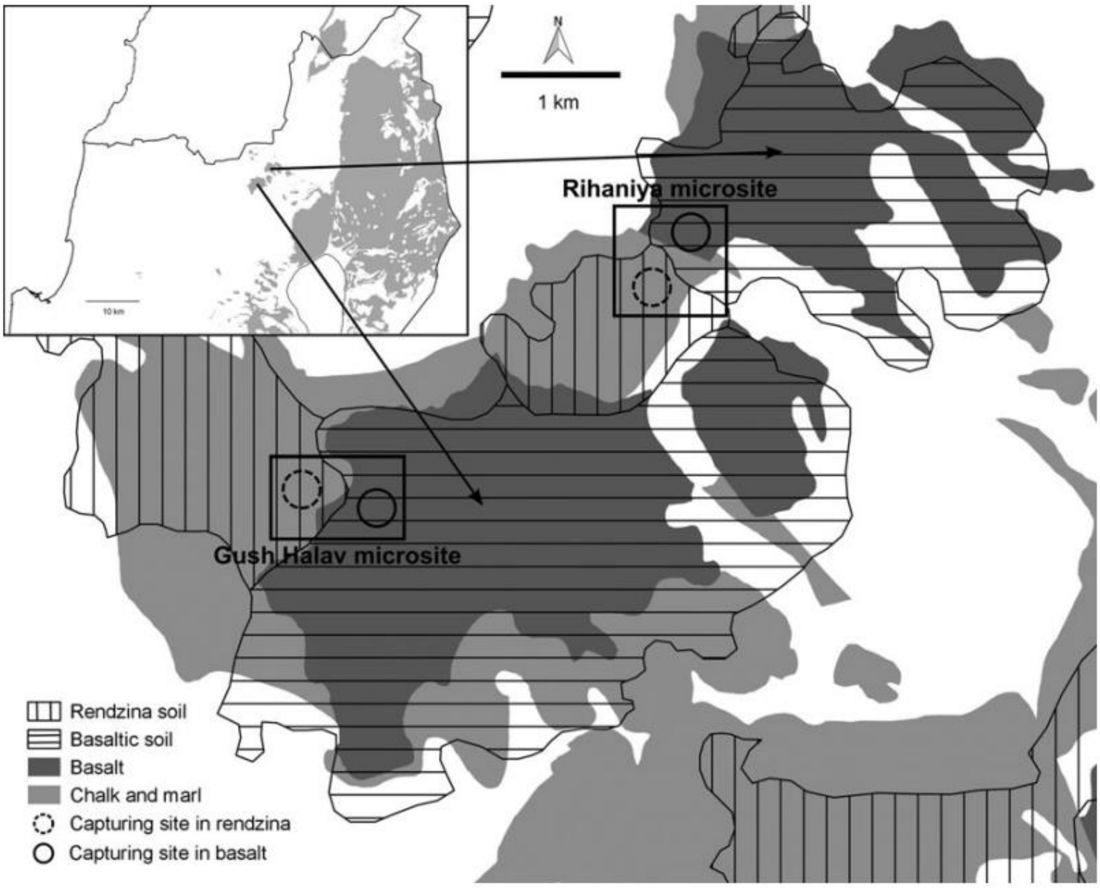

Figure 4. Geological and soil map of the capture sites in Israel (taken from Lövy et al. 2017).

The captures took place during the transition period between rainy and dry seasons (November – December or March – April), which is when the conditions for conducting the fieldwork and setting the traps are most favourable since the mole rats are still very active, and the soil is not as wet as in January and February. Their presence was determined by observing fresh mounds of excavated soil.

The animals were captured from two localities, in both of which two different types of soil are present and abutting each other (Figure 4). The first locality near the village of Gush Halav (33.023°N, 35.454°E, altitude 800 m) is partly situated on the Dalton plateau covered by basaltic soil, and partly on surrounding rendzina soil of chalk and marl bedrocks. The other locality is near the village of Rihaniya (33°02.5'N, 35° 29.2'E, altitude 760 m), where basaltic soil covering the Alma plateau abuts pale rendzina on chalk and marl rocks (see e.g. Levitte 2001).

3.2. Animals

In total, 57 animals have been used. The captive group of animals (8 females and 1 male) was captured using modified Hickman traps near the village of Gush Halav (33.023°N, 35.454°E, altitude 800 m) from November to mid-December of 2015. One female originated from rendzina, the others from basaltic soil. Since then, they have been housed individually in plexiglass tunnels connected to a central nesting box and kept at the Department of Zoology of the Faculty of Science, University of South Bohemia in České Budějovice. The tunnels are 8 cm wide, 8 cm tall, and filled with horticultural peat. Tissue paper is provided regularly and used by the animals as nesting material. The room is maintained under constant temperature (24±1°C) and humidity (40 – 60%), at a photoperiod of 12L/12D. They are fed *ad libitum* three times per week with a mix of dry pellets and vegetables such as carrots, sweet potatoes, beetroot, potatoes, lettuce and onions. Water is not provided, since mole rats obtain water from their food.

The wild group (23 males and 25 females) was captured using modified Hickman traps in March 2022 and housed individually in plastic boxes (42 × 26 × 18 cm) filled with wood shavings, and tissues and paper rolls for enrichment, nesting material and shelter. They were kept under constant conditions (temperature: 20±1°C; humidity: 40 – 60%; photoperiod 12L:12D) and fed *ad libitum* with carrots, sweet potatoes, lettuce and oat flakes. 34 animals (16 males and 18 females) were captured near the village of Gush Halav, and 14 of them (7 males and 7 females) near the village of Rihaniya. Since in both localities, two different types of soil are present – rendzina and basaltic soil – animals were captured from both. In total, 24 originated from rendzina (17 from Gush Halav, 7 from Rihaniya) and 24 from basaltic soil (17 from Gush Halav, 7 from Rihaniya). All animals were sexed, weighed and measured (body and hind-foot length). After the experiments, the mole rats were transported to the Faculty of Science, University of South Bohemia in České Budějovice for further research purposes.

3.3. Ethics statement

Since *Nannospalax galili* is not listed as a protected or an endangered species in Israel, no special permissions were required for capturing or handling animals in the wild. Mole rats are, in fact, considered to be an agricultural pest in that region. The study was approved by the University of South Bohemia (Permit MSMT-35731/2019-4).

3.4. Experimental design

The laterality tests with the captive and the wild group were conducted at the Faculty of Science, University of South Bohemia in České Budějovice in June and July 2021 and in the eastern Upper Galilee mountains in March 2022, respectively.

The two apparatuses used for the experiments are depicted in Figure 5. In case of the captive group, it was a Perspex Y-maze consisting of a starting tunnel (8 x 8 x 50 cm) connected to two arms (8 x 8 x 50 cm) under an 80 degree angle. Because of a low sample size (9 animals), the test was repeated 9 or 10 times with each individual. The animals from the wild group were tested in a T-shape Perspex maze, which consisted of a starting box (18,5 x 18,5 x 12 cm) connected by a short tunnel (8 x 8 x 10 cm) to two arms (8 x 8 x 40 cm each). In this case, the test was repeated 5 times per individual.

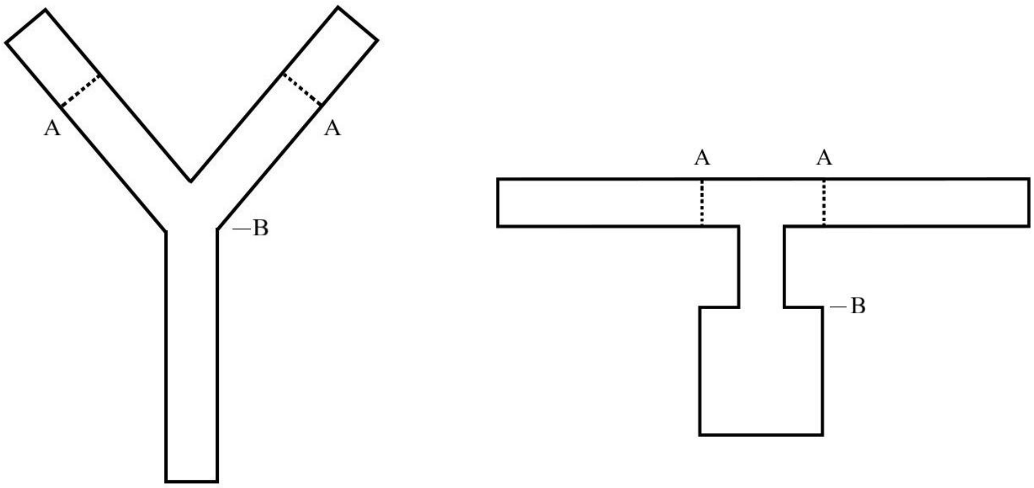

Figure 5. Y-maze (left) and T-maze (right) used for testing the captive and wild-caught groups of blind mole rats, respectively.

Before the start of a trial, an animal was placed in the starting part of the maze and kept away from the rest of the maze by a removable partition for five minutes. After the acclimatization period, the partition was removed and the animal was allowed to freely explore the rest of the maze. This version of maze testing was spontaneous, meaning the animal did not recieve any reinforcement to increase activity and go through the maze, neither positive nor negative. The trial was considered over when the animal would pass point A of an arm (see Figure 5). The maze was randomly rotated between trials to eliminate the possible influence of the Earth's electromagnetic field on the mole rat's decision. After one animal, the maze was disenfected with 35% ethanol, rinsed with water and thoroughly dried with paper towels. Each individual had a break of at least an hour between two trials. Since blind mole rats are diurnal, tests were always performed between 8am and 8pm, which encompasses their main activity period in the wild (Rado et al. 1993; Šklíba et al. 2016b). All the experiments were video recorded using a Sony camera placed above the maze, and the videos were later used for data analysis (see below).

3.5. Data acquisition

Data was manually extracted from the videos after the completion of the experiments. Two measures of asymmetry were used – first turn of the head immediately after entering the junction part of the maze, and the final choice, once the animal passed the point A of the maze with its front legs. The relative laterality index (L_I) was calculated for each individual, for both measures of laterality, according to the following formula:

$$L_I = \frac{\text{number of left turns} - \text{number of right turns}}{\text{total number of trials}} \text{ x } 100$$

In case the turn could not be determined (for example, the animal took longer than 30 minutes to go through the maze, fell asleep in the starting box, or it entered the maze backwards), the trial was disregarded, marked as non-valid and the total number of trial for that individual was reduced. L_I ranges from -100 to 100, with -100 meaning the individual turned right in all trials and 100 meaning it turned left in all trials. Absolute laterality (L_A) is the absolute value of L_I and represents the strength of laterality, regardless of whether the preference is to left or right. The L_A value of 0 indicates that the individual equally turned to both sides and shows no side preference, while the value of 100 indicates the individual chose the same direction in all trials and shows a strong preference. Trial duration was measured as time elapsed from the moment the animal left the starting part or realised the partition was removed (meaning it passed point B with the tip of its head) until it made the final choice (see Figure 5).

3.6. Statistical analyses

The relative laterality index for both measures of lateralization (i.e., first turn of the head after entering the maze junction, and final turn of the animal) was used in testing lateralization. Each mole rat was tested for individual laterality in a two-tailed binomial test. A two-tailed t-test was used to determine whether all the individuals together show a non-random turning bias. The same test was repeated by pooling the individuals by their characteristics, i.e. separately for wild and captive animals, males and females, Gush Halav and Rihaniya animals, and basalt and rendzina animals. A prerequisite to the t-test was either a sufficiently large sample size (>30 individuals), or normality of the data not being rejected by the Shapiro-Wilk test. In case neither one of these assuptions was met, the Wilcoxon rank-sum test (Mann-Whitney U test) was used as a non-parametric equivalent to the t-test. The relative indices (L_I) were compared between the beforementioned groups in an unpaired two-samples t-test if 1) the sample sizes were sufficiently large or the data met the assumption of normality, and 2) the F-test did not reject that the variances of the two groups were equal. In case one of those assumptions was rejected, the Mann-Whitney U test was used as a non-parametric equivalent. Since the absolute laterality indices (L_A) were not normally distributed, the Mann-Whitney U test was used as a non-parametric equivalent of an unpaired two-samples t-test to compare the degree of lateralization among the beforementioned groups. The Mann-Whitney U test was also used in comparing reaction time between the groups; it was not possible to use the t-test as the F-test rejected that the variances between the groups were equal. The data were analysed using the RStudio software (R Core Team 2023).

4. Results

In total, 57 animals went through 323 trials. 83 trials were conducted with the 9 individuals from the captive group, and 240 trials with the 48 wild-caught individuals. 253 and 70 trials were done with animals from Gush Halav and Rihaniya, respectively. Mole rats from basaltic and rendzina soil performed 194 and 129 trials, respectively. 16 of the trials were marked as non-valid, meaning the experiment was terminated and disregarded because the animal fell asleep during testing, did not go through the maze within half an hour, or entered the maze backwards.

Table II. Individual turning biases of *Nannospalax galili*. For each individual, the total number of left and right full and head turns is given, along with the two-tailed binomial probability for individual lateralization (p-value or ns for not significant). One, two and three asterisks indicate a significance level at 0.1, 0.05 and 0.01, respectively. Reaction time represents a period from the moment the animal left the starting box until it made the final turn (for details see Methods).

Mole rat ID	Wild/ captive	Sex	Locality	Soil type	Left turn	Right turn	Binomial probability	Head left	Head right	Binomial probability	Reaction time (s)
JB01	wild	F	Gush Halav	basalt	3	0	ns	1	2	ns	172.4
JB02	wild	M	Gush Halav	basalt	1	4	ns	3	2	ns	19.6
JB03	wild	M	Gush Halav	basalt	0	3	ns	2	1	ns	60.1
JB04	wild	M	Gush Halav	basalt	1	4	ns	2	3	ns	25.5
JB05	wild	F	Gush Halav	basalt	2	1	ns	1	2	ns	52.8
JB06	wild	M	Gush Halav	basalt	3	2	ns	2	3	ns	73.9
JB07	wild	F	Gush Halav	basalt	0	5	p=0.0625*	3	2	ns	721.4
JB08	wild	F	Gush Halav	basalt	0	5	p=0.0625*	4	1	ns	15.6
JB09	wild	M	Gush Halav	basalt	2	3	ns	3	2	ns	382.8
JB10	wild	F	Gush Halav	basalt	0	5	p=0.0625*	3	2	ns	6.6
JB11	wild	F	Gush Halav	basalt	1	2	ns	1	2	ns	16.7
JB12	wild	F	Gush Halav	basalt	2	2	ns	1	3	ns	7
JB13	wild	F	Gush Halav	basalt	3	2	ns	3	2	ns	13.9
JB14	wild	M	Gush Halav	basalt	1	4	ns	3	2	ns	26.3
JB15	wild	F	Gush Halav	basalt	4	1	ns	3	2	ns	18.2
JB16	wild	F	Gush Halav	basalt	1	2	ns	0	3	ns	7.2
JB17	wild	F	Gush Halav	basalt	2	3	ns	4	1	ns	29.4
JR01	wild	M	Gush Halav	rendzina	2	3	ns	3	2	ns	4.9
JR03	wild	F	Gush Halav	rendzina	1	4	ns	1	4	ns	64.2
JR04	wild	F	Gush Halav	rendzina	4	1	ns	1	4	ns	7.7
JR05	wild	F	Gush Halav	rendzina	1	4	ns	5	0	p=0.0625*	6.6
JR06	wild	M	Gush Halav	rendzina	0	5	p=0.0625*	1	4	ns	2.8
JR07	wild	M	Gush Halav	rendzina	4	0	ns	4	0	ns	4.8
JR09	wild	M	Gush Halav	rendzina	1	3	ns	2	3	ns	19.7
JR10	wild	F	Gush Halav	rendzina	3	2	ns	2	3	ns	7
JR11	wild	M	Gush Halav	rendzina	2	3	ns	3	2	ns	9
JR12	wild	F	Gush Halav	rendzina	4	1	ns	3	2	ns	7
JR14	wild	F	Gush Halav	rendzina	4	1	ns	0	5	p=0.0625*	215.1

JR15	wild	F	Gush Halav	rendzina	2	3	ns	0	5	p=0.0625*	5.6
JR17	wild	M	Gush Halav	rendzina	1	4	ns	5	0	p=0.0625*	7
JR19	wild	M	Gush Halav	rendzina	5	0	p=0.0625*	4	1	ns	9
JR20	wild	M	Gush Halav	rendzina	2	3	ns	3	2	ns	3.5
JR21	wild	M	Gush Halav	rendzina	2	3	ns	2	3	ns	10.6
JR22	wild	M	Gush Halav	rendzina	3	2	ns	2	2	ns	6.9
RB01	wild	F	Rihaniya	basalt	2	3	ns	4	1	ns	6.3
RB02	wild	M	Rihaniya	basalt	1	4	ns	2	3	ns	8.3
RB03	wild	F	Rihaniya	basalt	2	3	ns	2	3	ns	14
RB04	wild	F	Rihaniya	basalt	5	0	p=0.0625*	4	1	ns	7.9
RB05	wild	M	Rihaniya	basalt	2	3	ns	2	3	ns	10.7
RB06	wild	F	Rihaniya	basalt	0	5	p=0.0625*	1	4	ns	3.7
RB07	wild	F	Rihaniya	basalt	1	3	ns	2	2	ns	34.8
RR01	wild	M	Rihaniya	rendzina	2	3	ns	0	5	p=0.0625*	14.4
RR02	wild	M	Rihaniya	rendzina	3	2	ns	5	0	p=0.0625*	11
RR04	wild	M	Rihaniya	rendzina	1	4	ns	2	3	ns	11.2
RR05	wild	F	Rihaniya	rendzina	4	1	ns	3	2	ns	10
RR06	wild	M	Rihaniya	rendzina	3	2	ns	5	0	p=0.0625*	6.1
RR07	wild	F	Rihaniya	rendzina	2	3	ns	1	4	ns	6
RR08	wild	M	Rihaniya	rendzina	3	2	ns	3	2	ns	45.7
S125	captive	F	Gush Halav	rendzina	4	5	ns	4	5	ns	7.7
S137	captive	F	Gush Halav	basalt	3	5	ns	0	8	p<0.01***	35.1
S142	captive	F	Gush Halav	basalt	3	5	ns	5	3	ns	43.2
S149	captive	M	Gush Halav	basalt	2	8	ns	8	2	ns	12.9
S150	captive	F	Gush Halav	basalt	5	5	ns	5	5	ns	9.6
S155	captive	F	Gush Halav	basalt	4	4	ns	8	1	p=0.0391**	57.3
S157	captive	F	Gush Halav	basalt	3	7	ns	2	8	ns	41.6
S158	captive	F	Gush Halav	basalt	2	7	ns	2	7	ns	20.6
S174	captive	F	Gush Halav	basalt	5	4	ns	6	3	ns	13.2

The relative laterality index (L_I) was used in determining individual and group-level turning biases. Seven mole rats showed a side preference for the final turn, and nine different mole rats showed a preference for the head turn at the significance level of 0.1 (Table II). All individuals grouped together showed a significant right-side turning bias – in total, 178 trials finished with the animal choosing the right arm of the maze, while the left arm was chosen in 129 trials (see Table III for statistical details). A statistically significant side bias was found for the captive population for the final turn – 50 turns in total were made to the right side, while 31 turn was to the left. A significant side preference was found for the basaltic-soil population in case of the final turn. In total, basaltic-soil animals made more right (114) than left turns (66), while rendzina mole rats had a similar number of turns to both sides (64 and 63, respectively). A higher number of trials was marked as non-valid in the basaltic than in the rendzina group – 14 trials among the basaltic-soil group (7.2% of total basalt trials) compared to 2 from the rendzina group (1.6%). A significant preference to the right was found for males in case of the final turn – in total, males made 74 turns to the right and 48 to the left. A marginally significant right-side preference was found for the group originating from Gush Halav (140 right-side turns, 98 left-side turns). No statistically significant preference was found for the final turn in the wild group, females, animals originating from Rihaniya, and rendzina-soil mole rats. No significant side preference was detected for the first head turn in any of the groups.

Table III. Group-level turning biases. Results of two-tailed t-tests/Mann Whitney U test, used to determine whether certain groups of mole rats show non-random turning biases. One, two and three asterisks indicate a significance level at 0.1, 0.05 and 0.01, respectively.

Group	Final turn		Head turn	
	t (df) / W	p-value	t (df) / W	p-value
All individuals	-2.02 (56)	0.04771**	0.079 (56)	0.9375
Captive	-2.72 (8)	0.02638**	-0.17 (8)	0.8678
Wild	-1.54 (47)	0.1298	0.17 (47)	0.8685
Males	77.5	0.03616**	1.56 (23)	0.1328
Females	-0.98 (32)	0.3364	-1.13 (32)	0.2663
Gush Halav	-1.87 (42)	0.06915*	-0.077 (42)	0.9392
Rihaniya	-0.77 (13)	0.4529	0.27 (13)	0.7929
Basalt	-2.75 (31)	0.00975***	-0.061 (31)	0.9518
Rendzina	-0.004 (24)	0.9967	155.5	0.8848

When comparing individual laterality biases between groups, a marginally significant difference was detected between males and females for the head turn, and between the basaltic-soil and rendzina groups for the final turn (see Table IV for statistical details). No significant difference was found between captive and wild individuals for either of the measures of lateralization, males and females for the final turn, mole rats from the two localities for either of the measures, mole rats from the two different types of soil for the first head turn.

Table IV. Comparisons of turning biases between groups. Results of the unpaired two-samples t-tests/Mann-Whitney U test, used to test whether there is a difference in relative laterality indices (L_R) between the groups of mole rats. One asterisk indicates a significance level at 0.1.

Groups compared for L_R	Final turn		Head turn	
	t (df) / W	p-value	t (df) / W	p-value
Captive/wild	0.51	0.6096	0.23 (55)	0.8161
Males/females	0.82	0.4130	-1.89 (55)	0.0640*
Gush Halav/Rihaniya	-0.29 (55)	0.7720	-0.29 (55)	0.7746
Basalt/rendzina	-1.82 (55)	0.0742*	-0.17	0.8689

A significant difference was found between the captive and wild-caught group in the degree of lateralization for the final turn when tested in a Mann-Whitney U test, with the wild-caught group showing a stronger lateralization bias than the captive group (Figures 6 and 7, Table V). No significant differences were found for the head turn, nor were they found among any of the other groups compared for either of the measures of lateralization. Reaction time was significantly different between basaltic-soil and rendzina animals – it took a significantly longer time for the mole rats from basaltic-soil to go through the maze than it did for rendzina ones (Figures 8 and 9). No significant differences were found between reaction times among the other groups. Detailed results are shown in Table V.

Table V. Comparisons of the degree of lateralization and reaction times between groups. Results of the Wilcoxon rank-sum test (Mann-Whitney U test), used to compare groups of mole rats regarding their absolute laterality indices (L_A), and the reaction times. Two and three asterisks indicate a significance level at 0.05 and 0.01, respectively.

Groups compared	L_A final turn		L_A head turn		Reaction time	
	W	p-value	W	p-value	W	p-value
Captive/wild	122.5	0.0361**	232.5	0.7161	286	0.1299
Males/females	387	0.8875	459	0.2941	439	0.4952
Gush Halav/Rihaniya	329	0.5995	292.5	0.8776	371	0.2000
Basalt/rendzina	427.5	0.6547	341.5	0.3327	610	0.0005***

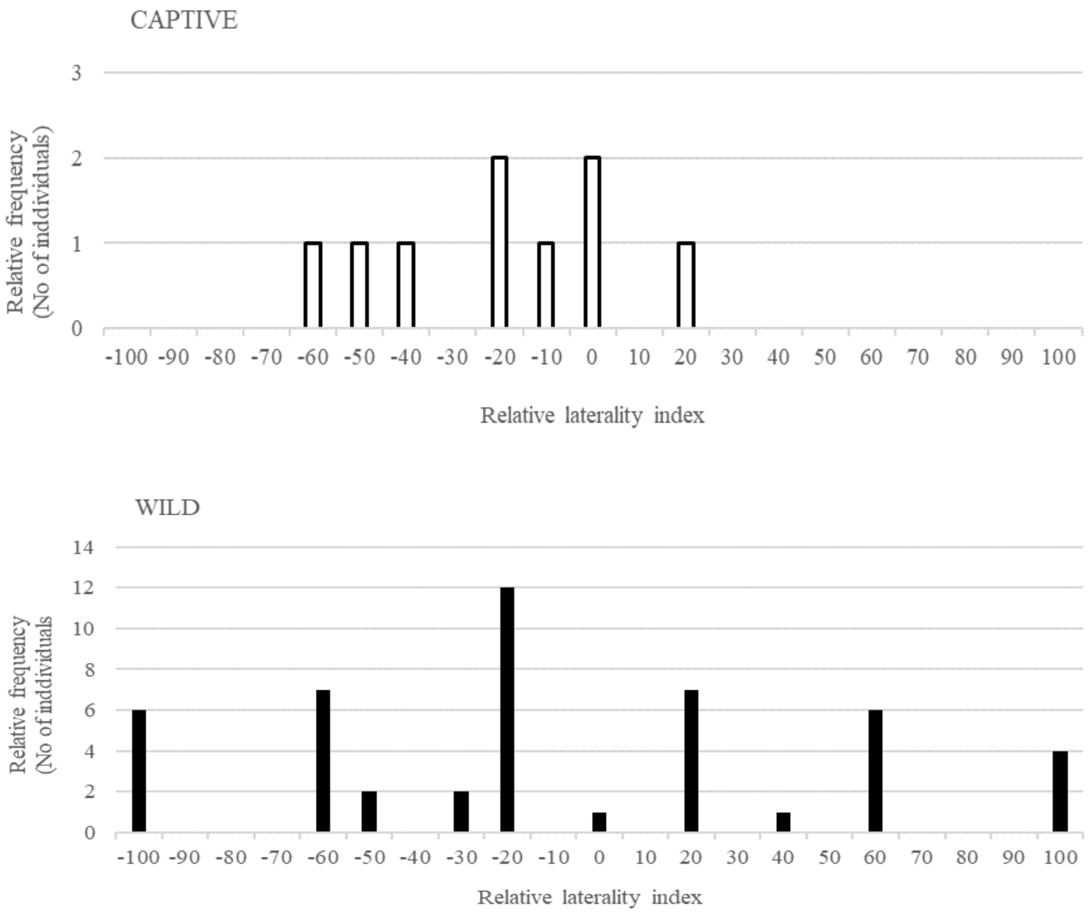

Figure 6. The relative frequency of individual laterality for captive (n=9; white bars) and wild-caught (n=48; black bars) *Nannospalax galili* along a relative laterality index. The Y-axis represents the number of individuals with the same relative laterality index, and the X-axis represents right-turning on the left side, with increased turning to the left as the index increases. A relative laterality index value of 0 means the individual shows no side preference, turning an equal number of times to the right and to the left.

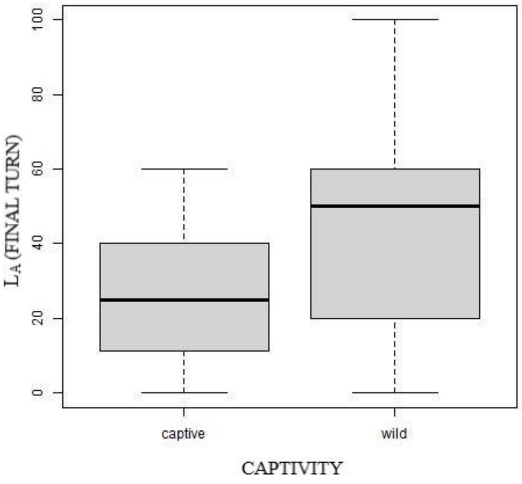

Figure 7. Absolute laterality index (L_A) for the final turn of captive and wild-caught *N. galili*. Higher values represent a stronger preference, while zero represents no side preference.

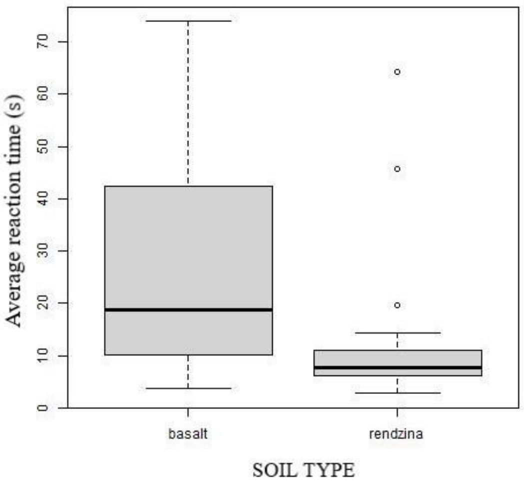

Figure 8. Average reaction time of basaltic-soil and rendzina *N. galili*. Individuals with a reaction time higher than 80 seconds (one rendzina and three basaltic-soil animals) were omitted for a clearer graphical representation.

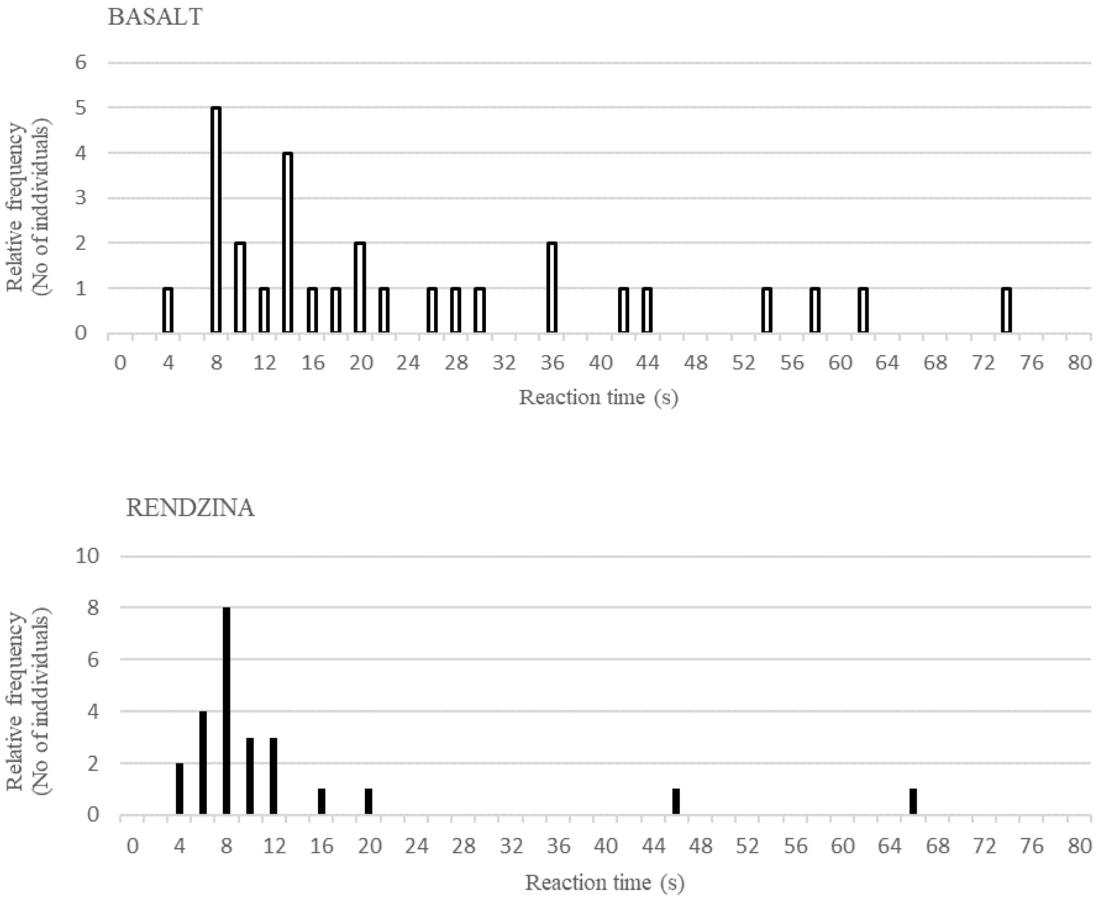

Figure 9. The relative frequency of reaction times for basaltic-soil (n=29) and rendzina (n=24) *Nannospalax galili* populations. The X-axis represents the time which was necessary for an animal to make its turning choice after leaving the starting box, categorized into two second intervals, and the Y-axis represents the number of individuals who fall into the same category. Individuals with a reaction time higher than 80 seconds (one rendzina and three basaltic-soil animals) were omitted for a clearer graphical representation.

5. Discussion

Laterality tests can be used as preliminary tests to check if there is a certain level of functional asymmetry in an individual before conducting experiments in a T- or Y-maze setup where an animal must choose between two options. Such experiments have already been performed in subterranean rodents and include, for example, sensory tests (Heth et al. 2002; Lange et al. 2005; Vitámvás 2013), soil preference tests (Lövy et al. 2017), mate-choice trials (Lövy et al. 2019), studies on reference memory and recognition of novel spaces (Oosthuizen et al. 2013). To illustrate, an individual may turn left not because it necessarily decided to choose the left option, but because it simply had an inherent preference to turn left. In that case, the results might be misleading and the interpretation inaccurate. Another way to overcome this problem could be to repeat the trial with each individual several times, while randomly switching the left and right option. However, the experimental question sometimes does not allow repeated testing of an individual. Manns et al. (2021) conclude that "brain asymmetries are a basic characteristic of the rodent brain, which should not be neglected as it has consequences for planning and analysing experimental manipulation in basic and translational research". Potential lateralization biases (on individual, population or species level) should be taken into account while designing maze tests and analysing obtained data.

To my knowledge, individual and population-level laterality of two *N. galili* populations originating from different soil types and potentially undergoing ecological speciation has not been examined before. Furthermore, this study is also the first specifically designed to investigate laterality in any species within the *Nannospalax ehrenbergi* complex in captive conditions. Kimchi and Terkel (2003) studied 10 individuals of the chromosomal form 2n=58 (*N. carmeli*) in the wild, conducting a series of tests blocking their tunnels with obstacles of different types and shapes and investigating their ability to detour the obstacles and rejoin the disconnected sections. They noticed that, when encountering a symmetrical obstacle, either solid or an open ditch, the mole rats showed no laterality preference and would dig a bypass equally from both sides. A side preference was also not found among *N. galili* from the current study when all the animals were tested together.

In the current study, only 7 and 9 out of 57 animals showed a side preference for the final and the first head turn, respectively (surprisingly, these were different animals in each of the measures), but that was if the significance level of p = 0.1 was considered. Due to the wild-caught mole rats being tested only five times, it is impossible to reach the significance level of p = 0.05 even if the individual turned to the same side in all the trials using a two-tailed binomial test, which is commonly used in laterality experiments (e.g. Jacobs & Oosthuizen 2021, Jacobs & Oosthuizen 2023). It should be noted, however, that it is indeed possible the animal turned to the same side in all the trials (only four mole rats turned to the same - left - side in all the trials) purely by chance, not because of an inherent bias. For future studies, more trials per individual are needed to make a more definite conclusion.

A right-side preference has often been reported in rodents, mainly rats and mice as they are most commonly used in laboratory research (Glick & Ross 1981; Ali et al. 1994; Schwarting & Borta 2004; Maciejewska et al. 2015). Jacobs and Oosthuizen (2021) and Jacobs and Oosthuizen (2023) noticed a left-turning bias in two species occupying the underground niche as *N. galili* – the Cape mole-rat *Georychus capensis* and the Damaraland mole-rat *Fukomys damarensis*. In addition, *G. capensis* is, like *N. galili*, highly aggressive, territorial and solitary (Bennet et al. 2006). Aggressive and agnostic behaviours are mainly right-hemisphere dominant processes (which are reflected by a left-turning bias), along with responses to novel and unexpected environments and processing of spatial information (Rogers 2002; Rogers et al. 2013). All of this considered, one would expect similar side preferences in Israeli mole rats like in those two species, but no left-side preference has been found in any of the groups tested in this study. On the contrary, when it came to the final turn in the maze, a significant right-side preference was discovered in all the *N. galili* individuals grouped together, separately in the basaltic-soil population, captive group, and in the males, while a marginally significant right-side preference was found in the mole rats originating from Gush Halav. It is unclear why a higher usage of the left-brain hemisphere is found in *N. galili*, as that side of the brain is specialised in processing emotions with a positive association, foraging and responses to familiar situations (Rogers 2002; Rogers et al. 2013); none of those seem applicable to the current case, i.e. a highly aggressive, xenophobic rodent placed in an unfamiliar maze. However, spalacids are closely related to rats and mice (all are classified under the Myomorpha suborder), unlike African mole-rats who are hystricognath rodents (Nevo 1999). This might signify an unknown genetic nature of left-side preference in Murid rodents, which would deserve further investigation.

The wild-caught mole rats showed a relatively stronger lateralization bias than the captive ones, regardless of direction. The average absolute laterality index for the wild group of *N. galili* was 49, while for the captive group it was only 25. Similar patterns were obtained for the solitary Cape mole-rats (Jacobs & Oosthuizen 2021), and for the social Damaraland mole rats (Jacobs & Oosthuizen 2023), although in the former study, the difference was not significant. In addition, Oosthuizen et al. (2013) have found that captive Cape mole rats showed less variability in behaviour and were less active than their wild counterparts. Right and left individual laterality is thought to be influenced by the environment, while the strength and consistency of the bias regardless of direction seems to be genetically determined (Collins 1977; Glick et al. 1977; Glick & Ross 1981). However, that would not explain the difference in strength of lateralization in this study as the captive group of blind mole rats was not captive-bred (as was the case in Jacobs and Oosthuizen (2023)) but born in the wild and originating from the same area and population as the basaltic-soil animals from the wild-caught group, implying the same genetic origin. *Nannospalax*, being a solitary and highly aggressive rodent genus, is nearly impossible to breed in captivity – to this day, there has been only one instance of a successful breeding, pregnancy and rearing of the blind mole rat pups (Gazit et al. 1996); thus, it is currently not possible to test captive-bred individuals. Nevertheless, they have been kept in captivity for over six years before the start of the experiments. Thus, it seems that the main factor distinguishing a captive from a wild group and that can possibly influence the strength of laterality is the time spent in captivity. It is well known that animals in captivity experience much more stable and in many aspects more mundane environment (without predation, climatic and food availability changes etc.) than their wild counterparts (Mason 2010). It has been proved that captive conditions may result in changes in cognitive performance (Bibost & Brown 2014; van Schaik et al. 2016). In contrast to studies on subterranean rodents, comparisons in laterality between wild and captive individuals have been made for several other vertebrate species, such as chimpanzees (Hopkins et al. 2009), aye-ayes (Lhota et al. 2009), parrots (Brown & Magat 2011), rainbowfish (Bibost & Brown 2013) and manatees (Tyler-Julian et al. 2016), but none have shown any significant differences. The main difference between these studies and studies on subterranean rodents is that in the former, the laterality of the species was studied by observing limb use or eye preference, and in neither was the animal directly handled. The methodology of the current study was quite similar to the design of the studies by Jacobs and Oosthuizen (2021) and Jacobs and Oosthuizen (2023), all of which involved handling of the animals, lifting them by the skin on the back and transferring to the experimental maze. Importantly, handling can induce aggression, stress and anxiety (Balcombe et al. 2004; Hurst & West 2010), and each one of those factors can influence laterality (Casperd & Dunbar 1996; Santin et al. 1996; Rogers 2002; Rohlfs & Ramírez 2006; Rogers 2010; Austin & Rogers 2012; Byrnes et al. 2016). For this reason, an acclimatization period of 5 minutes in the starting box preceded the experiment itself, to allow the animals to relax and get used to the new conditions (although in some individuals, the starting box seemed to have caused even more stress, as they were constantly biting, scratching and pushing the walls and the ceiling). Several authors recommend the use of tunnels when handling and transferring laboratory rodents, as it can reduce anxiety, increase willingness to explore and therefore improve performance in a behavioural test, without the negative impact of stress on results (Hurst & West 2010; Gouveia & Hurst 2013, Gouveia & Hurst 2017). This could especially apply to strictly subterranean rodents such as blind mole rats and African mole-rats, who spend virtually entire lives in underground burrows (Nevo 1999). Indeed, Kimchi and Terkel (2004) found that *N. ehrenbergi* preform significantly better in narrower than in wider tunnels. Considering the natural environment of subterranean rodents, I would expect handling by the use of tunnels rather than lifting would markedly reduce their aversion and stress. Prior to the experiments, the wild-caught group of *N. galili* has been handled only while being caught, examined and sampled, while the captive group is regularly being handled at least once a week for weighing.

Laterality is often tested regarding predator avoidance, escape behaviour and predator-prey interactions. Brown et al. (2004) have suggested that high predation pressure may increase the strength of lateralization in a population. Blind mole rats are severely hunted by jackals, foxes, mustelids, birds of prey such as kestrels and owls (Németh et al. 2016 and references therein; Šklíba et al. 2016b; Lövy, personal communication). There have been no documented instances of reptiles predating on Israeli mole rats, but judging by their size and geographic range, *Daboia palaestine*, *Dolichophis jugularis*, *Malpolon insignitus*, *Hemorrhois nummifer* and *Pseudopus apodus* might be able to hunt them (Bar et al. 2021). Birds of prey catch mole rats during periods of surface activity (Németh et al. 2016), leaving the blind rodents with not much chance to run away. However, mustelids seem to hunt them inside their burrows (Németh et al. 2016), and judging by the hunting strategy of the mole snake *Pseudaspis cana* which preys on mole-rats from southern and eastern parts of Africa (Finn et al. 2020), I presume Israeli squamates would also be able to enter the burrows through fresh mounds and hunt mole rats from within the burrows. The tunnel systems of subterranean rodents are incredibly complex structures (see e.g. Šklíba et al. 2012; Šumbera et al. 2012) and can serve an anti-predatory role, providing ample opportunities for the mole rat to confuse the predator and escape into one of many branches of the tunnel (Šklíba et al. 2008; Németh et al. 2016). In this case, having a lateralized brain could be a substantial benefit while escaping a predator, because if one brain hemisphere is specialized for a function, the duplication of functions on both sides is avoided, and the animal is enabled to simultaneously perform two tasks using separate hemispheres, for example paying attention to the predator and searching for an escape route (Vallortigara 2000). Since captive individuals are, undoubtedly, not exposed to predators, predation pressure may be another factor explaining the weaker side bias of captive blind mole rats as opposed to wild-caught ones. With the progress of captivity (and depending on its length), the awareness of predation is reduced to zero, which might influence laterality reaction in animals (Griffin et al. 2000). Subterranean rodents living in the wild are exposed to dramatically different environmental conditions and pressures than the ones kept in captivity, and Jacobs and Oosthuizen (2021) note that "the influence of captivity (…) may result in less extreme lateralized behaviour in captive animals".

Contrary to my hypothesis, the basaltic population of blind mole rats has shown a significantly higher reaction time than the rendzina population. From the moment they noticed that the barrier blocking the starting box was removed, they needed an average of 62 seconds to leave the starting box, go through the tunnel and make their final turning choice, fully entering either one of the maze arms. The rendzina mole rats were much faster and mostly seemed to simply rush through the maze – it took them an average of 12 seconds to complete the trial. Out of the 14 animals whose reaction time was longer than half a minute, only three (21%) were from rendzina soil, while the rest

(79%) were from basaltic soil. Those animals might have been too stressed out by the handling or too cautious in the unfamiliar surroundings. In several instances it also happened that the animal would fall asleep in the starting box. Some individuals were biting, scratching and pushing the walls and the ceiling of the starting box during the acclimatization period and overall showing high levels of aggression, but would instantly freeze after the barrier blocking the rest of the maze was removed, continuing slowly and carefully through the maze, or returning to the starting box multiple times – such was the male JB09 (basaltic-soil, wild group) during his first trial, in which he had the overall highest reaction time of nearly 31 minute. The female JB07 (basaltic-soil, wild group) with the highest average reaction time (12 minutes) had the second highest reaction time in a single trial – 28,5 minutes in her first trial. She had just been woken up when she was taken out of her housing box and placed into the maze. Her movements were slow and cautious, she was keeping close to the walls and the ground, sniffing and using her vibrissae to sense the environment. The higher reaction time of the basaltic-soil population could also be connected to a higher number of non-valid trials in that group – 14 trials by basaltic-soil animals, but only 2 by rendzina-soil ones were terminated and disregarded, one of the reasons being that they did not reach the end of the maze even after half an hour.

One factor explaining the idea of more cautious behaviour of basaltic-soil mole rats comes from the comparison of population densities between basaltic-soil and rendzina-soil populations. Basaltic soil has a significantly higher population density of mole rats than rendzina in the two studied localities in northern Israel (Hadid et al. 2013; Lövy et al. 2015; Lövy et al. 2017). In subterranean rodents, aggressive interactions are more likely to occur under higher population densities (Nevo et al. 1986; Busch et al. 2000) due to competition for resources (Nevo et al. 1982). Nevo et al. (1986) have noted that aggressive behaviour increases with diversity of the environment and a higher resource availability in *Nannospalax ehrenbergi* accross Israel, which applied to this case would be basaltic soil as it offers a larger food supply than rendzina (Lövy et al. 2015; Lövy et al. 2017). Furthermore, the basaltic-soil mole rats were more active and more soil was being pushed into the mounds aboveground, indicating that the rate of digging new tunnels is higher (Šklíba et al. 2016b). Such behaviour could be explained by a stronger need of territory defence. An individual blind mole rat is indeed aware of the presence of others living around its own territory (Šklíba et al. 2016b). It can therefore be presumed that animals from basaltic soil are more aggressive than rendzina-soil ones and might be expecting conspecifics in their vicinity, which could explain their slower movements and cautious behaviour. Rendzina-soil animals may not be so cautious/vigilant since they live under much lower population density, implying that the chance to meet an opponent is much lower than for basaltic-soil mole rats. Blind mole rats use seismic signals to communicate – they patrol their burrow systems and mark the territory by thumping their heads against tunnel ceilings regularly, producing vibrations which are effectively transmitted over relatively long distances (Heth et al. 1987; Rado et al. 1987). Conspecifics detect those signals by pressing their lower jaws to the walls of the tunnel (Rado et al, 1987). Such 'jaw-listening' behaviour has been observed in Perspex tunnels during laterality experiments, more often in the basaltic than rendzina population. The frequency of 'head drumming' is the highest near potential confrontational areas, which are sections of their tunnels bordering with other conspecifics' territories (Zuri and Terkel, 1996). Nevo et al. (1986) also report that blind mole rats in captivity often thump during aggressive interactions and territory defence. Direct encounters are highly likely to result in a serious injury, or even death for one or both, regardless of their sex (Guttman et al. 1975; Gazit et al. 1996; Lövy, personal communication). Therefore, more cautious behaviour would be highly advantageous in basaltic mole rats living under a higher population density.

The sensory cues used by the mole rats while passing through the maze and consequently choosing a side most likely may have been olfactory or tactile (Francescoli 2000). Visual cues were unlikely to be used, as blind mole rats, lacking external eyes, are thought to be able to detect light but not visual images (Cooper et al. 1993; Francescoli 2000). Auditory cues were not probable either, given that this species' auditory abilities are adapted for capturing low-frequency sounds underground (Francescoli 2000). Most importantly, the intensities of both light and sounds in the environment were consistent throughout the experiments. No vibrations were being produced on the surface of the maze, therefore seismic signals were not available. Orientation of the maze could have been a confounding cue for the animals as they orient themselves using the Earth's magnetic field (Kimchi & Terkel 2001), but that effect was accounted for by randomly rotating the maze between the trials.

Subterranean rodents construct their burrow systems to match the diameter of their own body, which in case of adult blind mole rats can be around 5 to 7 cm (Kimchi

& Terkel 2004; personal observation). That way they are constantly in touch with the tunnel walls and under ample tactile stimuli. The morphology of a blind mole rat is entirely adapted to locomotion in a narrow space – fur that can be combed both ways, reduction in size of extremities (short legs, lack of external ears and tail under the skin), and an elongated, flexible body allow them not only to swiftly and easily move both forwards and backwards, but also to turn on the spot by 180 degrees in a tunnel just slightly larger than their body width (Burda et al. 1990; Nevo 1999). In a series of experiments by Kimchi and Terkel (2004), it was shown that individuals of *N. ehrenbergi* preformed significantly better when tested in a narrow maze with a diameter of 6 cm than in a wider maze (diameter 10 cm). Furthermore, while moving through the wide maze, they tended to keep their bodies in tight contact with the sides of the maze as much as possible, alternating between left and right, and were often stopping and pressing their lower jaws and vibrissae to the maze walls. A similar 'wall-seeking' behaviour was also observed in animals from experiments described in this study, where tunnels with a width of 8 cm were used. The behaviour was especially extreme and frequent in individuals from basaltic soil, while it was often absent in rendzina mole rats. When placed in an open space, blind mole rats would either stop and freeze entirely, or would move very slowly and carefully. While conducting experiments with subterranean rodents in artificial tunnel systems, it is definitely advisable to use Perspex tunnels with dimensions adapted to a specific species. Using narrower rather than wider tunnels might help the animals feel more comfortable and under a lower level of stress, in addition to improving their performance in the test.

There are many ways to improve animal performance in maze tests. For example, some researchers decide to use different kinds of reinforcement, either positive or negative, in order to increase animals' activity levels and motivate them to go through the maze. Food rewards being placed at the ends of arms (Kimchi et al. 2005), or openings at the ends of arms providing an escape to a potentially less anxiogenic environment (Oosthuizen et al. 2013) would represent a positive reinforcement. On the other hand, the animal can be forced to move by filling the bottom of the maze with water (Santin et al. 1996; Jacobs & Oosthuizen 2021), or stimulating a predator attack by producing noise at the beginning of the maze (García-Muñoz et al. 2013). Their activity levels can also be increased by depriving them of food before the start of experiments (Andrade et al. 2001; Schwarting & Borta 2004; Maciejewska et al. 2015).

Maciejewska et al. (2015) note that starvation may alter the animals' purpose of being in the maze as they are no longer simply exploring but searching for a food source. Non-reinforced repeated tests may be tricky if there is no break for the individual between the trials – it is likely the animals' activity levels will decrease as they gain nothing for passing through the maze, but are constantly being placed back at its beginning after reaching the end (Schwarting & Borta 2004). Such a problem did not seem to apply for the current study, since a single mole rat had at least an hour between two trials, during which it was in its housing box. Vitámvás (2013) has concluded that for subterranean rodents conditioning could be a useful solution to overcome a low sample size of experimental animals in a preference test, where a simple single trial might not give significant results. Teaching them to connect the completion of a trial with a food reward strengthens their motivation to go through the maze. From personal experience with blind mole rats, conditioning was not successful and food was simply not an adequate reward – even after a 24-hour period of food deprivation, they seemed more interested in digging in the peat they were placed in than in their preferred foods.

Another compelling option is treatment with psychostimulatory drugs, such as amphetamines, cocaine or phencyclidine, as it is said they can induce, reveal or enhance already existing asymmetries (Jerussi & Glick 1975; Glick & Cox 1978; Glick et al. 1983; Ali et al. 1994). Amphetamines work by releasing more dopamine from the more active of the two dopaminergic pathways on different sides of the brain (von Voightlander & Moore 1973), therefore enhancing neurological asymmetry (Glick et al. 1974; Jerussi & Glick 1976). It is precisely the difference in the levels of dopamine between the two hemispheres that is related to spatial preferences – animals are shown to turn in the direction opposite from the side which contains more dopamine (Zimmerberg et al. 1974). Ho et al. (2004) have tested rats in an elevated plus maze after treating them with MDMA. The effect of the drug was dose-dependent, with a lower dose (7,5 mg/kg) having anxiety-inducing and psychostimulatory effects and resulting in increased levels of activity within closed arms but a higher reluctancy to enter the open arms. Since the basaltic population of *N. galili* tested in a T-maze already showed quite a high latency to exit the starting box, I wouldn't advise using the lower dose of MDMA for this species, especially in that kind of an experimental setup where the aim is for the animal to go through the maze entirely. Some of the individuals already were quite active inside the starting box, but reluctant to proceed to the junction and further. On the other hand, a higher dose (15 mg/kg) acted as an anxiolytic and led to increased activity in the open arms, although the drug caused severe loss of coordination. However, higher doses of drugs should be applied with caution as there is a chance of lethality which needs to be considered. Ambient temperature needs to be taken into account as well due to the fact that amphetamines can cause hyperthermia and heatstroke by affecting thermoregulation, therefore increasing the risk of lethality (Gordon et al. 1991; Bowyer et al. 1994), and *N. galili* seem to be especially vulnerable to high rather than low temperatures (Šumbera et al. 2023).

Lateralization is often connected to personality in animals, behavioural tendencies, learning abilities and information processing. In many cases, left and right lateralized individuals (or lateralized compared to non-lateralized individuals) have shown to exhibit different behaviours, personality traits, and performance in tasks (McDowell et al. 2016; Barnard et al. 2017; Tomassetti et al. 2019). In several instances, strongly lateralized individuals have shown to outperform weakly or non-lateralized ones (McGrew & Marchant 1999; Gűntűrkűn et al. 2000; Magat & Brown 2009; Dadda et al. 2015), especially when presented with two simultaneous tasks to solve (Rogers 2000; Dadda & Bisazza 2006; Piddington & Rogers 2013). Light/dark box tests are currently being conducted at the Faculty of Science, University of South Bohemia in České Budějovice with some of the *Nannospalax galili* individuals which have been subjects of experiments described in this paper, meaning mole rats from both the rendzina and basalt populations (Konečná, personal communication). The results obtained in the current study indicate that rendzina-soil mole rats may be bolder, more explorative and more willing to leave the box earlier than basaltic-soil ones.

6. Conclusions

This study was conducted with an aim to examine lateralization in the Upper Galilee Mountains blind mole rat *Nannospalax galili*, with a special emphasis on potential differences between two populations from different soil types, basaltic soil and rendzina. A significant right-side preference was found in all the animals together; it is unclear why the right side is preferentially chosen, as it indicates a higher usage of the left-brain hemisphere. The processes primarily controlled by that side of the brain are contradictory to the behaviour observed in the mole rats. The wild-caught group showed an overall stronger lateralization bias, regardless of the side, compared to the group that has been kept in captivity for several years. This may be due to captivity altering/reducing the animals' natural behaviours, along with the captive animals being relatively used to regular handling, causing a weaker lateralization response. An interesting idea to explore could be examining their turning biases in a maze filled with soil to make for a more natural environment, and test whether (and if so, how) it differs to their performance in an empty maze. A significant right-side turning bias was found in the basaltic-soil animals, but not in rendzina ones. Furthermore, the basaltic-soil population needed a significantly longer time to go through the maze and make a final choice. Since they are living under much higher population densities, basaltic-soil mole rats may be the more cautious and vigilant of the two populations, expecting a conspecific in their vicinity. Personality studies are currently being conducted with some of those same individuals at the Faculty of Science in České Budějovice – once the results of the study will be obtained, it would be interesting to connect them to the results of the current study and test whether there is a connection between personality, boldness and exploratory behaviour, and the strength and direction of lateralization in *N. galili.* Moreover, their research could provide further insight into potential differences in behaviour between basaltic-soil and rendzina populations, and consequently, into the possible ecological speciation of the Upper Galilee Mountains blind mole rat.

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