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Title: Hearing Ability Decreases in Aging Locusts

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Abstract

Insects display signs of aging, despite their short lifespan. We focus on the aging of adult locusts and their subsequent hearing ability. Our results indicate that younger adults (two weeks after maturity) have a greater neurophysiological response to sound, especially for low frequencies (<10kHz), as well as shorter latency to this neural response. Interestingly, when measuring displacement of the locust tympanal membrane, which the receptor neurons are directly attached to, we found the movement is not directly correlated with the neural response. Therefore we suggest the enhanced response is due to the condition of their tissues (e.g., elasticity). Secondly, we found that females and males do not have the same responses, particularly at four weeks post adult moult. We propose that this is due to female reproductive condition reducing the ability to receive sounds. Our results indicate older animals may therefore be less sensitive to the sounds of approaching predators.

Hearing Ability Decreases in Aging Locusts

Introduction

Many insects have a short life-span, especially during their adult stage. Even so, in this brief, final stage, insects may experience the effects of aging. For example, some insects show slower locomotion and lower activity levels with increasing age post maturity (Ridgel and Ritzman, 2005). Older moths, *Antheraea peryni,* 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, and therefore do not shed the hard outer cuticle used as the scaffold of their body. Indeed, as insects age, aspects of their cuticle harden and become less elastic, such as the cockroach tarsal pads (Ridgel and Ritzman, 2005). Adult insects have also been shown to thicken their cuticle by adding new layers in a circadian cycle (Neville, 1963).

To hear, 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 the receptor neurons that are directly attached to the membrane (Windmill et al., 2005). The ears are sensitive to nanometre level movements and such displacements are responsible for differing frequency sensitivities (Gordon et al., 2014). After emerging as an adult, some insect 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, female condition changes with time, as female *Schistocerca gregaria* may lay their first egg pod with as much variation as 33-54 days (Uvarov, 1966). With these factors in mind, we tested aging adult locusts and their hearing abilities, both through tympanal membrane deflection and neurophysiology.

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 SPL) finding similar patterns and so data are displayed for only the higher sound level. Male and female locusts did not have the same tympanal membrane displacement with sound as they aged (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 5kHz $F_{4,37} = 3.92$, $p = .010$; at 15kHz $F_{4,37} = 3.33$, $p = .021$). As males aged from two to eight weeks, there was no difference in their tympanal membrane movement for most of the frequencies (Fig 1B). Yet the older age groups, six and eight weeks, had significantly more displacement for the lower, ~4-5 kHz, frequencies, (e.g., at 4.5kHz $F_{4,43} = 3.65$, $p = .013$). Interestingly, when the displacement data was scaled to approximately the largest peak across all age groups (9.25-10kHz), the females--like the males--showed the same pattern for the displacement across the frequency spectrum.

At two 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 three and four week locusts had an intermediate level of difference, wherein they were significantly different at low frequencies but not high ones (Fig 1D-E) (e.g., 4 weeks: at 5kHz $F_{1,21} = 5.57$, $p = .029$; at 15kHz $F_{1,21} = 2.58$, $p = .124$). Old animals were significantly different across all frequencies (e.g., 8 weeks: at 5kHz $F_{1,12} = 6.87$, $p = .024$; at 15kHz $F_{1,12} = 8.23$, $p = .015$).

To understand the neurophysiological response relating to the membrane deflections, we analysed the neurophysiology of the locusts' hearing in several ways. First, across all frequencies (focusing on 70 dB SPL--the same sound level used for the displacement tests) the youngest, two week post maturity animals, had the highest neurophysiological response for lower frequencies in both sexes (under 10 kHz) (Fig 2A & B). This matches with the greater movement of the membrane at lower frequencies; for females, increased age decreased movement which is mirrored in the neurophysiology, but males show the same neurophysiology trend as the females (Fig 2) 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 2-5 kHz, though they were not significantly different at most frequencies (Fig S1).

Three frequencies (5, 10, & 15kHz) were examined in detail, across a 50 dB SPL range to determine any differences in neural activation and saturation with increasing sound levels. At 5kHz, female and male locusts had significantly higher neural responses for the lower sound levels (Fig 2C, S2 A-B). Between the sexes within each age group, there were practically no significant differences (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 kHz or at 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, S1 F-K, Suppl. 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 1-5 kHz with a similar trend as at 70 dB SPL (Fig 2D) (e.g., 5kHz $F_{2,14} = 6.79$, $p = .011$; at 15kHz $F_{2,14} = 1.31$, $p = .31$). The males only showed significant differences for the higher sound level between 4.5-5 kHz and there were few differences between the sexes at each age group (Fig S1 F-K, Suppl. Table S1). However, evaluating the trend from 50, 70, to 90 dB SPL several patterns are observed (Fig 2D, S1F-K). First, the neurophysiological response separated into two clear groups with the lower frequencies (2-10 kHz) having the higher value. 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 (Fig S1F-K).

Finally, we measured the latency for the neurobiological response (Fig 2E-L). 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 5kHz female $F_{2,14} = 9.28$, $p = .004$; male $F_{2,16} = 5.09$, $p = .022$). 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 2 I-L, Table S2). As expected for low frequencies (<10 kHz) increasing sound levels decreased latency times for two week old animals (red and orange colours, Fig 2I & K). Surprisingly, in contrast, for high frequencies the two week animals at 70 dB SPL actually had an increase in latency compared to both the 50 and 90 dB SPL (green and purple colours, Fig 2I & K). Male locusts of the older age groups followed a similar, yet less dramatic trend (Fig 2J). However, surprisingly, for older female locusts 70dB SPL was the slowest latency for most frequencies (Fig 2L).

Taken together, membrane movement and neurophysiology data show the effects of aging for adult locusts in their hearing response. At lower frequencies there is an increased neural-response between two 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 likely due to a change in the cellular physiology of the animals, but also due to animal reproductive status.

As two week old locusts of both sexes have greater and faster neurobiological responses despite equal changes in male tympanal membrane movement; we suggest the two 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 (Ridgel and Ritzman, 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 (Kumar et al., 1998) and an increase in the threshold for action potential generation (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 that increase their weight and decrease the volume available for air sacs to expand. We therefore measured their weight as they aged. We found females had a significant increase in weight with aging animals (2.15-2.8g, $F_{4,39} = 5.44$, $p = .002$) (Fig S4) while males displayed no significant differences, despite a slight decline with older ages (~1.6g, $F_{4,46} = 0.79$, $p = .54$) (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 weight with age. Under natural conditions at approximately four weeks of age females begin laying their eggs. This could explain why we see such a large difference between the four week female age group as their bodies are probably the most dense. In captivity, locusts do not always have proper reproductive fitness (Uvarov, 1966) which is perhaps why we did not see a decline of weight 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). Secondly, 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 Ritzman, 2005), that insects could be considered as a model of animal aging as they show aging 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.

Methods

Animals

Adult *Schistocerca gregaria* (Forskål) were obtained from Blades Biological Ltd, 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 hr light-dark cycle at 24°C.

Membrane Deflection

Animals were weighed before trials. Membrane deflection trials followed a similar protocol to previous work (Gordon et al, 2014). Briefly, their 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, USA) was placed at least 10cm away. 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 12.5Hz and measurements averaged at least 15 times per point measured and later binned to 500Hz categories. Gain (displacement/sound pressure level) values were used for analysis to account for any differences in sound signal amplitude. Final sample size included (week-male, female): 2w-8m, 8f; 3w-6m, 6f; 4w-9m, 15f; 6w-11m, 8f; 8w-10m, 3f. Weeks four and above contained animals from different shipments. Data was analysed with an ANOVA in SPSS (IBM, Armonk, 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 50µm silver wire, were placed under the auditory nerve in the metathorax and insulated using petroleum jelly. The final sample size was: 2w--5m, 5f; 4w--5m, 5f; 8w--7m, 5f.

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

Data were processed in LabVIEW, for details please see Gordon et al (2014). Briefly, the summed neural response of the auditory nerve was measured by calculating the root-mean-square (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 1-20kHz 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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References

Gordon S. D., Jackson J. C., Rogers S. M., Windmill J. F. C. (2014) Listening to the environment: hearing differences from an epigenetic effect in solitarious and gregarious locusts. Proc. R. Soc. B 281: 20141693. http://dx.doi.org/10.1098/rspb.2014.1693

Kumar G. L., Pophof B., Kumar S., Keil T. A. (1998) Age-related changes in the dendrites of olfactory receptor neurons in the male silkmoth *Antheraea peryni* (Insecta, Lepidoptera : Saturniidae). *Cell Biol Int* **22**, 445-455.

Malkin R., McDonagh T. R., Mhatre N., Scott T. S., Robert D. (2014) Energy localization and frequency analysis in the locust ear. *J R Soc Interface* **11** 20130857

Michel K., Petersen M. (1982) Development of the Tympanal Organ in Larvae of the Migratory Locust (Locusta-Migratoria). *Cell Tissue Res* **222**, 667-676.

Miller L. A. (1977) Directional Hearing in Locust Schistocerca-Gregaria-Forskal (Acrididae, Orthoptera). J *Comp Physiol* **119**, 85-98.

Neville A. C. (1963) Growth and Deposition of Resilin and Chitin in Locust Rubber-Like Cuticle. *J Insect Physiol* **9**:265-278.

Ridgel A. L., Ritzmann R. E. (2005) Insights into age-related locomotor declines from studies of insects. *Ageing Res Rev* **4**, 23-39.

Uvarov B.P. (1966) *Grasshoppers and locusts: a handbook of general acridology Vol 1*. Anti-Locust Research Centre. Cambridge, Great Britain

Windmill J.F.C., Gopfert M.C., Robert D. (2005) Tympanal travelling waves in migratory locusts. *J Exp Biol* **208**, 157-168.

Yeoman M. S., Faragher R. G. A. (2001) Ageing and the nervous system: insights from studies on invertebrates. *Biogerontology* **2**, 85-97.

Figure Legends

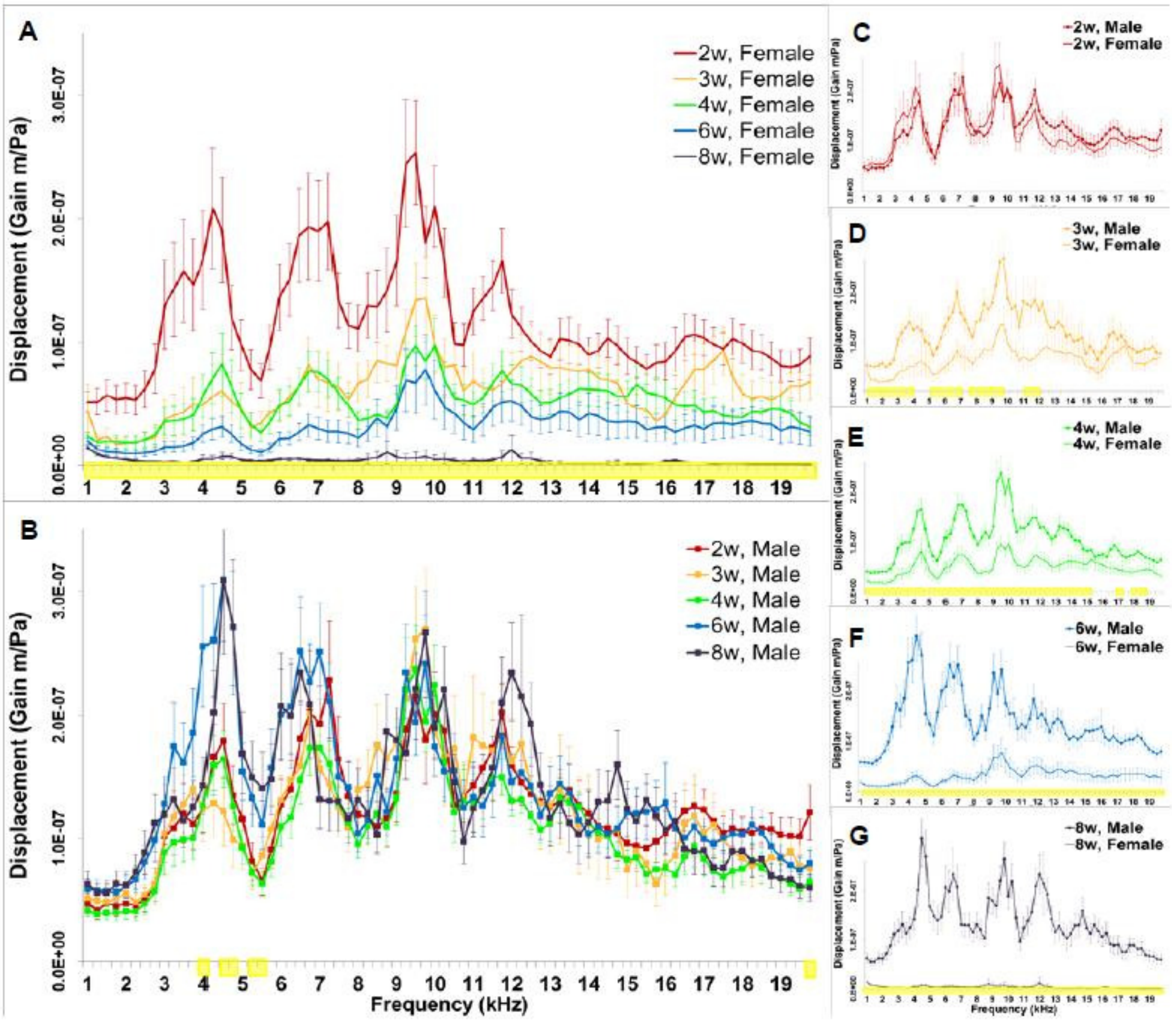

Figure 1. Displacement (gain m/Pa) of the tympanal membrane with sound (1-20 kHz) of among ages for (A) females, (B) males, and between sexes (C-G) at each age group: 2, 3, 4, 6, and 8 week post maturity locusts. Age in weeks is indicated by colour, red is two weeks. Males are represented with lines with squares. Yellow bar on the x-axis represents significance of 0.05 or less.

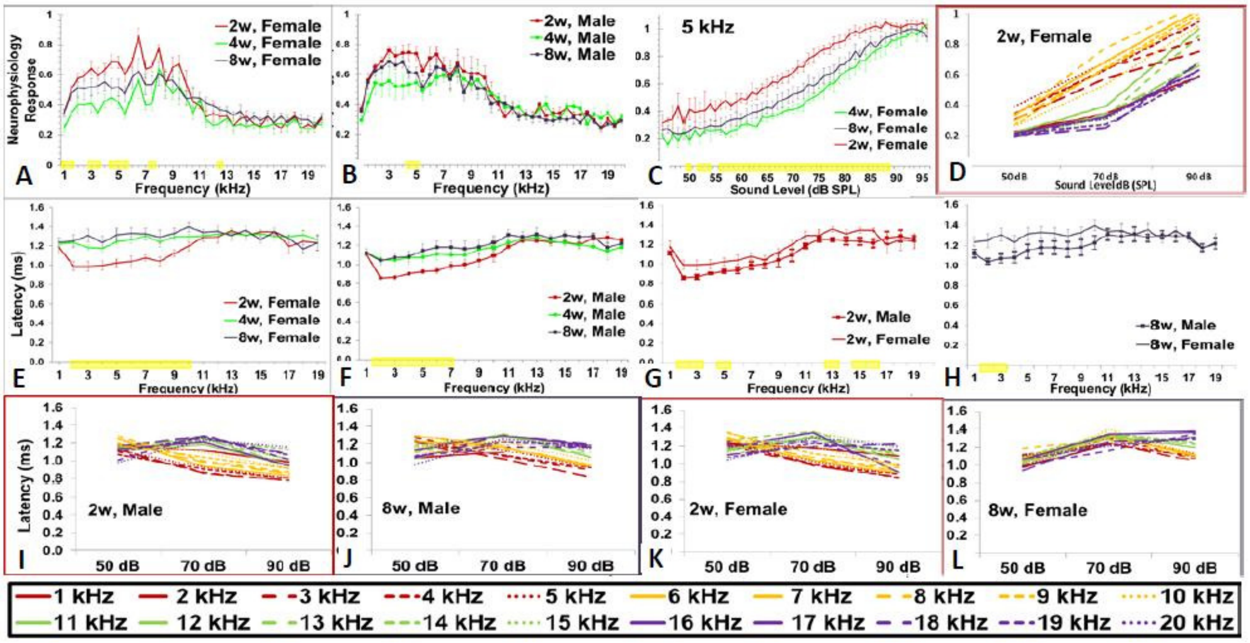

Figure 2. Neurophysiological response of locusts. (A) Female and (B) male relative neural responses across frequencies at 70 dB SPL. (C) Female response at 5 kHz across sound levels. (D) Female, 2 weeks post maturity, response across frequencies at 50, 70, 90 dB SPL. Latency responses for (E) females and (F) males at 70 dB SPL across frequencies. Latency response between males and females at 2 (G) and 8 (H) weeks post maturity. Latency responses at three sound levels for (I) 2 week males, (J) 8 week males, (K) 2 week females, and (L) 8 week females. Yellow bars indicate significance < .05 for A-C, E-H. Key at the bottom indicates the colour and pattern for each frequency for D, I-L.

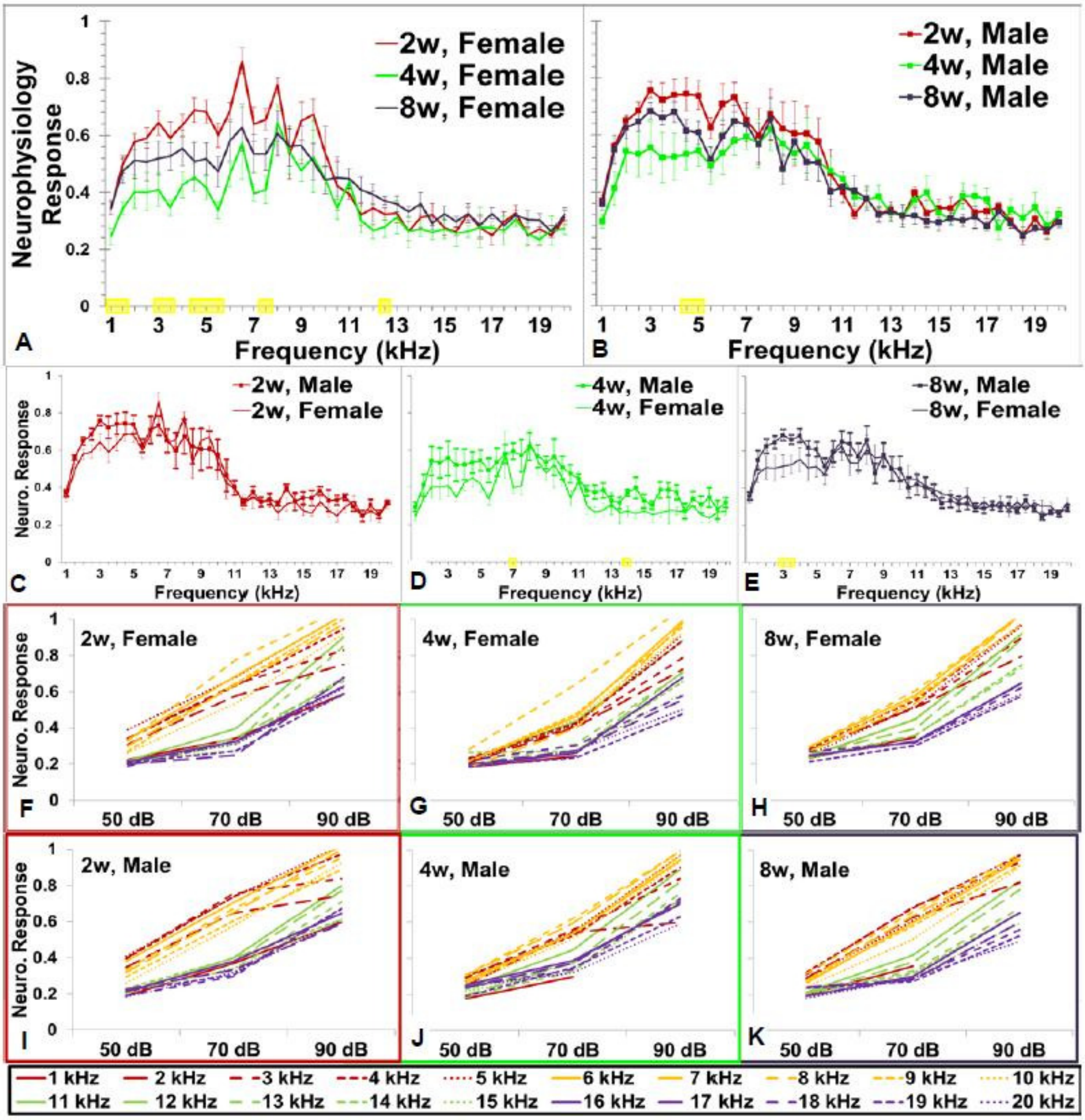

Figure S1. Relative neurophysiological response at 70 dB SPL for (A) females, (B) males, (C-E) 2, 4, 8 week post maturity locusts. Yellow bar on the x-axis represents significance of 0.05 or less. (F-K) Comparative neurophysiology response at 50, 70, and 90 dB SPL within each age and sex group. Frequencies are represented by colours: 1-5 kHz are red, 6-10 kHz are orange, 11-15 kHz are green, 16-20 kHz are purple.

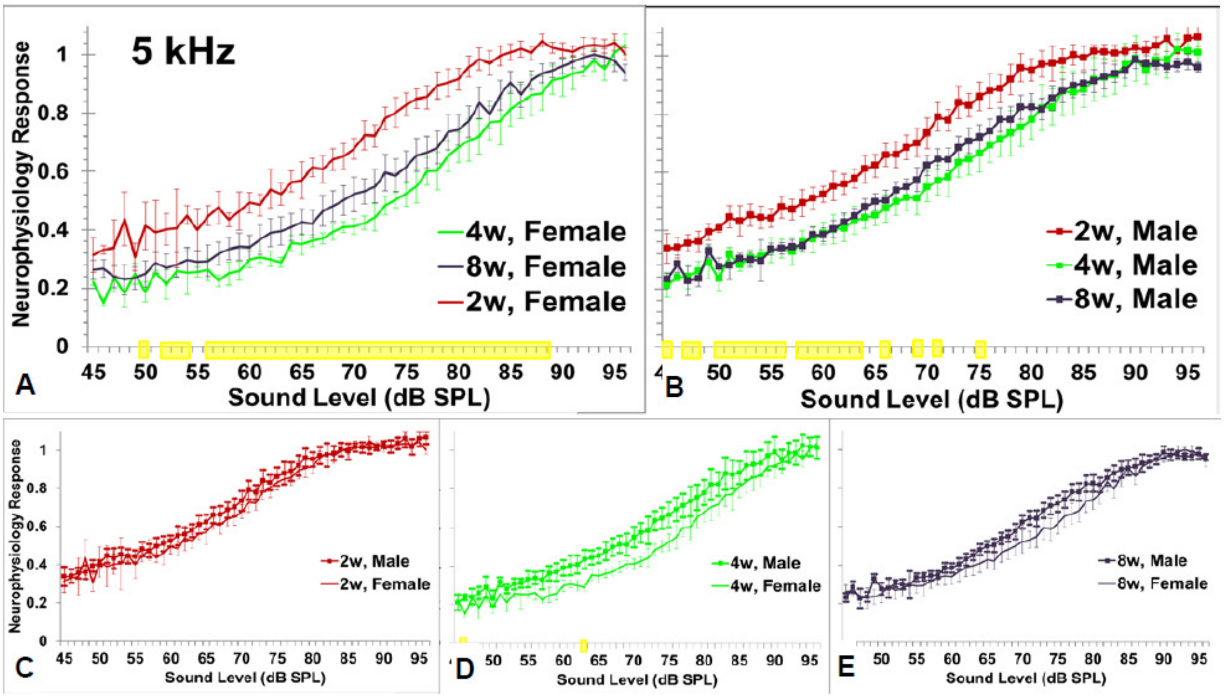

Figure S2. Sample relative neurophysiological response for 5 kHz for (A) females, (B) males, (C-E) 2, 4, 8 week old locusts. Yellow bar on the x-axis represents significance of 0.05 or less.

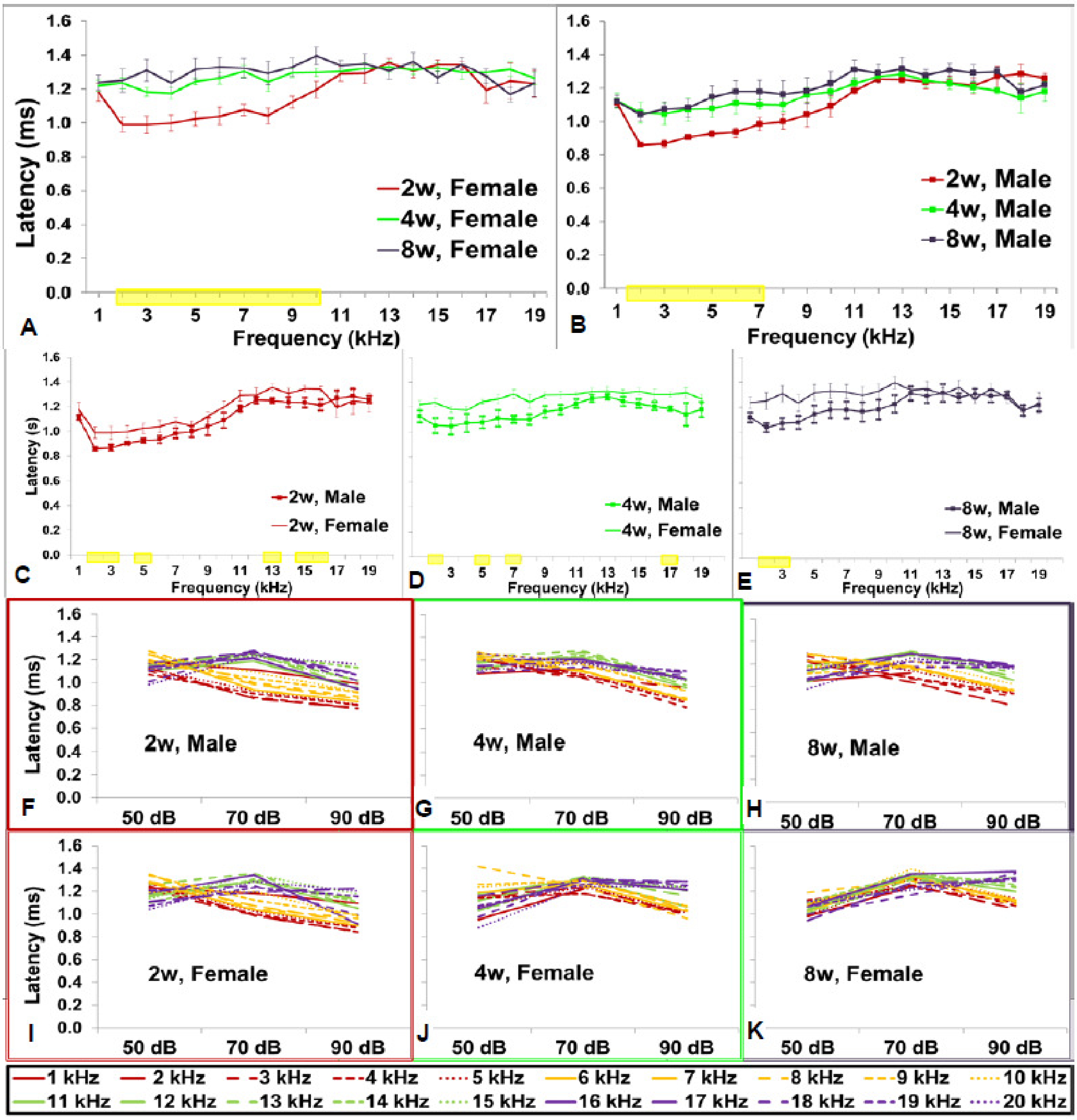

Figure S3. Latency to the neurophysiological response at 70 dB SPL for (A) females, (B) males, (C-E) 2, 4, 8 week post maturity locusts. Yellow bar on the x-axis represents significance of 0.05 or less. (F-K) Comparative latency response at 50, 70, and 90 dB SPL within each age and sex group. Frequencies are represented by colours: 1-5 kHz are red, 6-10 kHz re orange, 11-15 kHz are green, 16-20 kHz are purple.

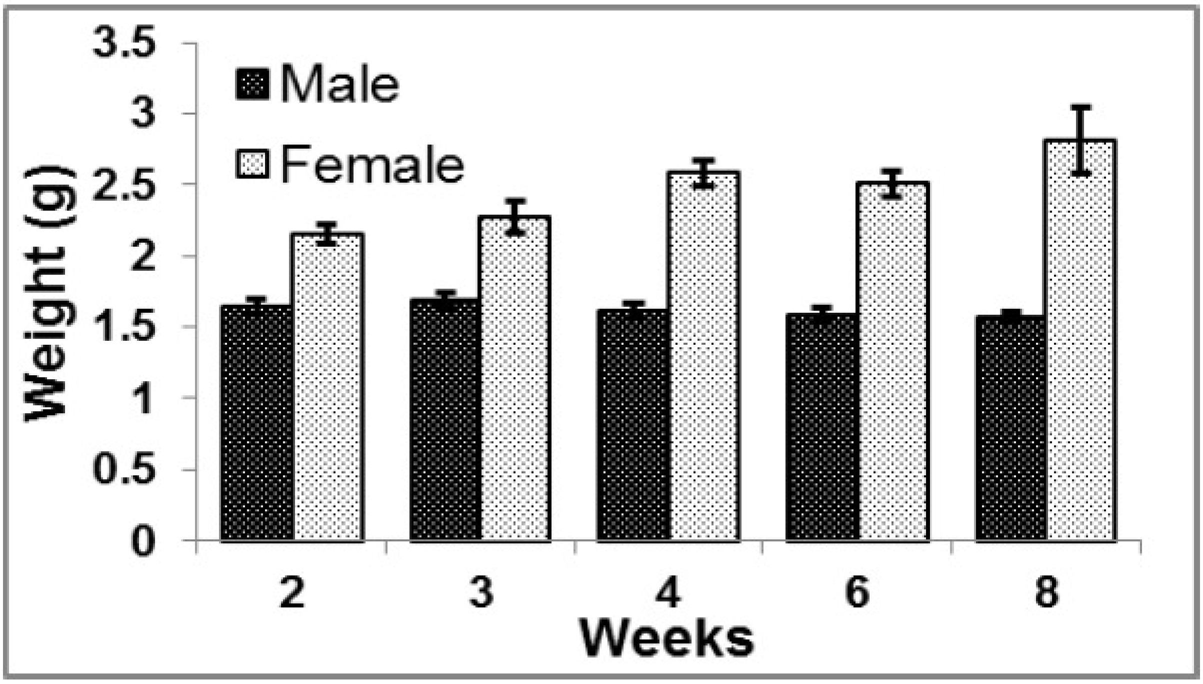

Figure S4. Weight (g) of males (dark bars) and females (light bars) for each age group. Weight significantly increased with female age ($F_{4,39} = 5.44$, p =.002) but not males ($F_{4,46} = 0.79$, p =.54).