Research Article

Hearing and Age-Related Changes in the Gray Mouse Lemur

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Received: 18 September 2013; Accepted: 1 July 2014; Online publication: 12 August 2014

ABSTRACT

In order to examine auditory thresholds and hearing sensitivity during aging in the gray mouse lemur (*Microcebus murinus*), suggested to represent a model for early primate evolution and Alzheimer research, we applied brainstem-evoked response audiometry (BERA), traditionally used for screening hearing sensitivity in human babies. To assess the effect of age, we determined auditory thresholds in two age groups of adult mouse lemurs (young adults, 1–5 years; old adults, ≥ 7 years) using clicks and tone pips. Auditory thresholds indicated frequency sensitivity from 800 Hz to almost 50 kHz, covering the species tonal communication range with fundamentals from about 8 to 40 kHz. The frequency of best hearing at 7.9 kHz was slightly lower than that and coincided with the dominant frequencies of communication signals of a predator. Aging shifted auditory thresholds in the range between 2 and 50.4 kHz significantly by 12–27 dB. This mild presbycusis, expressed in a drop of amplitudes of BERA signals, but not discernible in latencies of responses, suggests a metabolic age-related decrease potentially combined with an accompanying degeneration of the cochlear nerve. Our findings on hearing range of this

species support the hypothesis that predation was a driving factor for the evolution of hearing in small ancestral primates. Likewise, results provide the empirical basis for future approaches trying to differentiate peripheral from central factors when studying Alzheimer's disease-like pathologies in the aging brain.

Keywords: auditory thresholds, age-related hearing loss, Alzheimer's disease (AD), auditory-evoked brainstem response, primate, evolution

Abbreviations: AD – Alzheimer's disease; ARHL – age-related hearing loss; ABR – auditory brainstem response; BERA – brainstem-evoked response audiometry; EP – endocochlear potential; SPL – sound pressure level

INTRODUCTION

In humans, age-related hearing loss (ARHL, or presbycusis) is one of the top three most common chronic health conditions, the most common form of hearing loss, and the predominant neurodegenerative disease of aging, affecting individuals aged 65 years and older (Gordon-Salant and Frisina 2010; Ohlemiller 2004; Pleis and Lethbridge-Çejku 2007). Multiple factors may influence or cause ARHL, including various types of physiological degeneration throughout the auditory pathway, accumulated effects of noise exposure throughout life, medical disorders and their treatment, life style (e.g., drinking,

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smoking), as well as genetic traits (CHABA 1988; Dubno et al. 2013; Humes et al. 2012; Mazelová et al. 2003; Willott and Schacht 2010). Sensory impairments, such as deficiencies in hearing, are also known to affect cognition during aging (Valentijn et al. 2005) and could even be one of the causes for age-related cognitive impairment according to the sensory deprivation hypothesis (Lin et al. 2011a; Lindenberger and Baltes 1994; Sekuler and Blake 1987). To disentangle sensory from central factors leading to aging-related cognitive deficiencies, it is necessary to rapidly assess the auditory sensitivity of subjects used in cognitive test batteries.

Mouse lemurs are the smallest primates worldwide and discussed as a new primate model for brain aging and Alzheimer research (Bons et al. 2006; Fischer and Austad 2011). Some, but not all, aged mouse lemurs develop pathognomonic signs of Alzheimer's disease (AD) such as the presence of β -amyloid plaques (Mestre-Francés et al. 2000), abnormally phosphorylated tau protein aggregation (Bons et al. 1995; Delacourte et al. 1995), and cerebral atrophy (Dhenain et al. 2000; Kraska et al. 2011) as well as deficiencies in behavior and cognition (Bons et al. 1992; Joly et al. 2013; Nemoz-Bertholet and Aujard 2003; Trouche et al. 2010). Due to their small size, the maintenance and breeding of mouse lemurs is more cost-efficient than in larger primate species and they are not known for spreading any zoonotic disease. Lifespan is much shorter than in other primate brain aging models, with maximally 8 years in the wild (Zimmermann et al. unpublished) and maximally 18.5 years (Weigl 2005) in captivity. Bred gray mouse lemurs are considered as aged when older than 7 years in a normal photoperiodic regime (Zimmermann et al. unpublished). Mouse lemurs are one of the few primate species included in whole genome research (<https://www.hgsc.bcm.edu/content/mouse-lemur-genome-project>; <http://www.genome.gov/10002154>). Besides the importance of the gray mouse lemur for biomedical research, mouse lemurs are also important for evolutionary research since they model the ancestral primate condition (Kessler et al. 2014; Martin 1972; 1995). Mouse lemurs resemble ancestral primates in being small, nocturnal, and living in dispersed social systems in dense forest environments where they rely mainly on olfactory and acoustic cues for social communication (Buesching et al. 1998; Perret 1995; Zimmermann 2010) and prey and predator detection, localization, and recognition (Bunkus et al. 2005; Goerlitz and Siemers 2007; Kappel et al. 2011; Piep et al. 2008; Siemers et al. 2007; Sündermann et al. 2008).

Unfortunately, up-to-date published information on hearing abilities in mouse lemurs is restricted to one single study in which auditory cortical responses were used to assess auditory sensitivity (Niaussat and

Petter 1980). As electrodes were directly placed on the cortex, such an invasive method is not suitable as a screening method for rapidly assessing hearing abilities and deficiencies in primate colonies. Furthermore, the response characteristics and the obtained hearing thresholds are critically dependent on exact placement of the electrodes in the cortex. Brainstem-evoked response audiometry (BERA), a minimally invasive method commonly used in hearing research for both humans and animals (Ison et al. 2010), has been applied successfully to study auditory sensitivity in several larger non-human primate species, including lemurs (Chabert 1998; Ramsier and Dominy 2012; Ramsier et al. 2012a, b). Additionally, this method is less time-consuming than the measurement of absolute auditory thresholds by conditioning techniques that require the time-consuming training of the animals (e.g., Osmanski and Wang 2011).

In this study, we applied BERA as a rapid assessment tool to the gray mouse lemur, to gain first insight into the hearing sensitivities of two age groups of this small ancestral primate model, used in aging and Alzheimer research. According to literature findings on other mammals (Boettcher et al. 1993a, b), including primates (Torre and Fowler 2000), we postulated a significant effect of age on the amplitude and latency of a click-induced wave of the auditory brainstem (ABR) as well as on the tone pip-induced ABR thresholds.

MATERIALS AND METHODS

We measured the auditory brainstem response (ABR) using the minimally invasive brainstem-evoked response audiometry.

The conducted research follows the national guidelines of the German Society of Primatology (GfP) for research on non-human primates. It was approved by the State of Lower Saxony Office for Consumer Protection and Food Safety (approval date: 14 February 2012; number: 33.9-42502-05-12A205).

Subjects

Adult gray mouse lemurs (*Microcebus murinus*) of 12 males and 7 females were studied, housed and bred in the animal facility of the Institute of Zoology, University of Veterinary Medicine Hannover, Germany (for details of housing conditions see Wrogemann et al. 2001). Subject weight varied between 63 and 115 g. All subjects were born in captivity and ranged in age from 2 to 11 years. According to the age classification of Zimmermann et al. (unpublished), the studied subjects belonged to two different age categories: 10 adult animals (7 males and 3 females) were in the

young group (mean age=2.5 years; range=2–5 years) and 9 adults (5 males and 4 females) were in the old group (mean age=7.9 years; range=7–11 years).

Anesthesia and Monitoring

The anesthesia was performed by professional equipment used in human and veterinary medicine (Dräger Titus NMR System), operated by veterinary experts. Subjects were separated in their nest box and home cage shortly before the onset of their activity period and transported in their nest box to a commercial sound-attenuated chamber (IAC Comp., Germany) where the measurement was performed. Twenty minutes before anesthesia induction, the animals were premedicated with 0.02 mg kg⁻¹ of glycopyrrolate (Robinul®, Riemser Arzneimittel AG, Greifswald-Insel Riems, Germany) and 0.5 mg kg⁻¹ of midazolam (Midazolam B. Braun® B. Braun Melsungen AG, Melsungen, Germany) subcutaneously. Anesthesia was induced with 8 vol.% sevoflurane (SevoFlo®, Albrecht GmbH, Aulendorf, Germany) in 100 % oxygen for 1.5–2 min at a flow rate of 4 l min⁻¹ directly in their nest box. After loss of the righting reflex, the anesthetized animals were immediately placed on a heatpad (SnuggleSafe heatpad Lenric C21 Ltd., Littlehampton, UK) heated for 2 min at 800 W in a microwave and connected via a sealed face mask to a coaxial Bain breathing system. Anesthesia was maintained with an average inspired sevoflurane concentration of 3.4 vol.% at a flow rate of 1 l/min. Additionally, the animals received a subcutaneous depot of 1–2 ml of a balanced electrolyte solution (Sterofundin® B. Braun Melsungen AG, Melsungen, Germany) spiked with 2.5 % glucose. During the measurements, the rectal temperature, the blood oxygen saturation (SpO₂), the pulse rate, the respiratory frequency, and the gas concentration in the face mask were monitored via a Datex Ohmeda Compact Monitor (Datex Ohmeda, GE Healthcare, GE Healthcare Finland OY, Helsinki, Finland) and a digital camcorder (Handycam DCR-SR35, Sony Corp., Tokyo, Japan) in nightshot mode from outside the sound-attenuated chamber.

After the measurements, the animals woke up in their nest box and were brought back to their home cage.

Stimulus Generation and Calibration

Clicks and tone-pip stimuli were generated digitally by Audiology Lab data acquisition software (Otoconsult Comp., Frankfurt, Germany) running on a computer connected to a custom-built attenuator (PNS1, Otoconsult Comp., Frankfurt, Germany) and a DA/AD converter (National Instruments NI-USB-6251,

Austin, TX, USA) via an HK 980 amplifier (Harman/Kardon, Harman International Industries Inc., Stamford, CT, USA). High-frequency sound presentations were performed using a high-frequency loudspeaker (ribbon tweeter 923108, quadral GmbH & Co. KG, Hannover, Germany). Low-frequency sounds (<2 kHz) were presented via an ASCENT 250 speaker (quadral GmbH & Co. KG, Hannover, Germany) driven by the same amplifier. The speakers were facing the right ear of the subject and were placed at a distance of 75 cm and at an angle of 40° above animal level. The distance from the animal was chosen to guarantee an acoustic far-field and thus minimize interference effects in the sound field with stimulus frequencies above 500 Hz.

Calibration of the sound pressure level (SPL) reaching the gray mouse lemur's ear was performed using a calibrated free-field 1/4-in. microphone (type 4939, Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark), a preamplifier (type 2670, Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark), and an amplifier (Measuring Amplifier Type 2610, Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark; time constant "Fast"; linear frequency weighting; measuring range 22.4–200,000 Hz) connected to the National Instruments board. The microphone was placed at the external meatus of the right ear (ca. 2 mm, as close as possible), rostral from the pinna. The microphone did not displace the pinna.

The presented tone pips ranged from 500 to 80 kHz and were delivered at a rate of 10 s⁻¹ as trial-to-trial phase-alternating (inverted) stimuli to minimize the effect of cochlear microphonics on the measured signals. Low-frequency tone pips (500–2,000 Hz) were 6-ms long including a linear rise and fall of 1 ms. Tone pips between 3 and 40 kHz were 3-ms long including a linear rise and fall of 1 ms. Very high-frequency tone pips (>40 kHz) were 6-ms long including a linear rise and fall of 2 ms. Level was increased in 5-dB steps during the measurements. The click had a 50-μs duration, and at 0 dB, attenuation reached 101 dB SPL p.e. at the calibrated microphone positioned close to the right ear.

Evoked Potential Acquisition

Three silver chloride electrodes were positioned subcutaneously (vertex, retroauricular, neck). Via a preamplifier (AMP55, Otoconsult Comp., Frankfurt, Germany, amplification 40 dB), the electrodes were connected to the PNS1 which was connected to the NI data acquisition module. The preamplified signal was band-pass filtered (100–5,000 Hz, second order) and amplified by 60 dB, giving a total gain of 100 dB. Evoked potentials were obtained by averaging re-

sponses to 1,332 stimulus presentations (666 pairs of normal and inverted stimulus pairs).

Threshold Detection and Data Analysis

Thresholds were defined as the lowest stimulus level at which a signal was discernible from noise, regardless of the specific component of the brainstem response. Criteria for the response identification included the consistent presence of the identified components with gradual decrease in peak latency with increasing sound pressure level (Figs. 2 and 3).

For better threshold identification, recordings were additionally offline band-pass filtered (100–3,000 Hz, time-invariant IIR elliptic filter, fourth order) to further remove high-frequency noise from the signal. The amplitude and latency of each detectable component were determined using a custom-made software programmed in MATLAB 7.9 (Mathworks, Natick, MA, USA). Amplitudes and latencies for the different components were compared between the two different age groups using 2-way ANOVA followed by a two-tailed *t* test if data were normally distributed or a Mann-Whitney *U* test in case data were not normally distributed. Normality was tested using the Lilliefors test at $\alpha=5\%$. To test for an effect of sex or age on hearing thresholds across tested frequencies, we performed Mann-Whitney *U* tests. We used the Fisher's Omnibus test (Haccou and Meelis 1994, Schehka et al. 2007) to allow a global decision about the null hypothesis (age has no predictable influence on ABR thresholds). This test considered the multiple *P* values of the single tests to create an overall *P* value. This overall *P* value resulted in an acceptance or refusal of the null hypothesis and put aside α adjustments for each frequency which are necessary when testing the same null hypothesis several times. All statistical tests were conducted with α set at 0.05 in Statistica 6.1 (StatSoft, Inc., Tulsa, OK, USA) or SPSS Statistics 22 (IBM Corp., Armonk, NY, USA).

RESULTS

The auditory brainstem-evoked response is a stereotypical signal that is well reproducible between different animals and only weakly susceptible to the anesthetic state. The small signal is evoked by brief acoustic stimuli with a steep onset. In the present experiments, responses were recorded with clicks and brief tone pips.

ABR Waveform and Thresholds

The recorded click-evoked ABR showed a waveform consisting of five vertex-positive components occur-

ring in the first 10 ms after the stimulus reached the eardrum. At a moderate sound pressure level (50 dB above threshold), five or six components could be detected (Fig. 1). The recorded signal thus fitted the general form as well as the time window of brainstem-evoked responses observed in other species. The vertex-positive components were numbered with Roman numerals I–V; however, not all components could be identified in all animals. Component II could be identified in only six of the measured subjects, so that either a five-component ABR (Fig. 1, upper waveform) or a four-component ABR (Fig. 1, lower waveform) was systematically observed at high sound pressure levels. In some examples, instead of a clearly identifiable component II, a shoulder in component III was observed. Only the consistently occurring components I, III, IV, and V were further quantitatively assessed. Due to the 75-cm distance of the speaker to the animal, 2.187 ms delay of sound travel time (from speaker to the animal) was subtracted from latencies to obtain the “true” response latencies. Both the onset latencies of 1–2 ms as well as the amplitudes within 10 μ V correspond to the standard values of brainstem-evoked thresholds.

The appearance of tone-evoked ABRs, both in their morphology, threshold, amplitudes, and latency, depends on the frequency of the stimulus. However, they share the same general features as those obtained with clicks. Also here, component II could not be consistently identified in all recordings. The component that was most frequently identifiable at threshold was component III; however, in some animals and at some frequencies also, later components determined the threshold. The minimum threshold at which responses were detectable in young animals was 10–20 dB SPL at 7.9 kHz. Tone-evoked ABRs demonstrated a consistent decrease in latencies and increase in amplitudes with increasing sound pressure level (Fig. 2).

The waveforms were quantified for peak amplitudes and latencies (Figs. 3 and 4). Not all frequency-level combinations allowed the analysis of all ABR components: in some instances, some components were not detectable, while others were well discernible. Therefore, in the plots of Figures 3 and 4, a different number of data points and consequently some variation in the standard deviation can be observed. In the following text, we always compare click-evoked ABRs and low-, mid-, and high-frequency tone-evoked ABRs between young and aged animals.

The ABR amplitudes were within 1–10 μ V and monotonically increased with sound pressure level (Fig. 3). The largest amplitudes were observed for clicks (mean maximum amplitude of 6.3 ± 1.5 μ V) and smaller ones for tones (mean maximum amplitude for 7.9 kHz, 2.2 ± 1 μ V), whereas low-frequency stimuli

FIG. 1. Auditory brainstem response waveform from two young adult gray mouse lemurs. The waveforms were collected in response to a click. Five distinct components were detectable in the brainstem response (*upper waveform*). In about half of the animals, however, only four waves could be found (*lower waveform*). The time point at stimulus presentation is indicated as *first dashed line* (here at 5 ms) and the sound travel time

between the loudspeaker and the ear (here 2.187 ms) as *second dashed line*. Positive values are plotted. The stimulus was a 50- μ s condensation click, presented at 50 dB above threshold of the given animal. Recorded signal was band-pass filtered (100 Hz–3 kHz), amplified 100 dB, and 1,322 repetitions were averaged. Negative signals are plotted down.

elicited particularly small ABRs. The amplitude-level functions showed systematically lower mean amplitudes in the old compared to the young adults. The difference was statistically significant for the largest component (component III, Fig. 3) both with click and tone-pip stimuli ($\alpha=5\%$, Fisher's Omnibus test; click: $\chi^2=71.72$, $DF=14$, $P<0.05$; 15.9 kHz: $\chi^2=54.64$, $DF=12$, $P<0.05$).

Latencies of the waves were analyzed next (Fig. 4). Again, comparisons are shown for clicks, low-, mid-, and high-frequency tone pips. For all components, the latencies systematically decreased with increasing stimulus level; the decrements were in the submillisecond range. Both the young and the old age group showed, however, similar latency-level functions with an almost perfect overlap. Only few

FIG. 2. Level-dependence of tone-evoked auditory brainstem response waveform from a young adult gray mouse lemur. The waveforms of one animal collected in response to a tone pip. At higher stimulus levels, four distinct components were detectable in this case. The *arrow* marks component III. The sound travel time

between the loudspeaker and the ear (here 2.187 ms) is indicated as a *dashed line*. The stimulus was a 7.9-kHz tone of 3 ms in duration, 1 ms on/off. Recorded signal was band-pass filtered (100 Hz–3 kHz), amplified by 100 dB, and 1,322 repetitions were averaged.

FIG. 3. Amplitude-level functions of component III for young and old gray mouse lemurs in response to click and tone-pip stimuli (for sample sizes, see Table 1). Mean amplitudes and standard deviations for component III in the age groups are shown (diamonds young group, triangles aged group). **A** Click response, 50 μ s in duration, and alternating polarity. Abscissa: dB SPL p.e.; ordinate: component III amplitude in microvolts. **B** 4-kHz tone pip, 3 ms in duration, 1 ms on/off, and alternating polarity. Abscissa: dB SPL; ordinate: component III amplitude

in microvolts. **C** 7.9-kHz tone pip, 3 ms in duration, 1 ms on/off, and alternating polarity. Abscissa: dB SPL; ordinate: component III amplitude in microvolts. **D** 15.9-kHz tone pip, 3 ms in duration, 1 ms on/off, and alternating polarity. Abscissa: dB SPL; ordinate: component III amplitude in microvolts. Signal conditioning in all panels: filter 100 Hz–3 kHz, amplification 100 dB, and 1,322 repetitions averaged. Significant differences ($P < 0.05$) in amplitude between the groups are marked.

frequency-latency combinations showed any statistically significant difference at the 5 % level (Fisher's Omnibus test), and a consistent effect was not observable between the groups. This finding thus differed from the amplitudes.

In conclusion, the quantitative assessment of the ABRs corresponds to what has been previously observed in other species. Furthermore, the data demonstrate that the effect of aging is predominantly observed in amplitudes and less in latencies of the ABRs.

Hearing Thresholds

Mean ABR thresholds are presented here as a function of pure-tone frequencies for young adult and old mouse lemurs (Fig. 5; Table 1). As in other recent studies (Ramsier and Dominy 2012; Ramsier et al. 2012a, b), we used the 60-dB level to estimate the

low- and high-frequency limit of hearing from the ABR threshold function.

The such-defined hearing range in young adults covered 750–44.9 kHz, the frequency of best hearing was at 7.9 kHz. The old subjects had a hearing range from 2.3 kHz up to 37.4 kHz; the frequency of best hearing remained at 7.9 kHz also in this animal group. Thus, aging affects the lower and upper limit of hearing considerably but not the frequency of best hearing.

Within the same age group, there was no significant sex difference in the ABR thresholds for the best frequency of hearing (BF) (Mann-Whitney U test, BF_{young}: $N=6$ males, $N=3$ females, n.s., BF_{old}: $N=4$ males, $N=4$ females, n.s.) or for any other frequency (MWU, n.s.), so that thresholds of both sexes could be linked to the respective age group. In young adults, the mean threshold level across subjects varied from 14.4 ± 4.6 dB SPL at 7.9 kHz to 98 ± 6.3 dB SPL at 80 kHz (see Table 1). In old adults, it ranged from 31.9 ± 16.2 dB SPL at 7.9 kHz to at least 100 dB SPL at 80 kHz with high

FIG. 4. Latency-level functions for young adult and old gray mouse lemurs in response to click and tone-pip stimuli (for sample sizes, see Table 1). Mean latencies and standard deviations of the four measured waves (I, III, IV, V) in the two age groups are shown (diamonds young group, triangles aged group). The Roman numerals label corresponds to the component evaluated. **A** Latencies of click-evoked responses systematically shorten with increasing level for all components. No differences are discernible between young and aged groups. **A** 50- μ s click and alternating polarity. **B** Tone-evoked

responses, 4-kHz tone pip, 3 ms in duration, 1 ms on/off, and alternating polarity. No systematic difference between the young and aged group. **C** 7.9-kHz tone pip, 3 ms in duration, 1 ms on/off, and alternating polarity. No difference between the young and aged group. **D** 15.9-kHz tone pip, 3 ms in duration, 1 ms on/off, and alternating polarity. No systematic difference between the young and aged group. Signal conditioning in all panels: filter 100 Hz–3 kHz, amplification 100 dB, and 1,322 repetitions averaged.

interindividual variation. In an old male aged 11 years, no detectable responses were found for clicks and tone pips at the tested frequencies up to 100 dB SPL.

We found a predictable influence of age on mean ABR thresholds (Fisher's Omnibus test: $\chi^2=190.05$, $DF=34$, $P<0.05$). Old subjects showed significantly higher thresholds than young adults across almost all of the measured frequencies in the above-defined hearing range (500 to 1,500 Hz and 63.5 to 80 kHz were excluded because of small sample size in the old adult group). Mean thresholds in the old subjects were 12 to 27 dB (mean $\pm$ SD 17 ± 4 dB) higher than in young adults.

DISCUSSION

Our study demonstrates the objective hearing range of the gray mouse lemur using a well-established electrophysiological method in hearing research. The

hearing range was from 800 Hz to almost 50 kHz with best frequencies of hearing close to 8 kHz. Aging shifted auditory thresholds significantly. Aged animals had lower ABR amplitudes whereas the latencies showed no consistent group difference.

Methodological Issues

Our study describes four to five distinct components in the recorded ABR waveform of gray mouse lemurs. The emergence of the signal as well as the changes with increasing sound pressure level were consistent with previous findings in other species. In ABR recordings of gerbils (Boettcher et al. 1993a), guinea pigs (Ingham et al. 1998), or non-human primates like ring-tailed lemurs (Ramsier and Dominy 2010) or long-tailed macaques (Alegre et al. 2001), four distinct components have also been described. In contrast to that, in humans (Møller 2006), rats (Chen et al. 2010),

FIG. 5. ABR thresholds for gray mouse lemurs (for sample sizes, see Table 1). **A** Mean hearing threshold (thick black line) with standard deviation of the measured thresholds as determined from tone-evoked ABRs for the young adult gray mouse lemurs. The 60-dB level is marked by a dashed line. Hearing thresholds below 60 dB SPL represent the

hearing range. **B** Mean hearing threshold (thick black line) with standard deviation as determined from tone-evoked ABRs for the old adult gray mouse lemurs. The 60-dB level is marked by a dashed line. Hearing thresholds below 60 dB SPL represent the

or cats (Melcher et al. 1996), usually five components are present. However, when a highly synchronized stimulus (electrical pulse applied through a cochlear implant) was used in cats, a break-up of some components in up to three subcomponents with increasing current level was observed (Tillein et al. 2012). Consequently, the number of components is likely to be dependent on stimulus level (relative to hearing threshold) and neuronal synchrony. Given the fact that consecutive components can merge (Boettcher 2002; Tillein et al., 2012), the present results are consistent with previous findings and

indicate similar generators, including the auditory nerve, the cochlear nucleus, and the superior olivary complex (Biacabe et al. 2001; Boettcher 2002; Melcher and Kiang 1996).

The point of best hearing at 7.9 kHz has a distinct appearance in the audiogram. This could theoretically be the consequence of acoustic resonances rather than hearing sensitivity. ABR thresholds were therefore measured at the same level at three additional frequencies between 7.877 and 8.300 kHz. Findings were the same as for 7.9 kHz, making acoustic resonances in this range less likely.

TABLE 1

Comparisons of the mean hearing thresholds in young adult and old gray mouse lemurs

Young adults (N=9–10)			Old adults (N=2–8)			
Frequency (Hz)	Mean hearing threshold (dB SPL)	SD (dB SPL)	Mean hearing threshold (dB SPL)	SD (dB SPL)	U	P
500	68.9	10.5	90.0	0.0	–	–
630	66.0	8.4	80.0	28.3	–	–
790	58.0	7.9	75.0	7.1	–	–
1,000	55.6	8.8	80.0	14.1	–	–
1,200	53.3	7.1	73.3	15.3	–	–
1,500	47.5	7.2	65.0	12.9	–	–
2,000	39.0	5.2	62.9	12.5	1	0.0000
2,520	38.0	7.9	57.1	14.1	7	0.005
3,170	38.0	5.9	52.1	14.7	11	0.019
4,000	31.0	5.2	44.4	10.8	9	0.004
5,040	29.4	3.9	43.8	9.2	1.5	0.0000
6,300	28.3	7.1	44.4	8.6	5	0.002
7,900	14.4	4.6	31.9	16.2	10.5	0.011
10,080	26.7	4.3	40.0	10.7	6	0.002
12,700	26.1	7.0	41.9	11.0	7	0.004
15,870	29.4	7.3	46.3	9.5	6	0.002
20,000	25.0	7.1	43.8	13.0	7	0.0004
25,200	23.3	8.3	50.6	22.1	3	0.001
31,750	30.0	8.9	50.6	23.2	0.5	0.021
40,000	52.5	7.2	64.4	20.4	27	0.274
50,400	68.5	13.3	86.9	14.9	13.5	0.16
63,500	94.5	9.0	94.3	9.8	–	–
80,000	98.0	6.3	100.0	0.0	–	–

Mean hearing thresholds and standard deviation (SD) for each age group and tested frequencies are given. The *U* and *P* values are from Mann-Whitney *U* tests and the Fisher's Omnibus test revealed a significant effect of age on hearing thresholds ($P < 0.05$, see text). Young adults: number of animals varied between 9 and 10 for each frequency, dependent on the quality of recording; Old adults: the total number of investigated lemurs was 9. Seven to eight lemurs were used in the range between 2 and 50.4 kHz, dependent on the quality of recording. Statistical comparisons could not be performed in the range between 500 and 1,500 Hz and 63.5 and 80 kHz due to high thresholds/non-detectable responses at these frequencies in 6 old adult lemurs. One old adult male aged 11 years showed no auditory-evoked response for clicks and the tested tone pips at the maximum sound pressure level tested (100 dB). This male was thus excluded from any further analysis.

ABR thresholds are an estimation of hearing thresholds and often do not reach the behavioral thresholds (overestimate the thresholds). Comparison of the present data to behavioral thresholds presented for a large-bodied lemur species, the ring-tailed lemur, suggest an underestimation of the thresholds by about 10 dB (Mitchell et al., 1971). The lowest ABR threshold of about 14 dB SPL, measured in this study, is, however, similar to the lowest ABR threshold measured in a variety of other primates, including ring-tailed lemurs (Ramsier and Dominy 2012; Ramsier et al. 2012a, b).

Finally, it needs to be acknowledged that the presented thresholds were obtained with free-field stimulation, as in other primate studies, too (see Ramsier et al. 2012a). This corresponds to the natural hearing condition and to behaviorally assessed hearing thresholds, but the responses are not ear specific. This is unlikely to have affected the thresholds, as these are determined by the more sensitive ear, which is very likely the ear that was closer to the loudspeaker. We decided to use free-field stimulation to exclude problems that may result from the ear canal length and its very small diameter generating stronger reverberations in the artificial-closed field condition.

Calibration of the sound pressure level in closed condition with such high-frequency stimuli might be a confounding factor. Besides, as in other recent primate studies on hearing, we decided to use the less invasive free-field stimulation.

Behavioral Relevance of the Measured Hearing Range

The standard mammalian audiogram is characterized by a U shape with greatest sensitivity in a mid-frequency region, related to the species-specific communication range, and a progressive increase in thresholds for lower and higher frequencies (Ison et al. 2010). The audiograms of many primate species correspond to this general shape (Coleman 2009; Heffner 2004; Ramsier and Dominy 2012; Ramsier et al. 2012a, b). The presented mouse lemur's ABR thresholds for tone pips fit to this pattern as well. In contrast to anthropoid primates, mouse lemur's sensitivity to higher frequencies (above 20 kHz) is better, matching findings for other strepsirrhine primates (Ramsier and Dominy 2012; Ramsier et al. 2012a, b). The mouse lemur's hearing range, as determined in this study, corresponds

to its acoustic communication range (Leliveld et al. 2011; Zimmermann 2010) with about 8 to 40 kHz for the fundamentals of tonal communication signals. The mouse lemur's best frequency of hearing at 7.9 kHz is slightly lower than that and thus may represent an adaptation to frequencies of sounds emitted by prey or predators. Gray mouse lemurs use prey-generated rustling sounds from arthropods for prey detection and localization (Goerlitz and Siemers 2007; Piep et al., 2008; Siemers et al. 2007). However, these sounds are rather noisy and thus cannot explain the distinct sensitivity peak at 7.9 kHz. Thus, an adaptation to predator avoidance seems to be more likely. Mouse lemurs face the highest predation risk in primates (Scheumann et al. 2007) and mostly rely on olfactory and acoustic cues for predator detection and avoidance (Kappel et al. 2011; Rahlfs and Fichtel 2010; Sündermann et al. 2008). An important predator of mouse lemurs is the Madagascar harrier hawk (*Polyboroides radiatus*) (Fichtel and Kappeler 2002), and indeed, the frequencies of its vocalizations overlap with the mouse lemur's best frequency of hearing. This provides support for the hypothesis that predation drives the evolution of hearing sensitivities in small ancestral primates.

Effects of Aging

An age-related decrease in amplitudes of ABR components is in accordance with several studies in humans and animals (e.g., Sand 1991; Torre and Fowler 2000, or see Boettcher 2002 for review). Especially, the amplitudes of early components decrease with age (Sand 1991), which is in agreement with the present finding of a significantly lower amplitude in component III of old mouse lemurs. Boettcher (2002) suggested that the amplitude of the ABR is a direct function of the number of neurons and the synchrony of the neurons contributing to the response, as well as the value of the endocochlear potential (EP). Age-related changes in ABR amplitudes are suggested to be the consequence of a combination of a reduced number of neurons responding to the given stimulus, a reduced synchronization of activity of responding neurons and/or a reduction in the EP. The direct connection of neural degeneration of the cochlear nerve and decreased ABR amplitudes could be shown in mice and guinea pigs: A temporary noise-induced hearing loss could be reversed but caused an acute loss of afferent nerve terminals and delayed degeneration of the cochlear nerve (Kujawa and Liberman 2009; Lin et al. 2011b). These results suggest that one participating mechanism for the age-related decrease in ABR amplitudes of gray mouse lemurs could be an age-related neural degeneration.

In contrast to the significant age-related decrease in amplitude, our study did not reveal a difference in ABR latencies between the two age groups. ABR latencies should, however, be affected by threshold elevation (see Boettcher 2002 for review). These results suggest that either the threshold elevation was not large enough or that the main reason for the age-related decrease in ABR amplitudes of gray mouse lemurs with no changes in latencies is an age-related neural degeneration.

Since the description of four types of human presbycusis by Schuknecht (1974), research in this field has expanded and his framework has been widely discussed (Chisolm et al. 2003; Ohlemiller 2004; Schmiedt 2010). When comparing the general shape of the auditory thresholds of young and old gray mouse lemurs, it is noticeable that it does not change. The elevation of the hearing threshold in old subjects occurs across almost all frequencies. The mean threshold shift of 17 dB may not be considered as a hearing impairment according to the WHO grades (http://www.who.int/pbd/deafness/hearing_impairment_grades/en/index.html), but it is comparable to aging effects in other model species like chinchilla (McFadden et al. 1997) or mice (Henry 1982). Nonetheless, this threshold shift can already have an influence on the survival of the animals in the wild, since it likely impairs prey and predator detection as well as social communication.

A broadband age-related decrease in hearing sensitivity has also been described for the gerbil (Henry et al. 1980). In contrast to that, an age-related high-frequency hearing loss is common in many species, including humans (Schmiedt 2010). Nevertheless, there are different types of ARHL that are also characterized by different audiogram profiles (Schmiedt 2010). A flat audiometric loss like the one in our study can be ascribed to metabolic presbycusis (Dubno et al. 2013; Humes et al. 2012; Schmiedt 2010). However, it is difficult and not reliable to diagnose the form of ARHL solely based on the shape of the audiogram (Chisolm et al. 2003; Ohlemiller 2004). Thus, further studies are necessary to clarify the reasons and mechanisms of the decrease in hearing sensitivity described in this study.

CONCLUSION

In conclusion, auditory thresholds of mouse lemurs show typical mammalian characteristics. As in other primates, their hearing range covers the vocal communication range. The detection of a frequency of best hearing, matching the dominant frequency of communication signals of a predator more than those of conspecifics, indicates that predation is a previously

neglected selection factor shaping the evolution of hearing sensitivity in early primates.

For the first time, we could demonstrate an age-related change in hearing in this species, which is most likely of biological importance and presumably connected to metabolic presbycusis and an age-related neural degeneration in the cochlear nerve and auditory brainstem. This very distinct type of mild presbycusis was expressed in a drop of amplitudes but not discernible in latencies of responses.

Sensory changes are discussed as being related to cognitive impairments during aging and are even suggested as a factor inducing the phenomenology of Alzheimer's disease in humans (Lin et al. 2011a). Our study showed a high variation of ABR thresholds in the old adult group of mouse lemurs with one very old male, even found deaf. Previous neurobiological and cognitive approaches using mouse lemurs in aging and AD research (see [Introduction](#)) never examined whether brain pathologies or deficiencies in behavior and cognition were related to or caused by sensory pathologies. Focusing in the future on these important relationships may open up new perspectives for the early detection of aging-related diseases. Our study offers BERA as a minimally invasive, cost-, and time-efficient tool for screening hearing and aging-related deficiencies in colonies of this new primate brain aging model and provides the empirical basis for future approaches trying to disentangle peripheral from central factors when studying Alzheimer's disease-like pathologies in the aging brain.

ACKNOWLEDGMENTS

We would like to thank F. Sherwood-Brock for checking the English in the manuscript, H.-J. Sauer, L. Müller, and K. Nitschke for animal care and S. von den Berg and K.-H. Esser for technical support. This study was financially supported by a Georg-Christoph-Lichtenberg scholarship (CS) and in part by the DFG Cluster of Excellence Hearing4all (PH and AK) and the European Community's 7th Framework Programme (FP7/2007-2013) under grant agreement no. 278486 acronym "DEVELAGE" (EZ).

Conflict of interest The authors declare no actual or potential conflicts of interest.

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