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SHORT COMMUNICATION

Hearing ability decreases in ageing locusts

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ABSTRACT

Insects display signs of ageing, despite their short lifespan. However, the limited studies on senescence emphasize longevity or reproduction. We focused on the hearing ability of ageing adult locusts, *Schistocerca gregaria*. Our results indicate that the youngest adults (2 weeks post-maturity) have a greater overall neurophysiological response to sound, especially for low frequencies (<10 kHz), as well as a shorter latency to this neural response. Interestingly, when measuring displacement of the tympanal membrane that the receptor neurons directly attach to, we found movement is not directly correlated with neural response. Therefore, we suggest the enhanced response in younger animals is due to the condition of their tissues (e.g. elasticity). Secondly, we found the sexes do not have the same responses, particularly at 4 weeks post-adult moult. We propose female reproductive condition reduces their ability to receive sounds. Overall our results indicate older animals, especially females, are less sensitive to sounds.

KEY WORDS: Age, Sound, Insect, Laser vibrometry, *Schistocerca gregaria*, Neurophysiology

INTRODUCTION

Many insects have a short lifespan, especially during their adult stage (Ridgel and Ritzmann, 2005; Uvarov, 1966). Even so, in this brief, final stage, insects may experience the effects of ageing. Many studies of senescence focus on longevity and/or reproduction, especially in *Drosophila* (reviewed in Curtsinger et al., 1995). Additionally, some insects show slower locomotion and lower activity levels with increasing age post-maturity (Ridgel and Ritzmann, 2005). Older *Antheraea peryni* moths show a decrease with age in their pheromonal response as well as a decrease in their living dendrites (Kumar et al., 1998). As adults, most insects will not moult again; therefore, their cuticle may harden and become less elastic, such as the cockroach tarsal pads (Ridgel and Ritzmann, 2005). Adult insects have also been shown to thicken their cuticle by adding new layers in a circadian cycle (Neville, 1963), which could affect thin tissues, such as the ear's tympanal membrane.

To hear, locusts, like many insects, receive sound via a tympanum that is composed of a thin layer of cuticle – less than a micrometre thick in some regions (Malkin et al., 2014). This tympanum is very flexible and moves with sound. In locusts, there is a frequency-dependent travelling wave that forms, physically deflecting receptor neurons directly attached to the membrane (Windmill et al., 2005). The ears are sensitive to nanometre-level movements and such displacements are responsible for their frequency sensitivity (Gordon et al., 2014). After the locust emerges as an adult, some cuticle features continue to harden and sclerotize (Uvarov, 1966). The locust ear is not fully developed until it reaches its adult form (Michel and Petersen, 1982). Additionally, the maturation period of females varies with environmental condition; for example, lush versus dry plants affect the time that *Schistocerca gregaria* take to lay their first egg pod with a range of 33 to 54 days, respectively (Uvarov, 1966). With these factors in mind, we tested ageing in adult *S. gregaria* Forskål and their hearing abilities, through both tympanal membrane deflection and neurophysiology, to understand whether and how ageing affects hearing in locusts.

RESULTS AND DISCUSSION

Deflection of the tympanal membrane activates the mechanosensory receptor neurons directly attached to it; therefore, differences in deflection should result in differing amounts of neural activation. We measured deflections of the tympanal membrane at two sound levels (60 and 70 dB sound pressure level, SPL) across 1–20 kHz, finding similar patterns and so display data for only the higher sound level. Male and female locusts did not have the same tympanal membrane displacement with sound as they aged from 2 to 8 weeks (Fig. 1). With increasing female age there was an incrementally reduced amount of tympanal membrane displacement, significant across all frequencies measured (Fig. 1A) (e.g. at 5 kHz $F_{4,37}$=3.92, P=0.010; at 15 kHz $F_{4,37}$=3.33, P=0.021). As males aged, there was no difference in their tympanal membrane movement for most frequencies (Fig. 1B). Yet, the older age groups, 6 and 8 weeks, had significantly more displacement for lower frequencies (e.g. at 4.5 kHz $F_{4,43}$=3.65, P=0.013).

At 2 weeks post-adult moult, there were no significant differences between the sexes (Fig. 1C). In contrast, for the remaining age groups the males had greater movement in their tympanal membrane than the females (Fig. 1D–G). The 3 and 4 week locusts had an intermediate level of difference, wherein they were significantly different at low frequencies but not at high ones (Fig. 1D,E) (e.g. 4 weeks: at 5 kHz $F_{1,21}$=5.57, P=0.029; at 15 kHz $F_{1,21}$=2.58, P=0.124). Old animals were significantly different across all frequencies (e.g. 8 weeks: at 5 kHz $F_{1,12}$=6.87, P=0.024; at 15 kHz $F_{1,12}$=8.23, P=0.015).

To understand the neurophysiological response relating to the membrane deflections, we analysed the neurophysiology of the locusts' hearing in several ways, using the basic method of the RMS (root mean square) value of the summed electrophysiological response (see Materials and methods, and Gordon et al., 2014 for details). First, across all frequencies (focusing on 70 dB SPL – the same sound level used for the displacement tests) the youngest, 2 week post-maturity animals, had the highest neurophysiological response for lower frequencies in both sexes (under 10 kHz) (Fig. 2A,B). This coincides with the greater movement of the membrane at lower frequencies. For females, increased age decreased tympanal movement, which was mirrored in the neurophysiology, but males showed the same neurophysiology trend as the females (Fig. 2)

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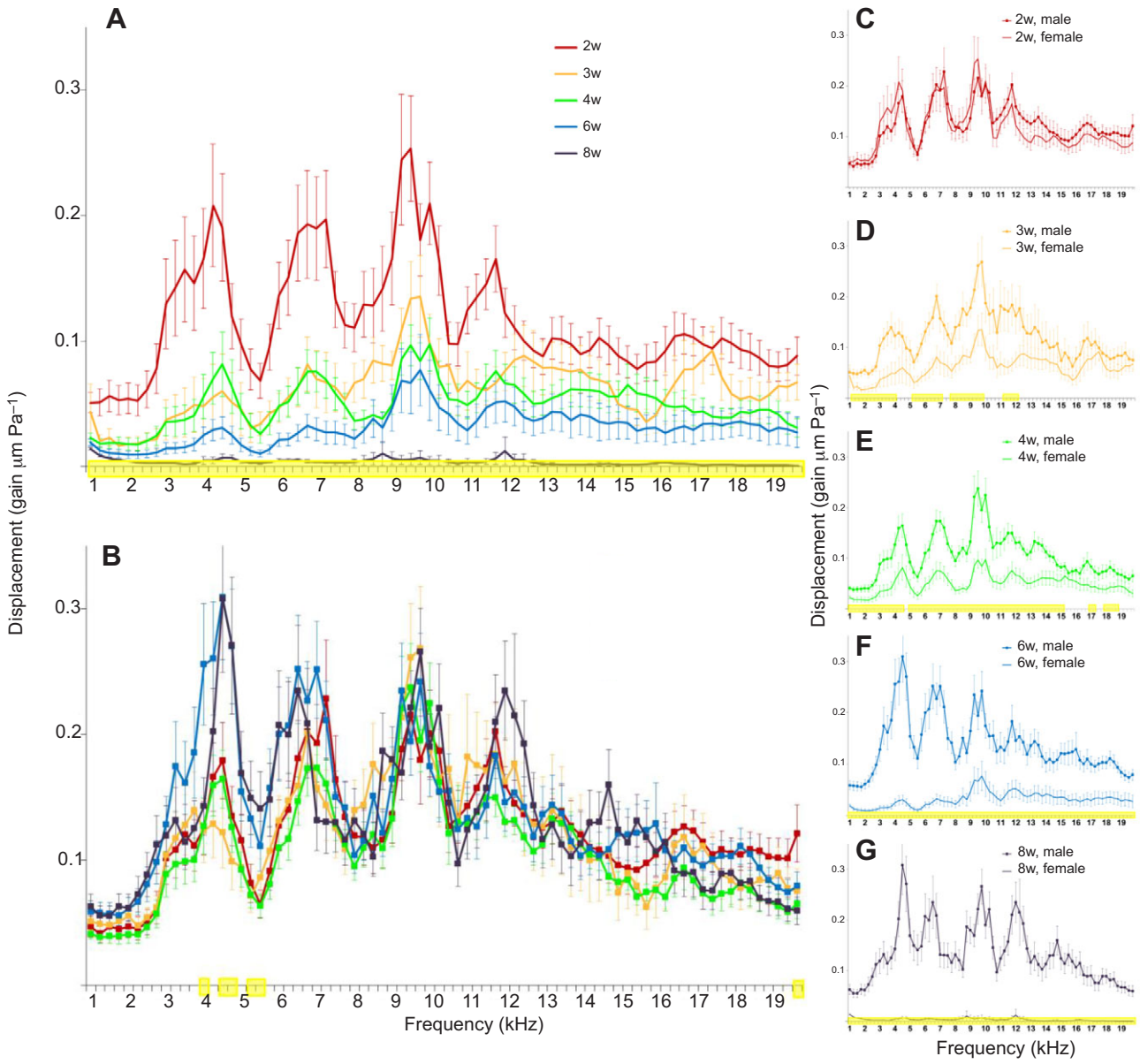

Fig. 1. Displacement (gain) of the tympanal membrane with sound (1–20 kHz) among *Schistocerca gregaria* of different ages. Data for females (A), males (B) and between sexes (C–G) at each age group: 2, 3, 4, 6 and 8 weeks post-maturity locusts. Age in weeks (w) is indicated by colour. Males are represented with lines with squares and females with smooth lines. Yellow bar on the *x*-axis represents significance of $P \leq 0.05$.

despite similar tympanal membrane movement among male age groups (Fig. 1B). Within age group, there was a trend for the males to have a relatively higher neurophysiological response than the females especially between 2 and 5 kHz, though they were not significantly different at most frequencies among ages or between sexes (supplementary material Fig. S1).

Three frequencies (5, 10 and 15 kHz) were examined in detail, across a 50 dB SPL range, to determine any differences in neural activation and saturation with increasing sound levels. At 5 kHz, female and male locusts had significantly higher neural responses for the lower sound levels (Fig. 2C; supplementary material Fig. S2A,B). Between the sexes within each age group, there were practically no significant differences (supplementary material Fig. S2C–E); however, there was a trend for the males to be more sensitive than the females at a given sound level. There were almost no significant differences at 10 or 15 kHz.

We then broadened our analysis to include low (50 dB SPL) and high (90 dB SPL) sound levels across all frequencies (Fig. 2D; supplementary material Fig. S1F–K and Table S1). At low sound levels, there were very few cases of any significant differences among age groups for either sex or between sexes within age groups. At high sound levels (90 dB SPL), among females there was a significant difference based on age between 1 and 5 kHz with a similar trend to that at 70 dB SPL (e.g. 5 kHz $F_{2,14}$=6.79, P=0.011; at 15 kHz $F_{2,14}$=1.31, P=0.31). The males only showed significant differences for the higher sound level between 4.5 and 5 kHz and there were few differences between the sexes at each age group (supplementary material Fig. S1F–K, Table S1). Evaluating the trend from 50 to 70 to 90 dB SPL, several patterns can be observed (Fig. 2D; supplementary material Fig. S1F–K). First, the neurophysiological response separated into two clear groups with the lower frequencies (2–10 kHz) having the higher value.

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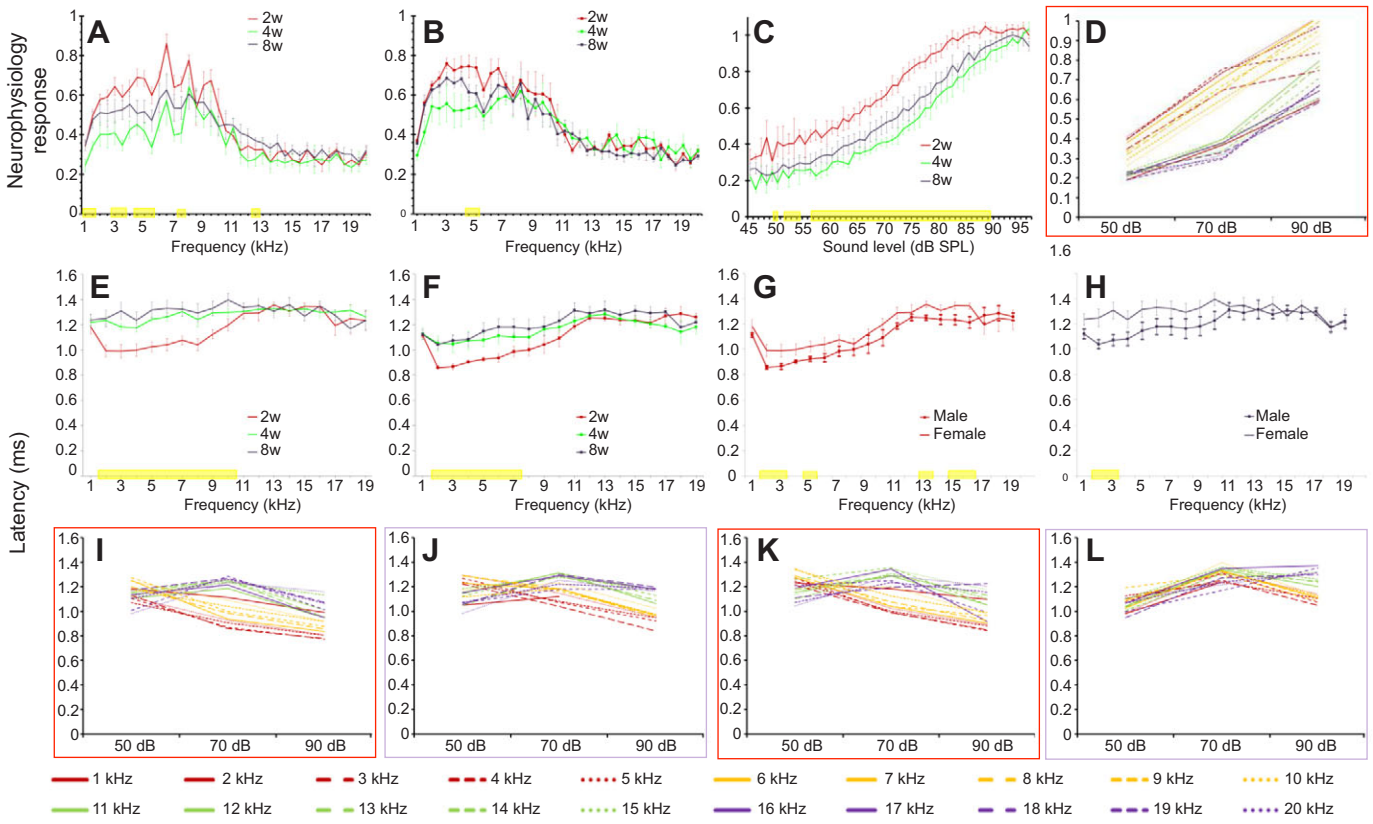

Fig. 2. Neurophysiological response of locusts. (A) Female and (B) male relative neural responses across frequencies at 70 dB SPL. (C) Female response at 5 kHz across sound levels. (D) Male response, at 2 weeks post-maturity, across frequencies at 50, 70 and 90 dB SPL. (E,F) Latency responses for females (E) and males (F) at 70 dB SPL across frequencies. (G,H) Latency response between males and females at 2 weeks (G) and 8 weeks (H) post-maturity. (I–L) Latency responses at three sound levels for 2 week males (I), 8 week males (J), 2 week females (K) and 8 week females (L). Yellow bars indicate significance $P \leq 0.05$. Key at the bottom indicates the colour and pattern for each frequency for D and I–L; frame colour is to help identify age group.

In addition, the lower frequencies had a more linear response, while the higher frequencies (11–20 kHz) had a greater increase in their neurophysiological response after 70 dB SPL. Finally, patterns were similar for each age/sex combination; however, the older animals had a lower high frequency response for the lower sound level (supplementary material Fig. S1F–K).

Finally, we measured the latency for the neurobiological response (Fig. 2E–L; supplementary material Fig. S3). Two week post-maturity adults responded significantly faster for the lower frequencies (under 8 kHz) at 70 dB SPL for both males and females (Fig. 2E,F) (e.g. at 5 kHz female 1.02±0.04 ms $F_{2,14}$=9.28, P=0.004; male 0.92±0.02 ms $F_{2,16}$=5.09, P=0.022). The males responded faster than the females, but this was only significant at a few frequencies (Fig. 2G,H). We next expanded the analysis to low and high sound levels (Fig. 2I–L; supplementary material Table S2). As expected, for low frequencies (<10 kHz), increasing sound levels decreased latency times for 2 week old animals (Fig. 2I,K, red and orange colours). Surprisingly, in contrast, for high frequencies the 2 week animals at 70 dB SPL actually had an increase in latency compared with both the 50 and 90 dB SPL (Fig 2I,K, green and purple colours). Male locusts of the older age groups followed a similar, yet less dramatic trend (Fig. 2J). However, surprisingly, for older female locusts, 70 dB SPL had the greatest latency for most frequencies (Fig. 2L).

Taken together, membrane movement and neurophysiology data show the effects of ageing for adult locusts in their hearing response. At lower frequencies there is an increased neural response between 2 week old adult locusts and the remaining age groups as well as shorter response latencies in the neural activity. Interestingly, these data were not a direct reflection of the tympanal membrane displacement. Males showed little difference in their tympanal membrane displacements with age, yet significant differences in their neurobiological response. Females showed a constant decrease in tympanal movement with age, though it was not directly proportional to the change in their neurobiological response. We suggest the decrease in the neuro-response initially is probably due to a change in the cellular physiology of the animals, but also to animal reproductive status.

As 2 week old locusts of both sexes have greater and faster neurobiological responses despite equal changes in male tympanal membrane movement, we suggest the 2 week old locust's tissues are in prime condition. Their ability to detect sounds at lower sound levels is superior to that of older animals, at low frequencies. As insects age, their tissues are known to lose elasticity and become harder (e.g. *Blaberus* cockroaches, Ridgel and Ritzmann, 2005), which consequently could affect the movement of internal air sacs and the neuronal attachment points involved in sensing tympanal movement. Additionally, some invertebrates are known to have a decrease in live dendrites with age (e.g. *Antheraea peryni* silkmoth, Kumar et al., 1998) and an increase in the threshold for action potential generation (e.g. *Lymnaea stagnalis*, Yeoman and Faragher, 2001), which could explain the observed results. Perhaps the

hormones present in both sexes during the height of their mating time period may reduce their neurophysiology response.

Another component that affects locust hearing is sound travelling through the body creating a pressure-gradient receiver for lower frequencies (Miller, 1977). As adult female locusts age they develop eggs, such that there is an overall increase in body mass; there is a likely decrease in the volume available for air sacs to expand, which would also affect hearing. We therefore measured their mass as they aged. We found females had a significant increase in mass with ageing (2.15–2.8 g, $F_{4,39}$=5.44, P=0.002) (supplementary material Fig. S4) while males displayed no significant differences, despite a slight decline with older ages (~1.6 g, $F_{4,46}$=0.79, P=0.54) (supplementary material Fig. S4). Previous work has shown that heavier animals have a higher threshold response to sound (Miller, 1977). Our work supports this, as females showed an increase in their body mass with age. Under natural conditions, at approximately 4 weeks post-maturity females begin laying their eggs. This could explain why we see such a large difference between the 4 week female age group. In captivity, locusts do not always have ideal conditions for reproduction (Uvarov, 1966), which is perhaps why we did not see a decline of mass with the oldest age groups.

The results of this study call attention to a number of important factors. First, there is a decline in hearing response with locust age. This means that the older locusts are more susceptible to being caught by predators. In addition, younger animals should be better at hearing each other (e.g. rustling of swarm mates). Second, animal reproductive condition should be a factor in studies. Our results clearly show that the female condition affects hearing more so than for the males. We suggest in this instance it is due to physical factors affecting the sound pathway. However, hormones and other factors may also create differences between sexes as they age. Furthermore, our results suggest, similar to others (Ridgel and Ritzmann, 2005), that insects could be considered as a model of animal ageing as they show ageing effects, develop on a much faster time scale than vertebrates, and can be studied under controlled conditions. Our study emphasizes the need to pay attention to the age of invertebrate research animals, as age differences could confound results.

MATERIALS AND METHODS

Animals

Adult *S. gregaria* were obtained from Blades Biological Ltd (Edenbridge, UK). The supplier provided animals with known final moult dates (either 1.5 or 3.5 weeks), keeping feeding conditions consistent between groups. Locusts were then fed organic lettuce and dried oats at the University of Strathclyde laboratory and were maintained on a 12 h:12 h light:dark cycle at 24°C.

Membrane deflection

Each animal's mass was taken before trials as an indicator of its condition. Membrane deflection trials followed a similar protocol to previous work, measuring identical locations on the membrane focusing on the neural attachment site called the pyriform vesicle (PV) (Gordon et al., 2014). Briefly, the right tympanal membrane was exposed to a micro-scanning Laser Doppler Vibrometer (PSV 300, Polytec, Waldbronn, Germany) with a close up unit (OFV 056). A loudspeaker (ESS Air Motion Transformer, South El Monte, CA, USA) was placed at least 10 cm away, to avoid operating in the near field. A microphone (Bruel & Kjaer 4138, Naerum, Denmark) was positioned to measure the sound pressure at the tympanal membrane. A broadband linear chirp was played at 60 and 70 dB SPL, generated by the laser vibrometer's control computer and then passed through an amplifier (TA-FE370, Sony, Tokyo, Japan). The FFT resolution was 12.5 Hz and measurements were averaged at least 15 times per point measured and later binned to 500 Hz categories. Gain (displacement/SPL) values were used for analysis to account for any differences in sound signal amplitude. Final sample size included (male, m; female, f): 2 weeks – 8m, 8f; 3 weeks – 6m, 6f; 4 weeks – 9m, 15f; 6 weeks – 11m, 8f; 8 weeks – 10m, 3f. Weeks 4 and above contained animals from different shipments. Data were analysed with an ANOVA in SPSS (IBM, Armonk, NY, USA) grouping by sex or age.

Neurophysiology

Neurophysiology experiments followed the same protocol as previous work (Gordon et al., 2014). Briefly, the locust was mounted ventral-side up in dental beading wax (Kedment, DWS307, Purton, UK). A pair of hook electrodes, made from 50 µm silver wire, was placed under the auditory nerve in the metathorax and insulated using petroleum jelly. We amplified the output signal (DAM50, World Precision Instruments Ltd, Hitchin, UK) before feeding it into the data acquisition program LabView (National Instruments, version 8.5.1, USB-6251, and BNC-2110, Austin, TX, USA) for processing and filtering. The final sample size was: 2 weeks – 5m, 5f; 4 weeks – 5m, 5f; 8 weeks – 7m, 5f.

Sound was controlled and calibrated in a similar manner to the membrane deflection trials. The sound stimulus was created with a custom-written LabView program (National Instruments, version 8.5.1), fed through a data acquisition system (National Instruments USB-6251 and BNC-2110). White noise (1–20 kHz) was played for 30 s before any of the experimental stimuli, to account for sensory adaptation. A sequence of tapered cosine-windowed (Tukey-windowed) pure-tone bursts was then played, ranging from 1 to 20 kHz at 1 kHz intervals over a 60 dB (SPL) range from 40 to 100 dB with 1 dB step sizes. Sound was played in a randomized order.

Data were processed in LabView (for details, see Gordon et al., 2014). Briefly, the summed neural response of the auditory nerve was measured by calculating the RMS of the signal for the duration of the sound stimulus. Latency was calculated to the time the signal first exceeded 20% of its maximum amplitude. Each individual dataset (several within an animal), comprising the responses to a full frequency range of 1–20 kHz sound stimuli across all sound intensities, was normalized to the maximum amplitude of the RMS electrophysiological response in the dataset, to control for any change in signal intensity (excluding latency data, which remained as absolute values).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

S.D.G. and J.F.C.W. worked together on experimental design. S.D.G. ran the experiments and analysed the data. Both authors contributed to organizing, writing and editing the manuscript.

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Supplementary material

Supplementary material available online at
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