

CORRECTION

Correction: Audiograms of three subterranean rodent species (genus *Fukomys*) determined by auditory brainstem responses reveal extremely poor high-frequency cut-offs

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The title was incorrect. The corrected title is below.

Audiograms of three subterranean rodent species (genus *Fukomys*) determined by auditory brainstem responses reveal extremely poor high-frequency hearing

We apologise to the authors and readers for any inconvenience this may have caused.

SHORT COMMUNICATION

Audiograms of three subterranean rodent species (genus *Fukomys*) determined by auditory brainstem responses reveal extremely poor high-frequency cut-offs

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ABSTRACT

Life underground has shaped the auditory sense of subterranean mammals, shifting their hearing range to low frequencies. Mole-rats of the genus *Fukomys* have, however, been suggested to hear at frequencies up to 18.5 kHz, unusually high for a subterranean rodent. We present audiograms of three mole-rat species, *Fukomys anselli*, *Fukomys micklemei* and the giant mole-rat *Fukomys mechowii*, based on evoked auditory brainstem potentials. All species showed low sensitivity and restricted hearing ranges at 60 dB SPL extending from 125 Hz to 4 kHz (5 octaves) with most-sensitive hearing between 0.8 kHz and 1.4 kHz. The high-frequency cut-offs are the lowest found in mammals to date. In contrast to predictions from middle ear morphology, *F. mechowii* did not show higher sensitivity than *F. anselli* in the low-frequency range. These data suggest that the hearing range of *Fukomys* mole-rats is highly restricted to low frequencies and similar to that of other subterranean mammals.

KEY WORDS: Hearing sensitivity, Subterranean mammals, Sound localization, Auditory fovea, Inner ear, Middle ear

INTRODUCTION

African mole-rats of the genus *Fukomys* spend their entire life in underground tunnel systems. The aphotic subterranean ecotope has led to manifold sensory adaptations (Burda et al., 1990). The underground acoustic environment is characterized by quick attenuation of high frequencies, whereas low-frequency airborne sound waves are less attenuated and under some conditions are slightly amplified in certain frequency windows (stethoscope effect at 200–800 Hz; Heth et al., 1986; Lange et al., 2007; Quilliam, 1966).

Several studies have addressed the hearing capabilities of subterranean and fossorial mammals of different genera (Begall et al., 2004, 2007; Brückmann and Burda, 1997; Heffner and Heffner, 1990, 1992, 1993; reviewed in Begall et al., 2017). In comparison to similar-sized epigeic mammals, audiograms of subterranean mammals are characterized by lower frequencies of most-sensitive hearing and extremely restricted high-frequency hearing. Furthermore, absolute sensitivities are low, which has been interpreted as either a degeneration of the auditory sense (Heffner

and Heffner, 1992, 1993) or an adaptation to the stethoscope effect (Burda, 2006; Lange et al., 2007). For example, while the most-sensitive frequency of hearing in the naked mole-rat *Heterocephalus glaber* was found at 4 kHz with a threshold of 35 dB sound pressure level (SPL) (Heffner and Heffner, 1993), epigeic animals of comparable body size typically have hearing ranges between 500 Hz and 70 kHz, with best frequencies well above 6 kHz and absolute sensitivities near to or even below 0 dB SPL (Vater and Kössl, 2011). The overall hearing range (at 60 dB SPL) in the naked mole-rat spans seven octaves between 65 Hz and 12.8 kHz (Heffner and Heffner, 1993). The blind mole-rat *Spalax ehrenbergi* has been shown to hear between 54 Hz and 5.9 kHz (<7 octaves, best frequency 1 kHz), representing the lowest high-frequency hearing limit found in a mammal to date (Heffner and Heffner, 1992). Interestingly, however, behavioural tests of the bathyergid mole-rat *Fukomys anselli*, which is closely related to the naked mole-rat, suggested an astonishingly broad hearing range from below 225 Hz (the lowest frequency tested) up to 18.5 kHz (Brückmann and Burda, 1997). Apart from the high-frequency limit, *F. anselli* exhibited the typical subterranean characteristics with high absolute thresholds, most-sensitive hearing at 800 Hz and an interesting anatomical adaptation for low-frequency hearing termed the acoustic fovea. While in other mammals the width of the basilar membrane increases more or less continuously from the cochlear base to the apex, in *F. anselli* around 50% of the basilar membrane length has a constant width, representing the cochlear place-frequency map from 600 Hz to 1000 Hz (Müller et al. 1992; Kössl et al., 1996). These cochlear place-frequency maps and electrophysiological recordings from the auditory brainstem did not support the suggested high-frequency limit of 18.5 kHz in *F. anselli*, as both found steep decreases above 5–12.6 kHz (Müller and Burda, 1989; Müller et al., 1992; note that when this work was published, *F. anselli* was referred to as *Cryptomys hottentotus*; the genus *Fukomys* emancipated from the genus *Cryptomys* in 2006; Kock et al., 2006). Findings from evoked otoacoustic emissions (Kössl et al., 1996) suggested that the deep anaesthesia used during brainstem recordings selectively affected high-frequency thresholds. It thus remained unclear whether high-frequency sensitivity (for a subterranean mammal) is an artefact produced by experimental techniques, or rather is an accurate reflection of the auditory sensory system and represents a specific trait of the genus *Fukomys*.

To date, no behavioural audiograms of mole-rats (genus *Fukomys*) with individuals individually tested in a well-defined sound field are available. As the animals do not drink water or readily accept liquid food, the gold standard conditioned avoidance procedures are difficult to apply, and therefore only a single behavioural audiogram based on group responses is available for *F. anselli* (Brückmann and Burda, 1997). The minimally invasive

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recording of auditory brainstem responses (ABRs) offers an alternative that allows for repeated testing of individuals in a controlled environment (Müller and Burda, 1989; Shvarev, 1994; Martin et al., 2012; Brandt et al., 2013). ABRs are early acoustically evoked potentials which contain all the retrocochlear information needed to establish an audiogram of a species (e.g. Lucke et al., 2016). Using this method, we set out to obtain additional audiograms from *Fukomys* mole-rats. To address the above-mentioned high-frequency limit issue, we retested *F. anselli* and compared it with two closely related species: *Fukomys micklemi*, which is of similar body size to *F. anselli*, and *Fukomys mechowii*, which is of a much larger body size and exhibits notable differences in middle ear morphology (Lange and Burda, 2005). We hypothesized that (1) our ABR measurements in the three *Fukomys* species, despite using far lower anaesthetic doses, resemble the previous data obtained by evoked potential recordings in *F. anselli* with a high-frequency cut-off much lower than 18 kHz, and (2) *F. mechowii* has a higher hearing sensitivity at low frequencies than *F. anselli* and *F. micklemi*, promoted by a larger middle ear cavity and a higher transformation ratio of the middle ear.

MATERIALS AND METHODS

Animals and housing conditions

We tested six Ansell's mole-rats [*Fukomys anselli* (Burda, Zima, Scharff, Macholán and Kawalika 1999)], five Micklem mole-rats [*Fukomys micklemi* (Chubb 1909)] and five giant mole-rats [*Fukomys mechowii* (Peters 1881)]. All animals were adult but not senescent (1–3 years of age; Table S1) and were visually inspected before testing, showing no signs of illness or auditory disorder. Note that the average lifespan is approximately 4–6 years in these species with maximum lifespans in reproductively active animals being even longer (Dammann and Burda, 2006; Dammann et al., 2011). The animals were born at the Department of General Zoology at the University of Duisburg-Essen, Germany. They were housed on a 12 h:12 h light:dark cycle, 24±1°C constant temperature and 40–50% humidity in terraria lined with wood shavings and enriched with clay pots. The size of the terraria varied with the size of the colonies. Carrots and potatoes were provided *ad libitum* and regularly supplemented with salad and dry rodent food. All animals were returned to their colonies immediately after the sessions.

Recordings of ABRs

The experiments took place between August and November 2015, except for animals F8 and F9, which were tested in July and August 2017. Animals were anaesthetized via intramuscular injection of ketamine and xylazine (see Table S3 for detailed doses; Garcia Montero et al., 2015) and placed in a custom-made anechoic chamber (see Malkemper et al., 2015). During recordings, body temperature was maintained by a non-electric Deltaphase isothermal heating pad (Braintree Scientific, Braintree, MA, USA) and regularly controlled with a rectal electrode. All procedures were approved by the North Rhine-Westphalia State Environment Agency (permit number: 84-02.04.2015.A383). Stimulus generation and presentation as well as the amplification and digitalizing of recorded responses were performed with a Tucker-Davis Technologies (TDT, Alachua, FL, USA) System 3 RZ6. Stimuli were presented via a Tannoy Arena Satellite speaker (frequency response 80 Hz to 54 kHz) positioned 15 cm from the left pinna of the animals at an angle of 90 deg (the angle of sound incidence was 0 deg; see Fig. S1). For frequencies below 125 Hz, a subwoofer (Punch HE Rockford Fosgate, Tempe, AZ, USA) was

used and placed on the foam-lined floor of the anechoic chamber below the level of the animal. Both speakers were driven by the built-in amplifier of the RZ6 multi I/O processor and calibrated with a ¼ in free field microphone (type 4939 with preamplifier 2669 C and conditioning amplifier Nexus 2692-A, Brüel & Kjær, Nærum, Denmark; frequency response 4 Hz to 100 kHz) placed at the position of the subject's ear. A digital oscilloscope (Picoscope 4224, Pico Technology Ltd, St Neots, UK) connected to the output of the conditioning amplifier served to check the frequency content of the stimuli. The built-in calibration tool of the BioSig RZ software (v5.7.0, TDT) was used to check the SPL. The BioSig RZ software system in turn was calibrated using a Brüel & Kjær 4230 sound level calibrator with a ¼ in microphone adaptor (B&K DB 0310). During sound field calibration, a dummy was placed at the position of the animal to simulate the acoustic environment during the recordings as closely as possible. Auditory stimuli were 5 ms (1 ms rise/fall times) pure tones presented 12 times per second. Alternating starting phases were used to reduce stimulus artefacts. During tests, the tone bursts were presented 256 times at each SPL and brainstem responses were averaged afterwards. Sound intensities were decreased in 10 dB steps between 80 dB SPL and 50 dB SPL and in 5 dB steps between 50 dB SPL and 20 dB SPL. All calibrations and measurements were performed within a grounded aluminium Faraday cage (23.5×23.5×20 cm) placed inside the anechoic chamber, housing the individual as well as the headstage and preamplifier. We tested 16 frequencies in a range from 50 Hz to 36 kHz (50 Hz, 125 Hz, 250 Hz, 354 Hz, 500 Hz, 630 Hz, 800 Hz, 1000 Hz, 1400 Hz, 2000 Hz, 4000 Hz, 8000 Hz, 12,500 Hz, 16,500 Hz, 18,500 Hz, 36,000 Hz). To pick up brainstem potentials, 27 gauge, 13 mm, subdermal, stainless steel recording electrodes (Rochester Electro-Medical Inc., Lutz, FL, USA) were used. The active electrode was placed at the vertex of the animal. The reference was placed at the brainstem and the ground at the mastoid (Fig. S1). The electrodes were coupled to a RA4LI low impedance headstage (TDT). The signals were pre-amplified by a Medusa RA4PA (TDT), fed to the RZ6 multiprocessor via a fibre optic cable and averaged within the BioSig software (v5.7.0, TDT).

Threshold determination

Averaged ABR waveforms for all tested intensities were printed for each frequency and thresholds were manually determined by three independent observers who were blind to the frequency condition. Threshold was defined as the mean between the lowest stimulus level at which a response could be visually detected and the next stimulus level below this (Fig. 1). The mean threshold of the three observers was calculated and only accepted as the threshold of a session if the standard deviation between the observers was less than 10 dB. If the standard deviation was higher than 10 dB, the recording for the respective frequency was repeated.

Statistics

Interspecies comparisons of threshold levels for the tested frequencies were performed with SPSS Statistics v24.0 (IBM Corp., Armonk, NY, USA). Normal distribution of threshold levels for each frequency was tested with the Shapiro–Wilk test. Multiple comparisons of normally distributed data were calculated with one-way ANOVA followed by the Bonferroni *post hoc* test for pairwise comparison. Data that did not follow a normal distribution were analysed with the Kruskal–Wallis test followed by the Dunn–Bonferroni *post hoc* test for pairwise comparison.

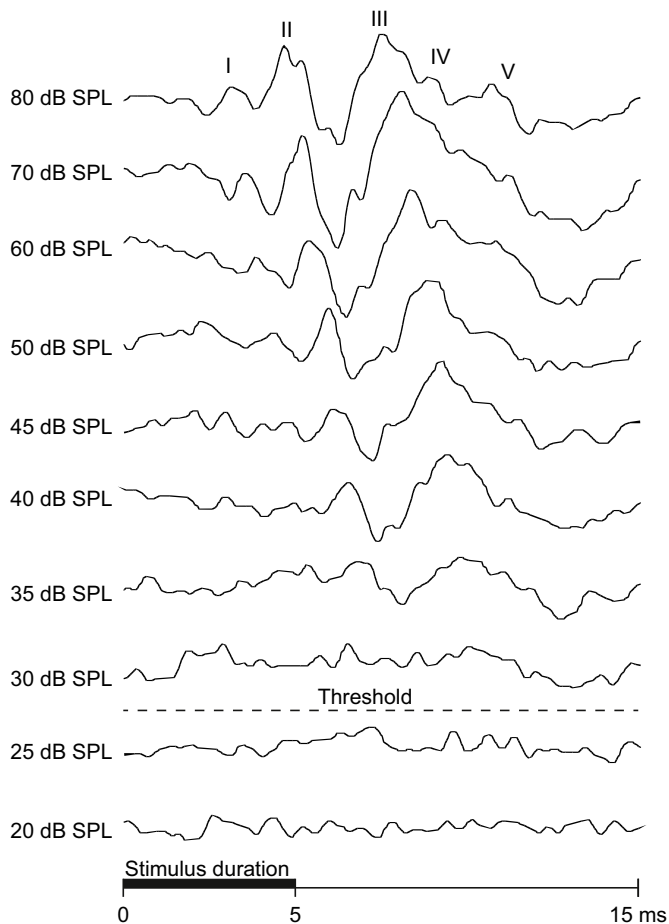


Fig. 1. Example traces of averaged brainstem responses of an individual *Fukomys anselli* (M3) recorded at different intensities of 800 Hz pure tones. The threshold was calculated as the average of the lowest sound pressure level (SPL) at which a brainstem response could be identified (mainly based on waves I and III) and the next stimulus level below this. Three independent observers blinded to the tested frequency identified the lowest SPL based on visual inspection. In the given example, a frequency of 800 Hz was tested and the threshold was set at 27.5 dB SPL (dashed line).

RESULTS AND DISCUSSION

Hearing ranges

The mean hearing range (at 60 dB SPL) of the three tested *Fukomys* species extended from 125 Hz to 4 kHz (Fig. 2, Table 1; for individual thresholds, see Table S2). Overall sensitivities were low: *F. anselli* and *F. micklei* showed highest sensitivity (*F. anselli*: 29 dB SPL, *F. micklei*: 37 dB SPL) at 1 kHz, and *F. mechowii* showed highest sensitivity at 1 kHz and 1.4 kHz (33 dB SPL). These hearing ranges, absolute sensitivities and frequencies of most-sensitive hearing are in good agreement with reported hearing parameters of other subterranean mammals (reviewed in Begall et al., 2017). High absolute thresholds compared with similar sized terrestrial mammals and most-sensitive hearing at low frequencies seem to be ecophysiological adaptations to the subterranean ecotope.

Variation between the individuals of each species was generally low and more pronounced at high frequencies. Only *F. micklei* showed inter-individual variation over the entire frequency range. *Fukomys micklei* was also the least sensitive of the three species. Its thresholds were significantly higher at 500 Hz (one-way ANOVA, $F=22.6$, $P<0.0001$), 630 Hz ($F=11.1$, $P=0.002$) and

1 kHz (only different from *F. anselli*, $F=4.1$, $P=0.042$). No significant differences were found between the thresholds of *F. anselli* and *F. mechowii*. We also tested all species at 36 kHz. None of the tested animals showed any measurable brainstem potentials as a response to these ultrasounds.

Comparison with existing audiograms

Our data are partially consistent with earlier studies of *F. anselli*, the subterranean rodent with the best-characterized auditory system to date. Both the frequency of the greatest hearing sensitivity (1 kHz) and the lowest perceived SPL at this frequency (28 dB SPL) are similar to the collective behavioural audiogram from previous studies (800 Hz, 24 dB SPL, Table 1; Brückmann and Burda, 1997). This finding is startling as thresholds determined by ABRs are usually considerably higher than behavioural thresholds (see below). This means either that our system is sensitive enough to pick up potential changes at the absolute threshold of perception in these animals or that the actual thresholds of *F. anselli* were underestimated by Brückmann and Burda (1997). Only well-controlled individual behavioural audiograms will solve this issue.

The high-frequency limit of *F. anselli*

These similarities demonstrate that our results are comparable with the collective behavioural audiogram, raising questions about the discrepancy in the higher frequency range. The high-frequency cut-offs of the three *Fukomys* species we studied are the lowest reported for mammals. Partly, this might be related to the applied method. In mammals, auditory thresholds determined by ABR are on average 10–30 dB SPL higher than those determined behaviourally (Gorga et al., 1988). However, the relationship is not linear across frequencies, generally being smallest at high frequencies, and in some cases ABR have even yielded lower thresholds than behavioural assessment (Gorga et al., 1988; Szymanski et al., 1999). Still, it is likely that the actual hearing range of the *Fukomys* species tested here is slightly broader than our ABR results, which agrees with an earlier ABR audiogram of *F. anselli* (Table 1; Müller and Burda, 1989). However, even if we liberally correct the thresholds to account for the lower sensitivity of ABRs by 15 dB SPL at each frequency, the 60 dB SPL high-frequency cut-off in *F. anselli* only shifts up to 12.5 kHz. This upper limit would be similar to that of the closely related naked mole-rat and would match cochlear place–frequency maps established for *F. anselli* (Müller et al., 1992), but still is in disagreement with the 18.5 kHz suggested by the collective behavioural audiogram of Brückmann and Burda (1997) and the otoacoustic emissions of Kössl et al. (1996). Two explanations have been suggested for discrepancies regarding the upper hearing limit of *Fukomys* mole-rats (Kössl et al., 1996). (1) Deep anaesthesia selectively influences high-frequency thresholds; with 90 mg kg⁻¹ ketamine, otoacoustic emissions quickly declined above 4 kHz, but after reducing the dose to 50 mg kg⁻¹, they were measureable up to 18 kHz (Kössl et al., 1996). However, in the current study, we used anaesthetic doses nearly tenfold lower than 50 mg kg⁻¹ (Table S3), and therefore it is more likely that (2) the outer ear of mole-rats with its tight meatus filled with hair and cerumen acts as a low-frequency filter that is bypassed in otoacoustic measurements, which are performed at the tympanic membrane (Kössl et al., 1996).

If true, how could we explain the 18.5 kHz upper hearing limit determined in the behavioural audiogram of Brückmann and Burda (1997)? We think this might not reflect the true auditory range for two reasons. First, there are general problems associated with the sound calibration in a collective audiogram as a result of the study

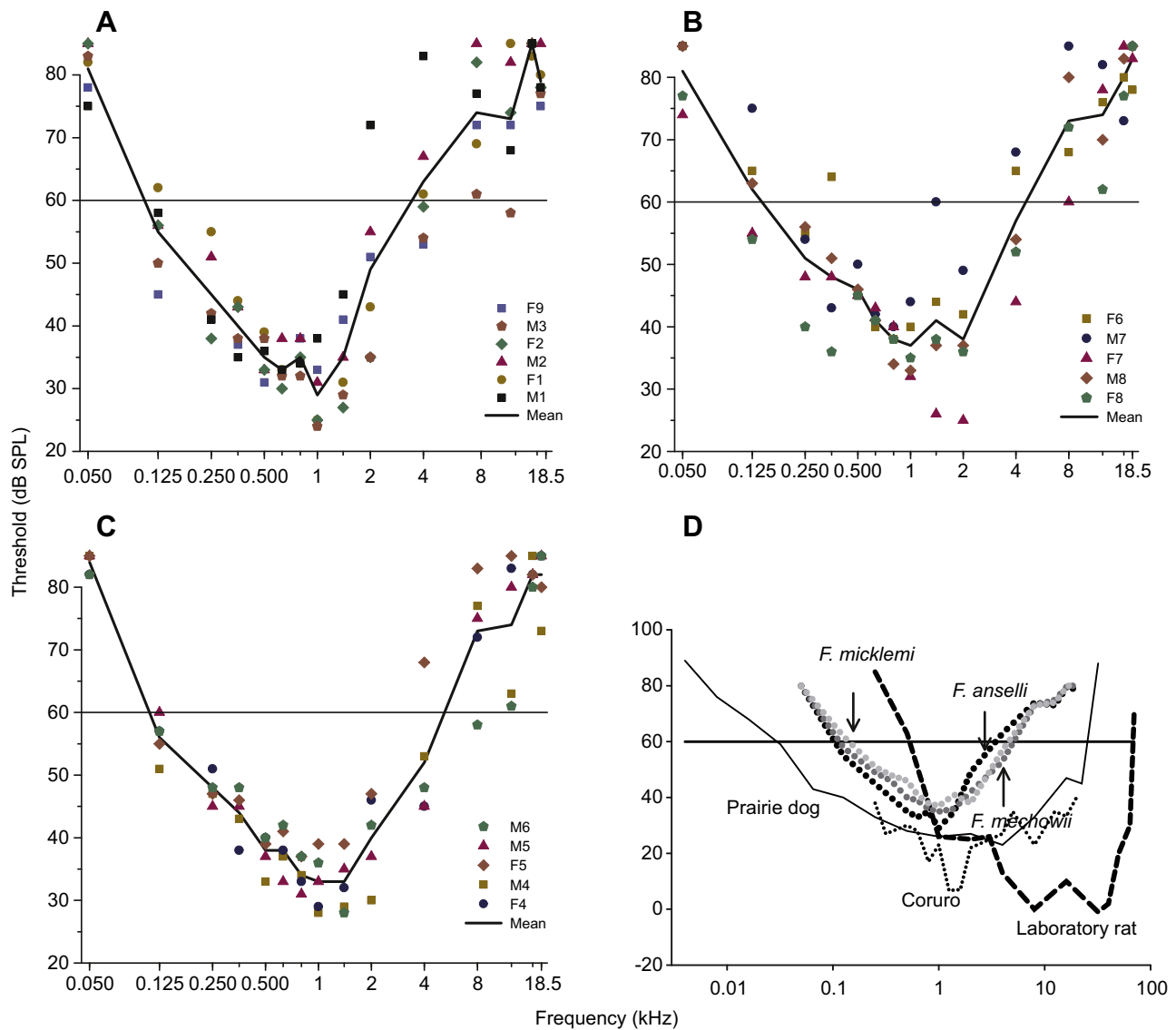


Fig. 2. Audiograms of the three tested *Fukomys* species. (A) *Fukomys anelli* ($n=6$). (B) *Fukomys micklei* ($n=5$). (C) *Fukomys mechowii* ($n=5$). Different symbols represent different individuals. (D) The three audiograms in comparison to those of other rodents demonstrate the restricted frequency range and low absolute sensitivity (adapted from Begall et al., 2004). Different symbols show the average values of two measurements of the different individuals; the black line represents the average hearing threshold of a species. Audiogram data for coruro from Begall et al. (2004), laboratory rat from Heffner et al. (1994a), prairie dog from Heffner et al. (1994b).

design. A group of animals is tested and the researcher uses the first response of an animal in a group as a measure of sound perception. The actual SPL at the ear of an animal is, however, highly dependent on the exact position and orientation of the animal within the sound field, two factors that are hard to control when group responses are measured. Furthermore, the microphone (B&K 4145) that Brückmann and Burda (1997) used to calibrate the sound field in the collective audiogram study had an upper frequency response limit at 18 kHz. Operating at or above the limit increases the likelihood of underestimating the actual SPL: differences in the angle of incidence can lead to differences of up to 20 dB when using this microphone to measure such high frequencies (the free field correction curves for the B&K 4145 are available at <https://www.bksv.com/-/media/literature/Product-Data/bp2032.ashx>). As these problems do not apply to our setup (the microphone B&K 4939 was never operating near the limit and the position of each animal was identical across all sessions), we conclude that high-frequency

hearing of *Fukomys* mole-rats is at least as restricted as in other strictly subterranean mammals.

Further corroborating our results, *Fukomys* mole-rats are highly vocal social mammals and the hearing ranges of the three mole-rat species obtained in the present study are in good agreement with the fundamental frequencies of their vocalizations (reviewed in Begall et al., 2017). A great majority of calls in the vocal repertoire of *Fukomys* have main frequencies between 0.4 kHz and 2.5 kHz (*F. anelli*: Credner et al., 1997; *F. mechowii*: Bednářová et al., 2013; *F. micklei*: Vanden Hole et al., 2013). Importantly, no calls with fundamental frequencies higher than 10 kHz have been reported, supporting the idea that higher frequencies may not be ecologically/functionally relevant to the animals.

Functional correlations with ear morphology

The morphology of the mole-rat auditory periphery has been well studied (e.g. Lange and Burda, 2005; Mason et al., 2016), allowing

Table 1. Mean auditory thresholds (in dB SPL) of *Fukomys ansellii*, *Fukomys micklemei* and *Fukomys mechowii* in comparison to previously reported values for *Fukomys ansellii*

<i>f</i> (kHz)	<i>F. ansellii</i>			<i>F. mechowii</i> (present study)	<i>F. micklemei</i> (present study)
	Brückmann and Burda, 1997	Müller and Burda, 1989	Present study		
0.05			>80	>80	>80
0.125			55	56	62
0.225	50	62			
0.25		61	45	48	51
0.354	40	55	40	44	48
0.5	52	42	35	38	46
0.63	38	39	33	38	41
0.8	24	38	35	34	38
1	39	40	29	33	37
1.4	36	47	35	33	41
2	38	58	49	40	38
4	39	72	63	52	57
5	41	82			
8	47	97	74	73	73
12.5	47		73	74	74
16.5	50		>80	>80	80
18.5	64		79	>80	>80

The lowest thresholds (best hearing) are indicated in bold. Red values indicate thresholds that are significantly higher in *F. micklemei* than in one or both of the other species (one-way ANOVA followed by Bonferroni *post hoc* test for pairwise comparison, $n=6$ for *F. ansellii*, $n=5$ for *F. mechowii* and *F. micklemei*). There were no significant differences between the thresholds of *F. ansellii* and *F. mechowii*. Values from Müller and Burda (1989) were estimated from the diagram in fig. 1 of the original publication as the raw data were not accessible.

us to make some predictions about the hearing abilities of our study species. In small mammals, the volume of the middle ear cavity is one of the main factors determining sensitivity in the low-frequency range (Mason, 2016a). Mammals adapted to low-frequency hearing, e.g. gerbils, possess enlarged middle ear cavities (Webster and Webster, 1975; Mason, 2016b). While the middle ear cavities of mole-rats in general are not particularly enlarged, the middle ear cavity of *F. mechowii* is more than two times larger than that of the two other study species (Lange and Burda, 2005; Mason et al., 2016). Furthermore, the transformation ratio, the product of the lever ratio of the malleus and incus and the area ratio of the tympanic and oval window membrane, often used as a proxy for the biomechanical transmission efficiency of the middle ear, is higher in *F. mechowii* (Lange and Burda, 2005). On this basis, we predicted *F. mechowii* to show higher sensitivity of hearing than *F. ansellii* and *F. micklemei*, especially at low frequencies. Instead, we found that the hearing thresholds of *F. mechowii* are similar to those of *F. ansellii* over the whole range of frequencies. This again demonstrates that morphological predictors of hearing ability need to be interpreted with extreme care (Mason et al., 2016). For mole-rats, it is likely that the hearing range is primarily restricted by the filter properties of the outer ear and perhaps the cochlea or higher order processing, while the middle ear seems to be a broadband transmitter (Mason et al., 2016; Gessele et al., 2016). Notably, the region of most-sensitive hearing around 1 kHz fits within the region of the auditory fovea in *F. ansellii* (Müller and Burda, 1992; Kössl et al., 1996; Pleštilová et al., 2016). Given the small differences in auditory sensitivity between the three species, we would expect *F. mechowii* and *F. micklemei* to also possess an auditory fovea. So far, no morphological or electrophysiological data have been published that would enable us to test this hypothesis.

Sound localization and high-frequency hearing in mammals

Small mammals rely on intensity differences created by the shading of the incoming sound by the head for sound localization. However, their small heads only attenuate high-frequency sounds effectively (Heffner and Heffner, 2016). Mole-rats with the functional head size (the minimal time a sound needs to travel from one ear to the other) of *F. ansellii* (mean±s.d.: $78\pm 7\ \mu\text{s}$, $n=10$) and *F. micklemei* ($80\pm 8\ \mu\text{s}$, $n=10$) would have to hear up to 70 kHz (Heffner and Heffner, 2016) to detect such intensity differences. The high-frequency limit of the larger *F. mechowii* ($115\pm 10\ \mu\text{s}$, $n=10$) would be expected to be around 60 kHz. Our data clearly demonstrate the independence of mole-rats from this relationship. Life in a more or less one-dimensional environment does not require accurate sound localization, thus releasing species from the need for sound localization, and therefore reduces the selective pressure for high-frequency hearing (Heffner and Heffner, 1992, 1993, 2016).

In summary, we present data on the hearing of three closely related subterranean *Fukomys* species, demonstrating low sensitivity and highly restricted high-frequency hearing.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.P.M.; Methodology: P.G., E.P.M.; Validation: Y.H., S.B., E.P.M.; Formal analysis: P.G., Y.H., S.B., E.P.M.; Investigation: P.G.; Data curation: P.G., Y.H., S.B.; Writing - original draft: E.P.M.; Writing - review & editing: P.G., Y.H., S.B., E.P.M.; Visualization: P.G., E.P.M.; Supervision: E.P.M.; Project administration: E.P.M.; Funding acquisition: E.P.M.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.164426.supplemental>

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SUPPLEMENT

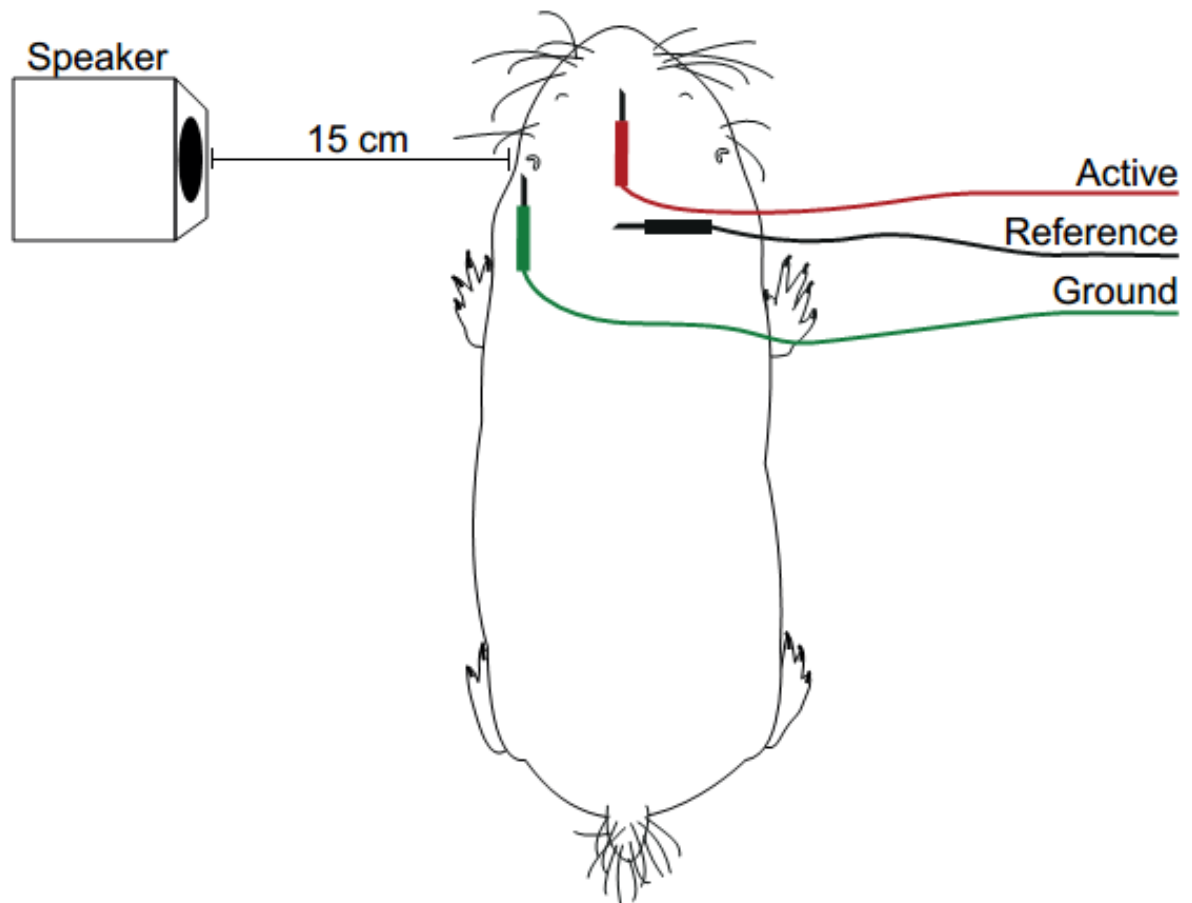


Figure S1: **Electrode and speaker positions during auditory brainstem potential recordings.** All recordings were performed within a grounded Faraday cage placed in an anechoic chamber.

Table S1: **Sex, age and weight of the tested individuals.**

Species	Individual	Sex	Weight (g)	Age (weeks)
<i>F. ansellii</i>	M1	male	112	152
	F1	female	76	83
	M2	male	121	74
	F2	female	97	113
	M3	male	124	175
	F9	female	71	109
<i>F. mechowii</i>	M4	male	150	55
	F4	female	176	71
	M5	male	228	55
	F5	female	180	102
	M6	male	437	81
<i>F. micklemi</i>	F6	female	78	174
	M7	male	186	161
	F7	female	73	131
	M8	male	105	115
	F8	female	73	114

Table S2: Individual thresholds and species' means (in dB SPL) at the tested frequencies.

<i>f</i> (kHz)	<i>F. anelli</i>							<i>F. mechowii</i>						<i>F. micklei</i>					
	M1	F1	M2	F2	M3	F9	Mean	M4	F4	M5	F5	M6	Mean	F6	M7	F7	M8	F8	Mean
0.05	75	82	85	85	83	78	>80	85	82	85	85	82	>80	85	85	74	85	77	>80
0.125	58	62	56	56	50	45	55	51	57	60	55	57	56	65	75	55	63	54	62
0.25	41	55	51	38	42	41	45	47	51	45	47	48	48	55	54	48	56	40	51
0.354	35	44	43	43	38	37	40	43	38	45	46	48	44	64	43	48	51	36	48
0.5	36	39	33	33	38	31	35	33	40	37	39	40	38	45	50	45	46	45	46
0.63	36	33	38	30	32	33	34	37	38	33	41	42	38	40	42	43	41	41	41
0.8	33	34	38	35	32	38	35	34	33	31	37	37	34	38	40	40	34	38	38
1	34	25	31	25	24	33	29	28	29	33	39	36	33	40	44	32	33	35	37
1.4	38	31	35	27	29	41	34	29	32	35	39	28	33	44	60	26	37	38	41
2	45	43	55	35	35	51	44	30	46	37	47	42	40	42	49	25	37	36	38
4	72	61	67	59	54	53	61	53	45	45	68	48	52	65	68	44	54	52	57
8	83	69	85	82	61	72	75	77	72	75	83	58	73	68	85	60	80	72	73
12.5	77	85	82	74	58	72	75	63	83	80	85	61	74	76	82	78	70	62	74
16.5	68	83	85	85	85	85	>80	85	82	82	82	80	>80	80	73	85	83	77	80
18.5	85	80	85	78	77	75	80	73	85	85	80	85	>80	78	85	83	85	85	>80

Individual frequencies of most sensitive hearing are highlighted in bold. >80 indicates that no brainstem potentials were detected even at the highest tested sound pressure level of 80 dB SPL. To determine the average 85dB SPL was used when no responses could be measured at 80 dB SPL. M = male, F = female

Table S3: Individual anaesthetic doses used during the experiments.

Species	Individual	Sex	weight (g)	Dosage of Ketamine (mg/kg)	Dosage of Xylazine (mg/kg)
<i>F. anelli</i>	M1	male	122	9.88 (+ 5.34)	2.47
	F1	female	76	9.27	3.31
	M2	male	121	6.61	2.48
	F2	female	97	9.62 (+ 6.2)	2.93
	M3	male	124	7.29	2.63
	F9	female	71	9.3	4.09
<i>F. mechowii</i>	M4	male	150	4.85	2.26
	F4	female	176	4.26	2.13
	M5	male	228	4.82	1.97
	F5	female	180	4.17	1.95
	M6	male	437	4.58	1.83
<i>F. micklei</i>	F6	female	78	5.13	2.56
	M7	male	186	5.38	2.69
	F7	female	73	5.38 (+ 4.11)	2.74
	M8	male	105	5.26 (+ 2.87)	2.39
	F8	female	73	6.85	3.19

The low dosages were based on a published protocol for anaesthesia in mole-rats (Garcia Montero et al. 2015). Some animals required a second injection of ketamine during a session, the dosage of which is shown in brackets.