

24 1989) but the traits described in these analyses are usually external morphological
25 characters taken from dried specimens. One living-tissue trait that has been used in
26 examining the evolution of the Lepidoptera is the number of receptor neurons in the
27 auditory organs of moths (Lewis and Fullard 1996; Yack *et al.* 1999). Within the
28 Noctuoidea, each ear of notodontid moths possesses a single receptor neuron (Eggers 1919;
29 Roeder 1974; Fullard 1984; Surlykke 1984) while noctuids possess two (Eggers 1919;
30 Roeder 1967; Surlykke and Miller 1982; Fullard *et al.* 1998), a trait which appears to be
31 sympleisio morphic with all other noctuid families that have been surveyed using recent
32 phylogenetic proposals (Miller 1991; Kitching and Rawlins 1998; Mitchell *et al.* 2005;
33 Fibiger and Lafontaine 2005). In addition to anatomical traits, there are physiological
34 differences between these two families with noctuids generally possessing more sensitive
35 ears (Roeder 1974) compared to notodontids although these characters are not as well
36 quantified.

37 Although molecular techniques have begun to clarify the phylogenetic organization
38 of the noctuoidean families (Weller *et al.* 1994; Mitchell *et al.* 2005) some clades remain
39 problematic. Miller (1991) proposed that an Australian genus of moths, represented by
40 *Oenosandra boisduvalii* Newman, 1856 which was previously considered a member of the
41 notodontid subfamily, Thaumetopoeinae (Turner 1903, 1922; Kiriakoff 1970) is
42 sufficiently unique to form a distinct family, the Oenosandridae. Miller (1991) considered
43 the Oenosandridae to be either a pleisiomorphic sister group to the Notodontidae or to the
44 quadrifid (i.e., modern) Noctuidae and emphasized the many shared characteristics of this
45 moth with both families. Recent revisions have added either three (Fibiger and Lafontaine
46 2005) or six (Mitchell *et al.* 2005) new families to the Noctuoidea with the Oenosandridae

47 placed as pleis iomorphic to the entire superfamily (Kitching and Rawlins 1998). Since
48 auditory receptor number is a dependable character separating notodontids from the rest of
49 the Noctuoidea, I performed auditory neurophysiological examinations on the Australian
50 species, *Oenosandra boisduvalii* to provide information about the heretofore undescribed
51 auditory condition of this group of moths and to present living-tissue data regarding the
52 phylogenetic status of the Oenosandridae with regards to the evolution of hearing within
53 the Noctuoidea.

54

55 **Methods**

56 *Animals*

57 *Oenosandra boisduvalii* were collected from street and store lights around the town of Port
58 Lincoln, South Australia, Australia from April 10 to 21, 2005 (Research Permits: South
59 Australia: permit not required for non-endangered species collected on private property;
60 Australia: Department of the Environment and Heritage Export Permit WT2004-7444).

61 Moths were kept in screened cages with water at ambient temperatures (20-25 °C) and used
62 within 12 hours of their capture.

63

64 *Auditory examinations and acoustic stimulus*

65 Auditory neural recordings were made using standard electrophysiological methods
66 (Fullard 1994). The tympanic nerve (IIIN1b (Nüesch 1957)) of the moths was exposed and
67 auditory action potential responses to acoustic stimuli recorded with a stainless steel hook
68 electrode referenced to another in the moth's abdomen. Neural responses were amplified
69 with a Grass Instruments P-15 preamplifier and stored in a PC laptop using an oscilloscope

70 emulating sampling board (50 MHz: (Pico Technology ADC 212/3) and analysis
71 programme (Pico Technology PicoScope 5.10.7). The ears were exposed to pulsed
72 synthetic sounds (20 ms, 500 μ s rise/fall time, 2/sec) generated by a customized MatLab
73 application programme running in a different PC laptop, amplified (Avisoft, Germany) and
74 broadcast from a Technics EAS-10TH400B speaker mounted 20 cm from the moths. The
75 auditory preparation and speaker were mounted in a sound-absorbing Faraday cage.
76 Intensities were recorded as mV peak-to-peak delivered to the speaker and converted to
77 peak equivalent sound pressure levels (dB peSPL) (rms re 20 μ Pa) (Stapells *et al.* 1982)
78 from equal-amplitude continual tones as measured with a Brüel and Kjær (B&K) type 4135
79 6.35 mm microphone and type 2610 B&K measuring amplifier. The entire system was
80 calibrated before and after the study with a B&K Type 4228 pistonphone.

81 I derived auditory threshold curves (audiograms) using sound pulses at 5 kHz
82 frequency increments randomly chosen from 5 to 100 kHz. A1 cell threshold was
83 determined as the stimulus intensity that evoked two receptor spikes per stimulus pulse
84 while A2 threshold was determined as that stimulus intensity first evoking the (usually)
85 smaller A2 cell spike. Intensity response curves were derived using pulses of 25 kHz sound
86 delivered at a range of intensities subthreshold to A1 threshold to approximately 5 dB
87 suprathreshold to the A2 cell.

88

89 **Results**

90 *Electrophysiology*

91 Six male and six female *Oenosandra boisduvalii* were collected for the neural
92 examinations. All provided stable auditory preparations that lasted for the one hour that
93 each moth was tested. Fig. 1 (A) illustrates the typical spiking response of a male moth to a
94 25 kHz stimulus pulse of increasing intensities and shows the presence of both A1 and A2
95 receptor cells. All moths, male and female, exhibited clear bi-neuronal auditory responses.
96 Fig. 1 (B) describes the firing pattern of one individual with its A1 cell response gradually
97 increasing to a plateau at approximately 70 dB and A2 commencing its firing at 70 dB
98 reaching a firing maximum at 80 – 90 dB.

99 Fig. 2 graphs the stimulus response curves of male and female *O. boisduvalii* to a 20
100 ms stimulus pulse of 25 kHz. All of the data are pooled and a curve has been fitted to each
101 graph. These curves reveal no obvious differences for the A1 cell between the genders
102 although there is some suggestion that greater maximum spike numbers are obtained in
103 males. A2 spiking for both genders also exhibits similar patterns with males showing a
104 greater tendency to level off than females. The median dynamic auditory range (the range
105 of stimulus intensities that evokes an increase in spiking) for the A1 cell in males was 24
106 dB *versus* 21 dB in females with no statistical difference between the genders (Mann-
107 Whitney $T = 44.5$, $P > 0.05$). A2 dynamic range also revealed no significant gender
108 differences (male: 9, female 13; Mann-Whitney $T = 29.0$, $P > 0.05$). After pooling the
109 genders the total auditory dynamic range of the two cells is 32 dB. The maximum firing
110 (spikes/stimulus pulse) attained by the A1 cell in males was 19 *versus* 16 in females
111 (difference not significant, Mann-Whitney $T = 48.5$, $P > 0.05$) and by the A2 cell, 14 in
112 males *versus* 13 in females (difference not significant, Mann-Whitney $T = 41.5$, $P > 0.05$).

113 Fig. 3 illustrates the median auditory sensitivity curves (audiograms) of both
114 auditory receptors in all males and females ($n = 12$). The curves indicate a broad tuning of
115 both the A1 and A2 cells at 25 to 65 kHz with approximately 10 – 20 dB difference in
116 thresholds between the two cells. There are no significant differences between males and
117 females at any frequency in either the A1 or A2 cell (Mann-Whitney Rank Sum Tests, all P
118 > 0.05). Superimposed upon the audiograms in Fig. 3 is a histogram of all the echolocation
119 frequencies emitted by 41 species and varieties (including undescribed species and vocal
120 dialects) of insectivorous bats from New South Wales (Pennay *et al.* 2004), a habitat where
121 *O. boisduvalii* exists (Common 1990). From this histogram it can be seen that the most
122 common echolocation frequencies are in a bandwidth from 30 to 60 kHz that matches well
123 with that of the most sensitive frequencies in the A1 and A2 cell audiograms of *O.*
124 *boisduvalii*.

125 Fig. 4 compares three audiogram characteristics of the A1 and A2 cell in males and
126 females: 1. median percent of all the stimulus frequencies tested that did not produce any
127 neural response at the highest intensity stimulus used; 2. median best frequency (kHz), the
128 frequency requiring the lowest intensity to elicit threshold and, 3. median threshold
129 intensity (dB peSPL) at best frequency, the stimulus intensity eliciting threshold at best
130 frequency. There were no differences between the genders for any of these characters
131 (Mann-Whitney Rank Sum Tests, all $P > 0.05$).

132 Fig. 5 compares the pooled male and female median A1 audiogram of *O.*
133 *boisduvalii* to that of 150 noctuids from 25 species sampled in another temperate habitat
134 (eastern Ontario, Canada (Surlykke *et al.* 1999)) and to that of the Neotropical Panamanian

135 notodontid, *Antaea lichyi* (Fullard 1987). In this figure it can be seen that *O. boisduvalii* is
136 10 - 20 dB less sensitive at all frequencies than the Ontario noctuids and more closely
137 resembles the audiogram of the one-celled Neotropical notodontid except at frequencies
138 higher than approximately 60 kHz where it is less sensitive.

139

140

141 **Discussion**

142

143 *Gender similarities and ecological considerations*

144 The fact that the auditory receptors of both genders exhibit similar intensity response
145 curves, dynamic ranges, firing maxima and audiograms implies that males and females
146 experience similar levels of predation pressure from echolocating bats. Degenerate ears are
147 common in flightless female moths of a number of species (Roff 1990), an auditory
148 condition that may be the result of the elimination of bat predation (Cardone and Fullard
149 1988). The auditory thresholds of male and female *O. boisduvalii* indicate that the type of
150 bat that would emit echolocation calls intense enough to activate their ears would be an
151 aerially foraging species (Holderied *et al.* 2005). This further implies that both genders
152 spend a similar amount of time in flight and have the same need for early-warning auditory
153 defences against the attacks of these types of bats. The audiograms of Figure 5 reveal a
154 surprising insensitivity in these moths compared to North American noctuids when
155 considering the different sympatric bat communities of the two areas (nine species of
156 insectivorous bats in Ontario *versus* 41 species in New South Wales (Pennay *et al.* 2004)).
157 Auditory comparisons suggest that the greater diversity of bats (and their echolocation call

158 frequencies) in tropical regions have resulted in sympatric moth ears with greater sensitivity
159 than those in temperate areas (Fullard 1982). The diverse bat fauna of southern Australia
160 would have similarly predicted higher auditory sensitivity in moths such as *O. boisduvalii*
161 but such an adaptation does not appear to exist. As discussed below, this moth shares
162 auditory similarities with notodontids, moths with relatively insensitive ears that
163 nevertheless coexist with diverse bat communities (e.g., Panamá (Fullard 1984, 1987)) so it
164 is possible that deafer moths, both temperate and tropical, may compensate with lifestyle
165 adaptations (e.g., reduced flight) that reduce their exposure to bats (Soutar and Fullard
166 2004).

167

168 *Phylogenetic considerations*

169 This study demonstrates that *O. boisduvalii* possesses an auditory organ with two receptor
170 cells and I have followed the convention of other noctuoid ears (Roeder and Treat 1957) by
171 naming the most sensitive cell as A1 and the least sensitive as A2. By reason of the
172 number of receptors, this moth would not belong in a notodontid subfamily as originally
173 proposed by Turner (1903). The pleisiomorphic, two-celled ear of *O. boisduvalii*
174 corresponds to its trifold forewing condition and agrees with the proposal of Kitchings and
175 Rawling (1998) and Fibiger and Lafontaine (2005) of it as a basal group to the rest of the
176 Noctuoidea. This observation leaves the Notodontidae as the sole noctuoid family with a
177 single auditory receptor. Yack *et al.* (1999) suggested that the notodontid ear was derived
178 from the two-celled state of other noctuoids and that cell reduction is an adaptive feature in
179 the evolution of a bat-detecting ear. Along this line, Lewis and Fullard (1996) suggested
180 that the four-celled hind wing-hinge receptor organ of the earless bombycoid moth,

181 *Malacosoma disstria* represents the metathoracic, pre-auditory condition and that cell
182 reduction occurred when this organ was replaced with the homologous auditory organ of
183 the Noctuoidea.

184 Physiological characters observed in *O. boisduvalii* suggest that affinities exist with
185 both noctuids and notodontids. One measure of the functional sensitivity of an ear is its
186 dynamic range, the decibels over which it can discern a difference in intensities by
187 increasing its firing. Roeder (1974) summarized the combined A1 and A2 cell dynamic
188 range for noctuids as 35 - 45 dB and the single A1 cell dynamic range for notodontids as 12
189 - 20 dB. The median A1 + A2 cell dynamic range of *O. boisduvalii* is 32 dB, rendering this
190 characteristic more like noctuids than notodontids. On the other hand, this moth's A1 and
191 A2 cells' insensitivity across all frequencies tested more resembles that of the notodontid
192 ear than the noctuid (e.g., Surlykke *et al.* 1999). For some moths, increased deafness is
193 likely a result of regressive changes in ears that are no longer needed (Fullard *et al.* 1997;
194 Muma and Fullard 2004) but *O. boisduvallii*'s nocturnal lifestyle suggests that its ear is
195 fully functional and, like that of Neotropical notodontids (Fig. 5) represents the minimum
196 sensitivity evolutionarily permissible in this sensory defence.

197 Living-tissue traits such as neuronal characteristics, although difficult to obtain can
198 serve a valuable role in phylogenetic analyses. Comparative studies of chordotonal sensory
199 cells and their connections to conserved central nervous system elements across a diversity
200 of taxa have provided insights into the general evolution of hearing in insects (Boyan
201 1993). Another group of noctuoids, the Doidae has proven to be even more challenging for
202 systematists who have placed them in no fewer than seven different lepidopteran families
203 (Miller 1991) that have from one to four auditory receptors. An examination of the ears of

204 the Doidae, in particular whether they share a one-celled auditory synapomorphy with the
205 Notodontidae with which they currently hold a close relationship (Fibiger and Lafontaine
206 2005), will provide grounds for clarifying the phylogenetic position of this family.

207

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214 Engineering Council of Canada.

215

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219 evolutionary specialization of the chordotonal system. *Journal of Insect Physiology*
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308 tympanal ear and its mesothoracic homologue in the Macrolepidoptera (Insecta).
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- 310
- 311
- 312

313 **Fig. 1.** (A) Representative trace of one specimen of *Oenosandra boisduvalii* (male)
314 illustrating the firing responses of the auditory receptors A1 and A2 to 25 kHz
315 sounds of increasing intensities. (B) A1 and A2 cell stimulus response curves of
316 same individual to increasing stimulus intensities describing the measurements
317 taken.

318

319 **Fig. 2.** Stimulus response graphs for the A1 and A2 cells of all moths tested (females = 6;
320 males = 6). Graphs illustrate individual responses (indicated by different symbols)
321 with sigmoidal curves fitted to all the data.

322

323 **Fig. 3.** Median audiograms of the A1 and A2 auditory receptor cells in male (n = 6) and
324 female (n = 6) *Oenosandra boisduvalii*. Histogram below the audiograms is of the
325 frequencies emitted by an entire community of insectivorous bats (New South
326 Wales) hypothetically sympatric with *O. boisduvalii*.

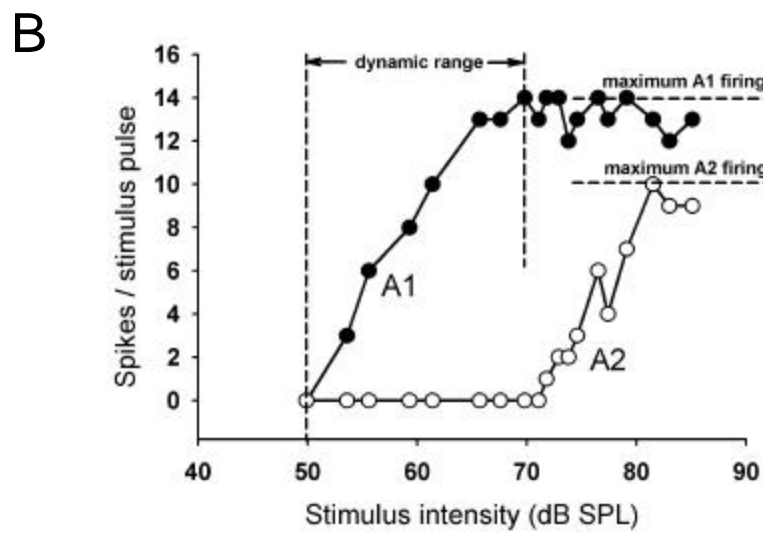
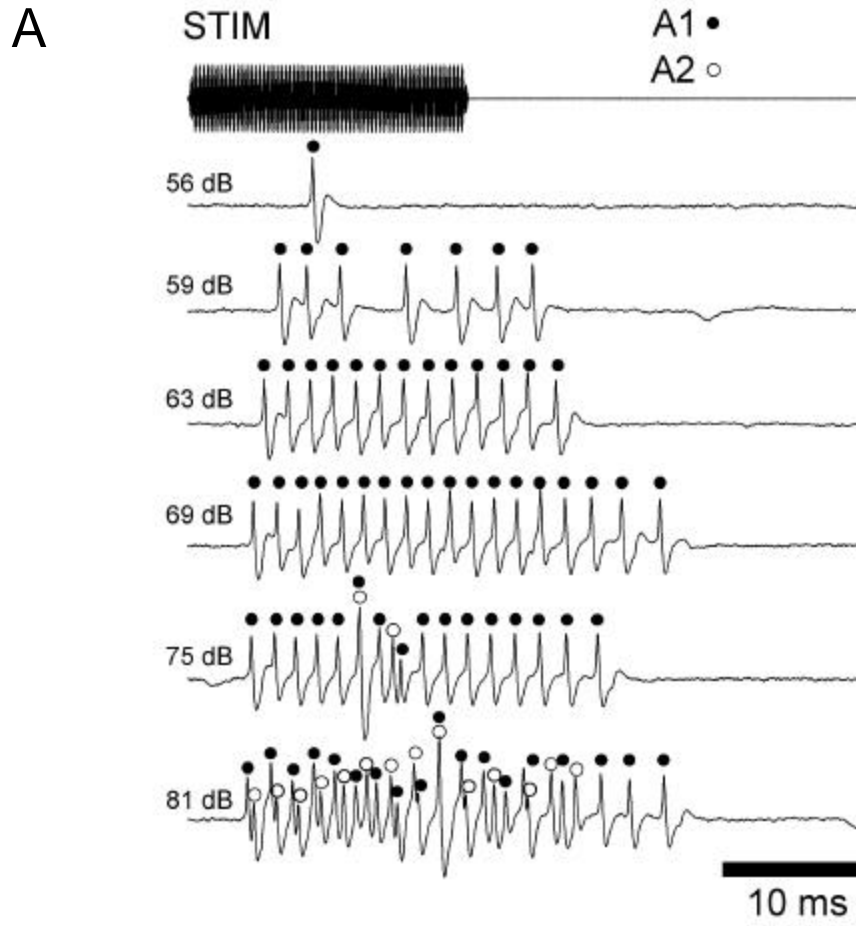
327

328 **Fig. 4.** Comparisons of the median percent of all frequencies tested with no response, best
329 frequencies and threshold at best frequencies of the A1 and A2 cells in males and
330 females. There are no significant differences between the genders in any of the
331 characters

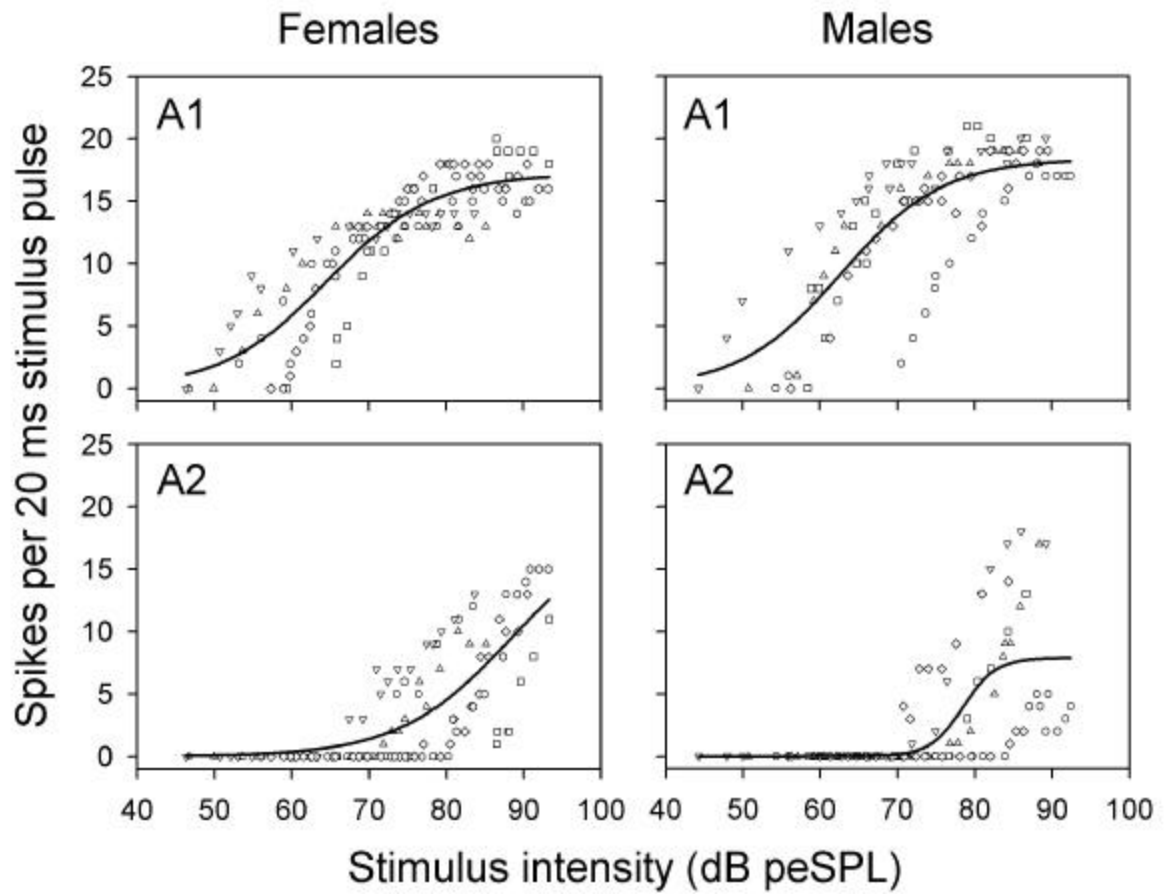
332

333 **Fig. 5.** Comparison of the median A1 cell audiograms of all individuals of *O. boisduvalii*
334 (n = 12), of noctuids from Ontario, Canada (25 species, 150 individuals) and of the
335 Panamanian notodontid, *Antaea lichyi* (n = 9), redrawn from Fullard (1987).

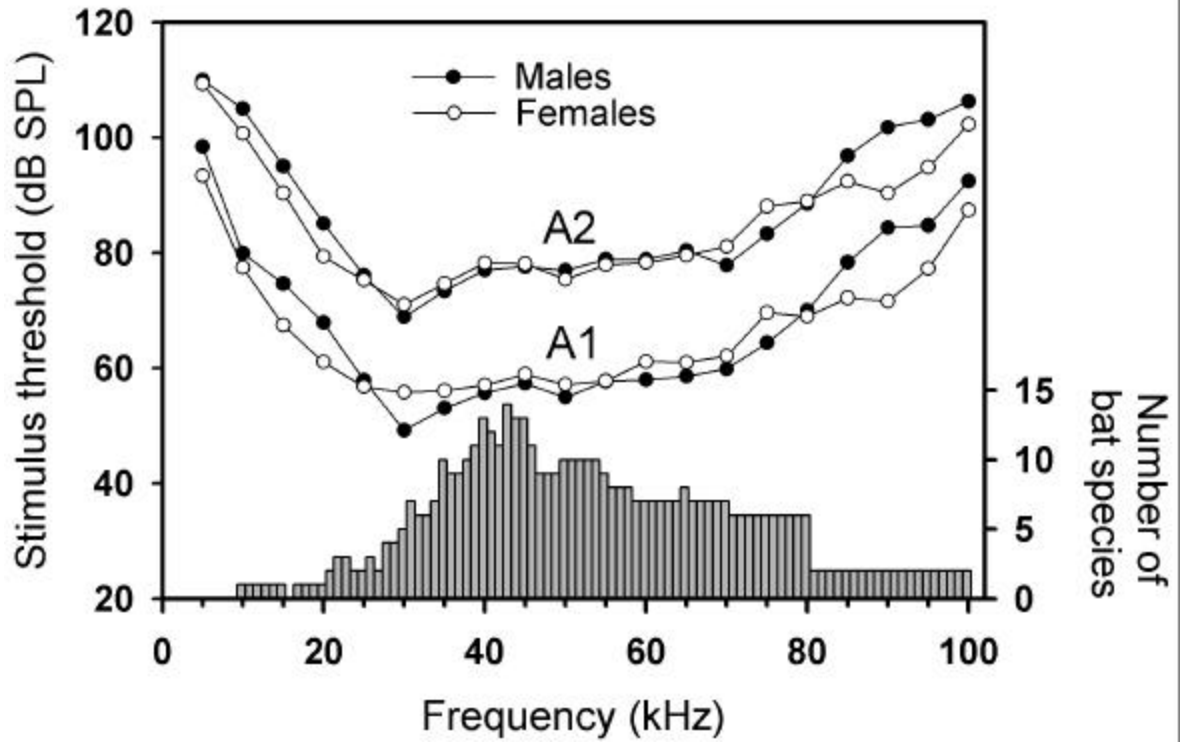
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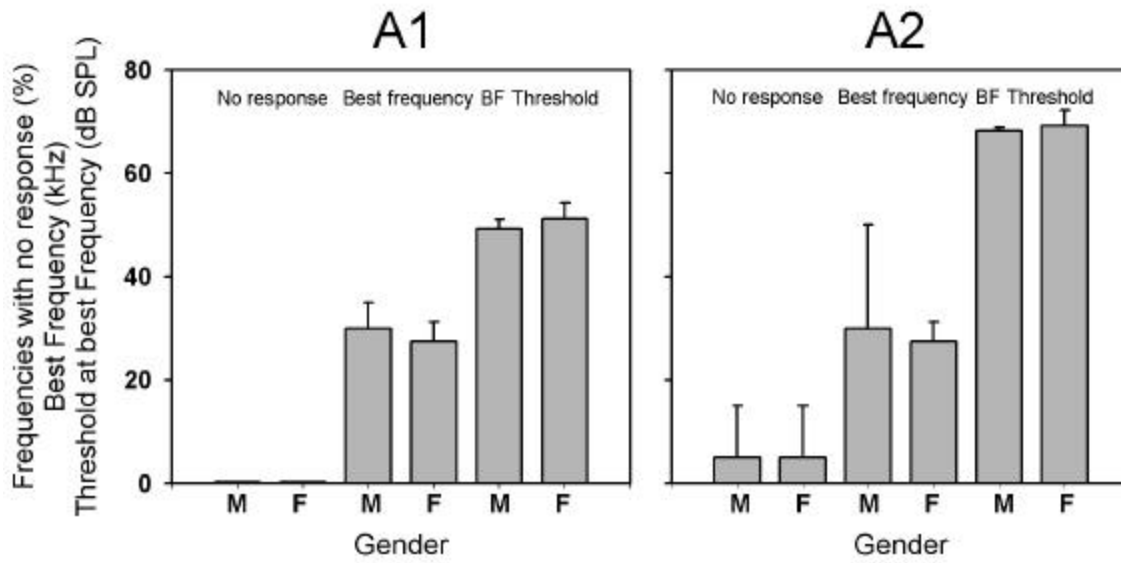
(Fig 2)



(Fig. 3)



(Fig. 4)



(Fig. 5)

