

1 **Phonotactic steering and representation of directional information in**
2 **the ascending auditory pathway of a cricket**

3
4 Lv¹, M., Zhang², X., and Hedwig^{2*}, B.

5 ¹China Agricultural University, Department of Entomology, Beijing, China

6 ²University of Cambridge, Department of Zoology, Cambridge, UK

7 * Author for correspondence, bh202@cam.ac.uk

8
9 **Running Title:** Phonotactic steering and directional information

10
11 **New & Noteworthy:** The ascending auditory interneuron AN1 has been
12 implicated in cricket auditory steering, but at small acoustic stimulation angles, it
13 does not provide reliable directional information. We conclude that either the
14 small bilateral auditory activity differences of the AN1 neurons are enhanced to
15 generate reliable descending steering commands, or more likely that directional
16 auditory steering is mediated via a thoracic pathway, as indicated by the reactive
17 steering hypothesis.

18
19 **ABSTRACT**

20 Directional hearing is crucial for animals depending on acoustic signals to locate a
21 mate. We focused on crickets to explore the reliability of directional information
22 forwarded to the brain by the ascending auditory interneuron AN1, which is
23 crucial for phonotactic behavior. We presented calling song from -45° to +45° in
24 steps of 3°, and compared the phonotactic steering of females walking on a
25 trackball with the directional responses of AN1. 40% of females showed good
26 steering behavior and changed their walking direction when the speaker passed the
27 body's longitudinal axis. The bilateral latency difference between right and left AN1
28 responses was small and may not be reliable for auditory steering. In respect to spike
29 count, all AN1 recordings presented significant bilateral differences for angles

30 larger than $\pm 18^\circ$, yet 35% showed a mean significant difference of 1-3 AP/Chirp
31 when the frontal stimulus deviated by 3° from their length axis. For small angles
32 some females had a very similar AN1 activity forwarded to the brain, but the accuracy
33 of their steering behavior was substantially different. Our results indicate a correlation
34 between directional steering and the response strength of AN1, especially for large
35 angles. The reliable steering of animals at small angles would have to be based on small
36 bilateral differences of AN1 activity, if AN1 is the only source providing directional
37 information. We discuss if such bilateral response difference at small angles can provide
38 a reliable measure to generate auditory steering commands descending from the brain,
39 as pattern recognition is intensity independent.

40

41 **Key words:** surface electrodes, AN1 interneuron, spike response, reliability,
42 binaural differences

43

44 **INTRODUCTION**

45 Insects, like other animals using intraspecific acoustic communication for the
46 localization of mates face two problems: They need to recognize the species-specific
47 signal and they need to localize the origin of the sound (Hoy 1978, Pollack 2000). For
48 the latter, directional hearing is fundamental as it forms the basis for auditory
49 orientation. The primary physical cues any animal can exploit to detect the direction of
50 an acoustic signal are interaural intensity differences and time differences of the sound
51 wave acting on the ears (Römer 2015; Robert, 2008; Lewis, 1983). Once processed by
52 the biomechanics and mechanosensory transduction in the auditory organ (Michelsen
53 and Larsen 2008; Oldfield et al. 1986), only bilateral differences in latency, discharge
54 rate and recruitment of auditory afferents provide the animal's central nervous system
55 with information regarding the direction of the sound source (Giovis and Pollack 2000;
56 Hennig et al. 2004; Hedwig and Stumpner 2016).

57 How is directional auditory information represented in the central nervous system?

58 We analyse this question in crickets, a well-established model for auditory processing
59 in insects (Hoy 1978; Huber and Thorson 1985). Female crickets use the male's calling
60 song for orientation while approaching a singing mate. When female *Gryllus*
61 *bimaculatus* walking on a trackball are exposed to the species-specific calling song,
62 they orient towards the acoustic signal and good trackers steer towards a sound source
63 that deviates by just 1-2° from the animal's long axis (Schöneich and Hedwig, 2010).

64 Hearing organs in crickets are located in the front legs and auditory afferents
65 terminate in the prothoracic ganglion, where the first stages of auditory processing take
66 place (Ball et al. 1989). Afferent auditory information is forwarded to the ascending
67 auditory interneurons AN1 and AN2 that project up to the brain (Wohlers and Huber
68 1982; Hennig 1988). Additionally, an inhibitory connection between the local omega
69 neurons ON1 and the corresponding contralateral AN1 and AN2 shapes the activity of
70 the ascending interneurons. The inhibition increases the directional response of the
71 ON1 neurons (Kleindienst et al. 1981; Selverston et al. 1985) and of the ascending
72 neurons and contributes to the acuity of sound localization (Horseman and Huber
73 1994a,b). In particular AN1 appears to be involved in calling song localization, as
74 modulating its activity in phonotactic walking crickets can reverse the walking
75 direction (Schildberger and Hörner 1988), and females start circling when one AN1 is
76 photoablated (Atkins et al. 1992). One point of view is that the left-right differences in
77 auditory AN1 activity are compared in the brain to guide auditory steering
78 (Schildberger and Hörner 1988; Horseman and Huber 1994b). AN1 activity has been
79 studied under natural conditions demonstrating substantial bilateral activity differences
80 for large stimulus angles (Kostarakos and Römer 2010). A detailed analysis of AN1
81 directional responses for small stimulus angles has not yet been obtained, although
82 female crickets demonstrate a high accuracy in phonotactic steering (Schöneich and
83 Hedwig 2010). Exploring the representation of directional information by AN1 will

84 provide insight into the reliability and precision of the auditory responses that are
85 forwarded to the brain.

86 Here we compared the phonotactic steering behavior of crickets walking on a
87 trackball with the directional neuronal responses of AN1. To monitor the activity of the
88 ascending AN1 neuron, we used surface electrode recordings from its terminals in the
89 brain (Kostarakos and Hedwig 2017). Walking behavior and neuronal recordings were
90 obtained in separate experiments, while systematically presenting acoustic stimuli from
91 different frontal angles.

92

93 **MATERIALS AND METHODS**

94 *Animals.* Female crickets (*Gryllus bimaculatus* deGeer) were isolated after the
95 final molt from the cricket colony at the Department of Zoology, Cambridge. Animals
96 were reared individually and fed on a protein and fat rich diet and water. Females with
97 intact silvery-white tympana were selected for experiments.

98 *Test design.* We used two approaches. In one set of experiments, we measured
99 female phonotactic steering behavior for one group of animals and compared it to the
100 AN1 response of another different group of females. In the second set of experiments,
101 we used the same females for phonotaxis experiments and subsequently recorded their
102 AN1 activity under corresponding conditions. Details are given in the Results.

103 *Acoustic stimulation.* The directional sensitivity of the animals was tested in in two
104 different subsequent experiments measuring their steering behavior and the responses
105 of the ascending auditory interneuron AN1. The same acoustic paradigm was used.
106 Sound stimuli were computer generated with Cool Edit Pro 2000 (Syntrillium, Phoenix,
107 USA, now Adobe Audition) and were delivered via a speaker (Sinus live, Neo13s,
108 Conrad Electronics, Hirschau, Germany). Stimuli had a carrier frequency of 4.8 kHz,
109 they consisted of 5 pulses (20 ms duration, incl. 2 ms rise and fall time, 20 ms intervals)
110 grouped in chirps which were repeated every 500 ms. Sound intensity was calibrated

111 with a 1/2'' free field microphone at the position of the cricket and adjusted to 75 dB
112 SPL (amplifier type 2610, microphone type 4191, Brüel and Kjær Nærum, Denmark).

113 The speaker was level with the cricket and was attached to a 57.3 cm long lever
114 fitted to the axis of a stepper motor (type 4490H048B K1155, controller MCNL3006S;
115 Faulhaber GmbH, Schönaich, Germany) so that a rotation by 1° corresponded to a
116 displacement of 1 cm. The motor axis was aligned with the center of the trackball (Fig.
117 1A). The control software allowed positioning the speaker with an accuracy of less than
118 0.5° at any angle to the cricket's length axis. We defined a speaker position of 0° as
119 frontal to the animal, left to the axis as minus and right as plus. The speaker moved
120 continuously in steps of 3° from -45° to +45° and then backward from +45° to -45°,
121 running for 2 to 3 cycles. At each step/angle the speaker stopped for 10 s while 20
122 calling song chirps were presented, i.e. in one forward-backward cycle 40 chirps were
123 presented at each tested angle. A silent interval of 2 s occurred between steps in which
124 the speaker moved to the next position. The stimulus regime was designed to cover the
125 range of angles in a reasonable time and to allow several repetitions while the recording
126 lasted. Speaker movements were monitored with a 360° smart position sensor (absolute
127 optical encoder type A2, US Digital, Vancouver, USA) with a resolution of 0.1°
128 coupled to the motor axis.

129 *Directional sensitivity of phonotaxis.* For testing the precision of phonotactic
130 steering, females were positioned on top of an open-loop trackball system with their
131 body length axis adjusted in line with the 0° speaker position. During walking the
132 tethered cricket rotated the trackball and the rotational movements of the ball were
133 measured with an optical mouse sensor (ADNS-2051, 2D Optical Mouse Sensor;
134 Agilent, Farnell Electronics, Oberhaching, Germany), which provided the forward
135 walking and lateral steering velocities of the animal. Velocity data were integrated to
136 calculate the animal's forward walking distance and the lateral deviation for any sound
137 sequence tested (see Hedwig and Poulet 2004, 2005 for details).

138 *Recording AN1 activity with surface electrodes.* Microelectrodes were pulled
139 (DMZ-Universal Puller, Zeitz-Instruments, Martinsried, Germany) from borosilicate
140 glass capillaries to an outer diameter of 60 μm . Electrodes were inserted into a custom-
141 made electrode holder using a platinum wire as contact (Isaacson and Hedwig 2017).
142 Electrodes and holders were filled with a solution of 4% of Tylose (Tylose H200 YG4,
143 ShinEtsu, Wiesbaden, Germany) dissolved in cricket saline with a composition (in g/l)
144 of 8.6 NaCl, 0.74 KCl, 0.76 CaCl₂, and 2.38 HEPES.

145 For the recordings, specimens were fixed with wax to a metal holder and placed in
146 walking position on top of a stationary trackball. The head capsule was opened frontally
147 to expose the brain; it was covered with Densiron Xtra (Geuder, Germany) to prevent
148 the tissue from drying. The electrode tip was gently attached to the ventral (according
149 to CNS development) surface of the brain where the ascending auditory interneurons
150 AN1 terminate. The tip position was altered until a good quality recording of AN1 spike
151 activity was obtained. The platinum reference electrode was placed next to the
152 mouthparts. Neuronal activity was amplified 10,000 \times and band-pass filtered between
153 300 Hz and 5 kHz using a differential amplifier (model 1700, A-M Systems, Carlsborg
154 WA, USA). All recordings were obtained in a sound proof room. In these experiments
155 the thoracic auditory trachea remained intact, ensuring the crucial bilateral interaction
156 of sound waves in the hearing system (Huber and Thorson 1985; Römer and Schmidt
157 2016).

158 *Data analysis.* Neuronal data was digitally recorded at a sampling rate of 21 kHz
159 per channel using either a CED Micro3-1401 controlled by Spike 2 software
160 (Cambridge Electronics Design, Cambridge, UK) or an A/D board (MIO 16E4,
161 National Instruments, Austin, TX, USA) controlled by custom-built software running
162 under LabView 5.01 (National Instruments, Newbury, UK). The systems recorded the
163 envelope of the sound stimuli, the angular position of the speaker and the neuronal
164 activity. Recordings were processed with Neurolab to calculate PST histograms,

165 instantaneous spike rates and the number of spikes/chirp (Knepper and Hedwig 1997).
166 The response latency was calculated and evaluated for the start of the chirp; for
167 comparison, it also was analyzed for all pulses. Representative recordings were selected
168 for presentation. Spike time data generated in NeuroLab was exported and further
169 statistical analysis was performed using a spreadsheet program (Microsoft Excel) or
170 GraphPad Prism 7 (Graphpad software, San Diego, California, USA). AN1 responses
171 are based on either 80 or 120 chirps presented at the tested angles. For each animal
172 mean responses at different angles were checked for statistical differences using a one-
173 way ANOVA with Tukey post-hoc test with $p < 0.05$ significance level.

174

175 **RESULTS**

176 *Directional steering during phonotactic behavior.* Female crickets (*G.*
177 *bimaculatus*) tethered on the trackball were exposed to calling song presented from -
178 45° to $+45^\circ$. The speaker moved in 3° steps and at each tested angle 20 chirps of calling
179 song were presented (Fig. 1 *A, B*). Crickets walked spontaneously or started walking
180 upon hearing the calling song. We tested 15 females, the lateral steering responses of
181 all females indicates the direction of acoustic stimulation, with animals steering to the
182 left or right when the speaker is left or right, correspondingly (Fig. 1*B*). Moreover the
183 animals reliably followed the change in speaker position, the responses however, show
184 a substantial degree of variation. The curves indicate females which are highly accurate
185 in following the incidence of sound and animals with rather broad steering responses
186 when the speaker crossed the midline. Based on the quality of the responses, we divided
187 the steering responses into three categories: very good steering animals, poor steering
188 animals and animals that did not show a proper phonotactic response, and provide
189 typical examples (Fig. 1*C*). The first example shows highly directional phonotactic
190 steering. The female clearly oriented to the sound source and changed its steering
191 direction from left to right when the speaker crossed the midline and the sound pattern

192 was presented from 3° to the right (Fig. 1C, left), 5 more animals behaved in a similar
193 way. The second example and another 4 animals performed phonotaxis, but they
194 discriminated the side of sound incidence only for larger angles of about ±15-24° (Fig.
195 1C, middle). The third group with 4 animals did not show a proper phonotaxis reaction
196 to the calling song, the females walked forward or stayed stationary, and showed only
197 minor lateral deviations not related to the acoustic stimuli (Fig. 1C, right).

198 We pooled the mean lateral deviation for bilateral corresponding angles of all
199 animals with steering responses (Fig. 2), excluding the data of the non-steering animals.
200 The mean values follow a 3rd order polynomial function, with the amplitude of steering
201 increasing with the stimulus angle, however also the variation of the steering response
202 increased. Although the polynomial function indicates steering for small angles, and
203 individual females clearly steered towards small angles, for the pooled data a statistical
204 significant difference of the lateral deviation from zero occurred only for angles larger
205 than 21° (1.69±1.30 cm).

206 *Directional characteristic of AN1.* In a different group of females, we obtained 20
207 single cell surface-electrode recordings of the ascending auditory neuron AN1 while
208 presenting a calling song pattern from different angles between -45° and +45° in steps
209 of 3° (Fig. 3A). Placing the electrode tip on the ventral surface of the protocerebrum,
210 where the AN1 axon terminates, reliably recorded its spikes, which clearly stood out
211 from the background activity; occasionally also the larger spikes of the AN2 neuron
212 were picked up (Fig. 3B, asterisks). Applying a filter algorithm that continuously
213 calculated the total voltage changes within a gliding time window of 0.8 ms (Knepper
214 and Hedwig 1997) increased the signal-to-noise ratio for further processing (Fig. 3B)
215 and a thresholding algorithm allowed a reliable separation of the AN1 spikes from AN2
216 activity and smaller amplitude background spikes. While presenting calling song, AN1
217 activity mirrored the temporal pattern of the sound in its spike activity and
218 instantaneous spike rate.

219 Over the range of angles tested, we calculated the latency of the AN1 response for
220 the onset of the chirps and also for all pulses of the chirps, and present 3 examples. For
221 the latency of the chirps, the first animal's mean value was rather short and only
222 16.77 ± 1.18 ms (*top, filled grey*), the second one had a mean latency of 22.99 ± 1.41 ms
223 (*middle, filled grey*) and the mean value of the third animal was 20.20 ± 1.39 ms (*bottom,*
224 *filled grey*), (Fig. 4A). The latency calculated for all pulses gave for the first animal a
225 mean value of 17.84 ± 1.21 ms (*top, grey outline*), for the second example it was
226 22.53 ± 1.95 ms (*middle, grey outline*) and the mean value of the third animal was
227 21.18 ± 1.12 ms (*bottom, grey outline*), (Fig. 4A). In case of the first example, the
228 latencies for the chirp and for all pulses exhibited slightly different distribution patterns.
229 When compared to the onset of the chirps, the latency distribution for all pulses was
230 broader including larger values, due to increasing latencies over the pulses within a
231 chirp. Overall 8 out of 20 animals showed similar shifts, whereas in the other cases the
232 latency distribution for the first pulse and all pulses very closely matched. For a more
233 detailed analysis we plotted the latency values for all chirps and pulses (not shown)
234 over the angles tested, each dot indicates the latency value of one chirp, dots might be
235 superimposed (Fig. 4B). The solid trend line gives the tendency of the latency values
236 for all chirps, while the dotted trend line gives the tendency of the latency values for all
237 pulses. In all cases both trend lines had similar values, and we choose the onset of the
238 chirps for further analysis. The latency values of all three examples and of the other 17
239 animals revealed a quite flat response curves. The data also indicate some very short
240 latency responses like 12.84 ms (arrow Fig. 4B, top), which were not excluded from
241 the analysis. These responses may be due to arbitrary background activity, or to AN2
242 spikes with reduced amplitude due to superposition with the background noise.

243 We analyzed statistically which latencies were different between any two angles
244 on one side of the animals and we analyzed which latencies of bilaterally corresponding

245 angles were different. The first test would indicate if the left and right responses are
246 symmetrical and the second test would reveal bilateral differences relevant for steering.

247 All the three examples (Fig. 4B) didn't show a significant difference in latency
248 between any two tested angles for the ipsilateral side of acoustic stimulation. Latencies
249 were also not significantly different for a wide range of angles on the contralateral side
250 (Fig. 4B). When comparing the latency values for bilaterally corresponding stimulation
251 angles, in these three animals none showed a significant difference over the range of
252 $\pm 45^\circ$.

253 For all experiments we also analyzed the instantaneous spike rate of AN1 and
254 calculated the mean number of AP/Chirp in response to the sound stimuli over all
255 stimulation angles; data are presented for the same 3 recordings (Fig. 5). The averaged
256 instantaneous spike rate of the three animals showed a sharp onset to each sound pulse.
257 The first AN1 responded with 29.68 ± 6.37 AP/Chirp throughout the range of angles, the
258 maximum of its averaged instantaneous spike rate was 370.64 AP/s (Fig. 5A, top). The
259 second AN1 generated 23.29 ± 3.97 AP/Chirp on the whole, and the maximum of the
260 averaged spike rate reached 319.03 AP/s (Fig. 5A, middle). The maximum spike rate of
261 the third animal was 314.56 AP/s (Fig. 5A, bottom), it generated 21.92 ± 1.79 AP/Chirp
262 over the range of tested angles.

263 Corresponding to the analysis of the latency values we plotted over the range of
264 angles tested, the number of APs generated by AN1 in response to each chirp, and
265 obtained three types of response patterns, which are all described by 3rd order
266 polynomial functions. The first example (Fig. 5B, top) shows a sigmoid shape in the
267 AP/Chirp over the range of tested angles with a rather steep change for small
268 stimulation angles. Its response was 29.94 ± 1.77 AP/Chirp when the speaker was in
269 front of the animal. Activity increased to 37.00 ± 1.20 AP/Chirp when the stimuli were
270 presented at $+45^\circ$ ipsilateral and it decreased to 21.39 ± 2.66 AP/Chirp when the stimuli
271 were presented at -45° contralateral. The data revealed a significant response difference

272 of 4 AP/Chirp for sound presented at an angle of -3° (28.37 ± 1.92 AP/Chirp) and $+3^\circ$
273 (32.37 ± 1.94 AP/Chirp) (Fig. 5B, top) and overall increasing differences for larger
274 angles. In the 20 recordings, we had six more animals showing similar results. Also the
275 response of the second example (Fig. 5B, middle) clearly depended of the stimulus
276 angle and revealed an asymmetric response pattern. Compared to the response at 0°
277 (24.05 ± 2.10 AP/Chirp), the response increased for sound presented at the ipsilateral
278 side to 25.91 ± 1.35 AP/Chirp at 45° and at -45° contralateral it decreased to 17.37 ± 2.40
279 AP/Chirp. For bilaterally corresponding angles the response became significantly
280 different with 1.31 AP/Chirp for -6° (23.03 ± 1.78 AP/Chirp) and 6° (24.34 ± 1.97
281 AP/Chirp) and was also different for larger angles (Fig. 5B, middle). Three more
282 recordings showed similar response properties. The data of the third example and 8
283 other AN1s showed a quite flat response curve. It generated 22.14 ± 1.59 AP/Chirp at 0°
284 and 22.28 ± 1.62 AP/Chirp at $+45^\circ$ and 20.57 ± 1.89 AP/Chirp at -45° . Regarding bilateral
285 responses, a significant difference of 1.1 AP/Chirp occurred between -18° (20.95 ± 1.43
286 AP/Chirp) and $+18^\circ$ (22.07 ± 1.63 AP/Chirp) (Fig. 5B, bottom).

287 Pooled over all AN1 recordings obtained, we subsequently plotted the mean
288 difference of the latency (Fig. 6A) and the AP/Chirp (Fig. 6B) for responses to
289 bilaterally corresponding angles. The mean latency difference for angles below 12° was
290 in the range of 0.07 ± 0.29 to 0.25 ± 0.48 ms, it increased with larger angles at 45° to
291 0.61 ± 1.11 ms. There is however, a considerable variance in the data and no mean
292 latency differences are significantly different from zero or different between each other.
293 A different response pattern occurred for the AP/Chirp, the difference in the response
294 strength increased with increasing angle. Following a 3rd order polynomic function, the
295 difference increased close to linear to a value of 4.18 ± 2.90 AP/Chirp at 12° and then
296 for larger angles more gradually to 8.22 ± 4.77 AP/Chirp at 45° . For 3° , 6° and 9° the
297 variance of the data decreased, and the calculated values based on the polynomic
298 function were, 0.99 , 2.03 and 2.98 AP/Chirp. Due to the variation of the data, when

299 compared to 0° the difference of AP/Chirp was significant only for 12° and larger
300 angles.

301 Based on these data we analyzed how the AN1 auditory responses related to
302 changes in sound intensity. To measure the AN1 intensity-response function we
303 positioned the loud speaker at 0° (frontal) and presented 60 chirps per sound intensity
304 with intensity increasing from 60 to 85 dB SPL in 5 dB steps; this sequence was chosen
305 to reduce effect of adaptation at high stimulus intensities. Under these stimulus
306 conditions, the latency of AN1 linearly decreased by about 2 ms from 21.83 ± 3.25 ms
307 at 60 dB SPL to a mean of 19.71 ± 2.45 ms at 85 dB SPL with a coefficient for the linear
308 fit of -0.0652 ms/dB over sound intensity (Fig. 6C). There was an increment in the
309 number of AP/Chirp over the range of sound intensity by 9.8 AP/Chirp, with a mean of
310 21.85 ± 4.95 AP/Chirp at 60 dB SPL and 31.67 ± 6.46 AP/Chirp at 85 dB SPL. The slope
311 of the linear regression function gave an increase by 0.3913 AP for an increase in sound
312 intensity by 1dB SPL (Fig. 6D). Thus the mean AP/Chirp of AN1 would show a
313 bilateral difference of 1 spike when the left-right sound intensity had a difference of
314 2.56 dB SPL. Based on the difference function of the AP/Chirp between bilaterally
315 corresponding angles and the intensity tuning, we calculated that the average difference
316 between +3° and -3° corresponded to a difference in sound intensity of about 3 dB,
317 while at $\pm 45^\circ$ the difference of 8.22 ± 4.77 AP/Chirp corresponded to an intensity
318 difference of 21 dB SPL.

319 For any two corresponding bilateral angles, we subsequently calculated the number
320 of animals which showed a significant difference ($p < 0.05$) in either latency (Fig. 7A)
321 or AP/Chirp (Fig. 7B), based on all 20 recordings of AN1. The latency values did not
322 show a significant difference for the frontal range of -3° to 3°, and only 10 recordings
323 showed a significant differences for angles larger than -30° and +30° (mean difference:
324 1.17 ms). With regard to spike number, 8 out of 20 recordings showed significant
325 differences between -3° and +3° (mean difference: 2.54 AP/Chirp), 10 recordings

326 provided notably differences between -6° and $+6^\circ$ (mean difference: 2.54 AP/Chirp)
327 and all AN1 presented significant bilateral differences for angles larger than -18° and
328 $+18^\circ$ (mean difference: 4.89 AP/Chirp), as reflected in the color pattern of the diagram.

329

330 *Correlation of directional steering and the strength of AN1 response.* Although
331 both data sets were obtained in different individuals, similar proportion of animals
332 showed good steering behaviors and good left-right discrimination based on AN1
333 activity, i.e. 40% for behavioral data and 35% for electrophysiological data. This
334 indicated a correlation between directional steering and the quality of the AN1
335 responses. We pooled the mean lateral deviation for bilateral corresponding angles of
336 very good steering animals (Fig. 8A) and the mean difference of the AP/Chirp for
337 responses to bilaterally corresponding angles of AN1 responses with steep changes for
338 frontal stimulation (Fig. 8B). Compared to all steering animals (Fig. 2) in the very good
339 steering females, the mean deviation was statistically significant also only for angles
340 larger than $\pm 21^\circ$, although with a higher value (2.15 ± 1.38 cm). The difference of
341 AP/Chirp was still significant only for $\pm 12^\circ$ and larger angles, however with an
342 increased value of 6.19 ± 2.50 AP/Chirp (compare to Fig. 6B).

343 For large angles, the spike information provided by AN1 might play a significant
344 role in sound localization; however, when the sound is presented from angles smaller
345 than 9° the mean response differences between the left and right AN1 will be well below
346 5 AP/Chirp. To check the link between AN1 responses and steering behavior in more
347 detail we compared for the same individuals the lateral steering response with the spike
348 response of AN1. We focused on small angles and presented calling song stimuli
349 repetitively between -9° and $+9^\circ$ in steps of 3° and successively recorded AN1 under
350 the same conditions, data were obtained for 10 animals (Fig. 9).

351 In 3 animals we recorded very good steering behavior, with directional changes in
352 phonotactic walking when the speaker frontally crossed the midline (Fig. 9A, left).

353 Another 3 animals presented poor steering and the other 4 did not show a proper
354 phonotactic response (Fig. 9B, left). After the behavioral tests, we recorded the AN1
355 neurons. As the latency always showed a flat response pattern, we present only data for
356 the AP/Chirp. We show two representative examples of a steering and a non-steering
357 animal. The animal with a very good steering response followed the changes in speaker
358 position and changed its steering direction with high precision when the speaker crossed
359 the midline. When comparing the AN1 spiking response for stimulation from bilateral
360 corresponding angles, the two examples gave similar results (Fig. 9A, B right). They
361 both showed a significant difference between -3° and $+3^\circ$ in the number of AP/Chirp.
362 For the good steering animal the difference was 1.15 AP/Chirp with a response of
363 19.89 ± 2.49 AP/Chirp at -3° and 21.04 ± 2.37 AP/Chirp at 3° . For the non-steering animal
364 the difference was 1.61 AP/Chirp with 23.08 ± 1.76 AP/Chirp at -3° and 24.69 ± 2.18
365 AP/Chirp at $+3^\circ$, and furthermore 3.25 AP/Chirp with 22.79 ± 1.55 AP/Chirp at -6° and
366 26.05 ± 1.92 AP/Chirp at $+6^\circ$.

367 We had two more good steering females. One had a similar pattern of AN1
368 response as the example given, when comparing the data for ipsilateral and contralateral
369 only. However, when we compared the bilateral responses, they only showed a
370 significant difference of 1.41 AP/Chirp between -9° (27.97 ± 2.14 AP/Chirp) and $+9^\circ$
371 (29.38 ± 1.81 AP/Chirp). The other female showed a significant difference of 1.51
372 AP/Chirp when comparing the response to -6° (26.95 ± 2.03 AP/Chirp) and $+6^\circ$
373 (28.46 ± 1.813 AP/Chirp). In three non-steering animals, one showed a significant
374 bilateral difference for $\pm 3^\circ$ of 1.51 AP/Chirp and two showed a significant difference
375 for $\pm 9^\circ$ with 1.79 and 5.55 AP/Chirp.

376 This indicates, that although steering and non-steering females can have a very
377 similar AN1 activity forwarded to the brain, the behavioral outcome can be very
378 different.

379

380 DISCUSSION

381 Our study provides an insight into the reliability of directional information
382 forwarded to the cricket brain by the auditory neuron AN1, and on its functional
383 implications for the neuronal control of phonotaxis.

384 *Responses to directional sound stimuli.* Female crickets walking on a trackball
385 followed the changing position of a calling song presented from -45° to $+45^\circ$ in steps
386 of 3° with a different degree of accuracy. A total of 40% females showed a highly
387 reliable change in walking direction when the speaker passed 0° , as described for
388 hyperacute steering crickets (Schöneich and Hedwig 2010), in 60% the directional
389 response became less accurate, when the speaker approached the midline (Fig. 1). The
390 pooled steering data follow a 3rd order polynomic function and increase up to 45° (Fig.
391 2). This is slightly different to directional auditory steering reported by Pollack and
392 Plourde (1982) for flying crickets, and by Stabel et al. (1989) and by Schöneich and
393 Hedwig (2010) for walking females, which report a decline of steering accuracy when
394 stimulation angles approach 45° . Although our pooled data show significant steering
395 only for angles equal/larger than 24° , 6 out of 15 females steered precisely to sound
396 stimuli deviating by 3° to 6° . In a similar way Pollack and Plourde (1982) report that
397 individual crickets steered to sound presentation at 5° , but the pooled data did not reflect
398 the accuracy of these animals. This may be seen as a fundamental dilemma, as the mean
399 may represent the population, whereas the best responses may be evolutionarily more
400 relevant, and may allow deeper insights into the underlying functional properties of the
401 system. Selecting for good tracking animals (Thorson et al. 1982) may be justified to
402 reveal the best performance.

403 Comparing the bilateral response differences with the frontal AN1 intensity tuning
404 (Fig. 6) demonstrates that a bilateral difference of 1 AP/Chirp corresponds to a mean
405 sound intensity difference of 2.56 dB SPL. In choice experiments crickets steer towards
406 the louder sound source if the intensity difference is just 1-2 dB SPL (Hedwig and

407 Poulet 2005) and also bush-cricket (Rheinlaender et al. 2006) and grasshoppers (von
408 Helversen and Rheinlaender 1988, Ronacher and Krahe 2000) reliably respond to
409 similar intensity differences. In the bush-cricket *Mecopoda elongata* interaural intensity
410 differences of just 1 dB SPL delivered by dichotic ear stimulation, lead with increasing
411 stimulus intensity to significant binaural discharge difference of 3 to 6 AP/Stimulus in
412 the Omega neuron (Stradner and Römer 2008) and in *Leptophyes punctatissima* at
413 stimulation from 15° the T-fibre shows mean bilateral response differences of about 2
414 AP/Stimulus (Kostarakos et al. 2007). In summary these data indicate that for
415 directional processing, at stimulus angles close to the midline cricket and bush-cricket
416 auditory pathways can operate with minute interaural intensity differences and with
417 very small differences in bilateral spike activity (see Römer 2015).

418 *Bilateral differences in AN1 activity.* We compared the steering behavior with the
419 directional information forwarded by AN1 and analyzed bilateral differences in
420 response latency and spike count. The directional responses of AN1 should be enhanced
421 by the inhibitory effect of the Omega neuron ON1, which increases the bilateral
422 auditory contrast (Wohlers and Huber, 1982; Kleindienst et al. 1981; Horseman and
423 Huber, 1994a). The ON1 directional tuning given by Wiese and Eilts-Grimm (1985)
424 however, shows a strong decrease in ON1 activity below 30°, indicating that the impact
425 of its inhibition on AN1 may decrease correspondingly and may be less effective for
426 frontal acoustic stimulation.

427 As the AN1 latency varied, the pooled latency differences (Fig. 6A) come with
428 considerable variability. The mean bilateral latency differences gave only small values
429 of 0.61 ms over the range of $\pm 45^\circ$, and only 10 out of 20 recordings showed significant
430 differences for angles larger than $\pm 30^\circ$ with 1.17 ms. For afferents, the bilateral latency
431 difference at $\pm 30^\circ$ reached about 1.3 ms resulting in a latency gradient of $42 \mu\text{s}/^\circ$
432 (Schöneich and Hedwig 2010). In our experiments however, the mean difference in
433 AN1 latency at $\pm 30^\circ$ was considerable lower and 0.34 ms. Also Kostarakos and Römer

434 (2010) report only small AN1 latency differences of 0-1 ms for stimulation angles of
435 $\pm 30^\circ$ and large variations with increasing speaker distance. In AN2 latency differences
436 occur of several milliseconds, but change with pulse rate and the duration of stimulation
437 (Samson and Pollack 2002). Similar to auditory processing in grasshoppers (Ronacher
438 and Krahe 2000), bilateral latency differences in crickets may not be as relevant for
439 directional auditory steering as differences in bilateral response strength (Samson and
440 Pollack 2002; Hedwig and Poulet 2005).

441 In comparison specimen showed significant bilateral differences in their spike
442 count. Our pooled data reveal an increase in the bilateral difference of the AN1 response
443 with increasing stimulation angle (Fig. 6B). For $\pm 30^\circ$ we recorded a difference 7.05
444 AP/Chirp over all tested animals and a difference of 10.43 AP/Chirp when only the best
445 responding animals were considered. This is in the same range to the directional error
446 signal of about 7.2 AP/Chirp reported by Horseman and Huber (1994b) and a difference
447 of about 8 AP/Chirp given by Kostarakos and Römer (2010). Our data follow a 3rd order
448 polynomial function, which increases sharply over the first 15° and then starts to level
449 off. This saturating course of the AN1 directional tuning is similar to previously reported
450 steering responses (Pollack and Plourde 1982; Stabel et al. 1989; Schöneich and
451 Hedwig 2010).

452 For our pooled data the difference in the bilateral spiking response is only
453 significant over $\pm 12^\circ$ at a level of 4.18 AP/Chirp. Based on previous measurements
454 (Horseman and Huber 1994b; Kostarakos and Römer 2010), which were performed at
455 intervals of 30° , a linear change of the bilateral response difference of about 0.25 AP/ $^\circ$
456 can be calculated for frontal stimulation. Our data reveal overall a polynomial function,
457 however, considering a close to linear relationship for angles up to $\pm 12^\circ$ a slope of 0.35
458 AP/ $^\circ$ describes the frontal directional tuning (Fig. 6B), similar to the previous results.

459 Measurements of directional steering within the range of $\pm 9^\circ$ with successive
460 recordings of AN1 in the same animals demonstrated precisely steering animals, in

461 which AN1 showed a significant bilateral response difference of 1.15 AP/Chirp for $\pm 3^\circ$
462 and 2.81 AP/Chirp at $\pm 9^\circ$. When tested with the same paradigm females, which did not
463 show a proper steering behavior, also revealed a corresponding bilateral difference in
464 the AN1 response (Fig. 9). Thus, small AN1 activity differences may underlie
465 directional auditory steering, but in these experiments AN1's activity is not a strict
466 predictor for the accuracy of the steering response.

467 *AN1 activity and directional steering.* In summary, AN1 may make a contribution
468 to sound localization for large angles, but based on the mean AN1 error signals for small
469 angles a crucial question is, whether these are sufficient to allow reliable directional
470 steering responses. Based on the best AN1 recordings encountered (Fig. 8B) up to 12°
471 the AN1 responses are not significantly different from the response at 0° , at 3° the mean
472 error signal will be about 1.56 AP/Chirp and at 9° it will be 4.7 AP/Chirp. In walking
473 crickets however, the auditory afferents respond to the leg movements and generate
474 non-auditory background activity that considerably reduces the reliability of auditory
475 coding in ON1 and AN1 interneurons (Schildberger et al. 1988) and the directional
476 information forwarded to the brain. Nonetheless female crickets show accurate and
477 reliable changes in walking direction, even when the frontal stimulus angle changes by
478 just $1-2^\circ$ (Schöneich and Hedwig 2010). If auditory steering is based on bilateral
479 differences in AN1 activity, it will require that these differences are enhanced or at least
480 maintained when processed in the brain, before forwarded as bilaterally different
481 steering commands to the thoracic motor system. Importantly however, the properties
482 of the pattern recognition circuit in the brain (Schöneich et al. 2015), may not preserve
483 bilateral differences of few AP/Chirp as the recognition process is independent of sound
484 intensity. Especially, the high-order feature detecting neurons show characteristics of
485 sparse coding, responding only with few spikes to a chirp (Schöneich et al. 2015;
486 Kostarakos and Hedwig 2012), and do not reflect minor variations in AN1 activity.
487 Currently there is no indication that small bilateral response differences of the AN1

488 neurons in the range of few spikes, would be maintained at this level of auditory
489 processing.

490 Male grasshoppers turn towards a female response song after listening to 3 subunits i.e.
491 250 ms of the song. Based on the variability of the activity in individual auditory
492 afferents, their interneuron system may need to integrate over 15-20 afferents to reach
493 a robust decision for the lateralization behavior (Ronacher and Krahe 2000). In the
494 cricket, AN1 integrates activity from auditory afferents and is assumed to be the only
495 neuron feeding activity into the pattern recognition network in the brain (Schildberger
496 et al. 1989). Once pattern recognition is activated, females will steer towards the
497 individual sound pulses of chirps, which are just 20 ms long (Hedwig and Poulet 2004),
498 indicating that auditory processing underlying steering does not integrate information
499 over long periods of time; such an integration rather may occur at the level of the motor
500 system (see below).

501

502 *Reconsidering the role of AN1 activity.* AN1 activity closely correlates with
503 directional phonotactic orientation (Kostarakos and Römer 2010) and the bilateral AN1
504 activity difference with the crickets' behavioral preferences in two-choice experiments
505 (Kostarakos et al. 2008; Trobe et al. 2011). Moreover, intracellular recording and
506 stimulation experiments indicate AN1 activity as crucial for auditory steering. Photo-
507 ablation of one AN1 leads to angular steering errors and ipsilateral circling during
508 phonotaxis (Atkins et al. 1992). Also the walking direction of females orienting towards
509 a sound source can be reversed by hyperpolarization of the AN1 with dendrites
510 ipsilateral to the side of the sound source (Schildberger and Hörner 1988). It has been
511 suggested that a comparison of the bilateral AN1 activity occurs in the brain, and
512 crickets subsequently steer towards the side with stronger AN1 activation, indicating
513 that AN1 is important and necessary for phonotactic steering (Schildberger et al. 1989).
514 However, a comparison of bilateral AN1 activity in the brain is not required if the

515 directional auditory signals are integrated directly with the motor activity at the thoracic
516 level (see also Stabel et al. 1989).

517 Crickets use two pattern recognition networks (Pollack 1986; Stabel et al. 1989),
518 one in each half of the protocerebrum (Schöneich et al. 2015). Detailed behavioral
519 analyses point towards reactive auditory steering responses and a modulatory, gating
520 effect of pattern recognition on auditory steering (Hedwig and Poulet 2004; Poulet and
521 Hedwig 2005; Gabel et al. 2015). The observed impact of inhibiting AN1 on
522 phonotactic steering (Schildberger and Hörner 1988) might well be an indirect effect,
523 mediated by a reduced or even abolished gating output of the pattern recognition
524 network, rather than a change in a steering command. This could be accomplished by
525 the organization of phonotactic behavior as suggested by Poulet and Hedwig (2005)
526 and outlined by Gabel et al. (2015; Fig. 6D). In this scheme, any modulation of the
527 auditory activity forwarded to the pattern recognition network, would have an indirect
528 impact on the modulatory signal, forwarded from the pattern recognition network to the
529 auditory-to-motor pathway controlling the steering behavior. This could be the basis to
530 explain the effects of intracellular AN1 hyperpolarization.

531 Auditory steering may be achieved via a more direct thoracic pathway. Boyan
532 (1978) describes the activity of a non-identified descending prothoracic auditory
533 interneuron with a high directionality, and discusses its possible role in forwarding
534 auditory activity towards thoracic motor centers. Furthermore, Imaizumi and Pollack
535 (2005) revealed a population of bifurcating auditory afferents that due to their axonal
536 branching pattern may have little influence on the ascending auditory information, but
537 rather may relay activity about the cricket song to descending ganglia, controlling the
538 motor activity. The descending auditory neuron DN1 that is directional sensitive and
539 copies the song pattern like AN1 (Wohlers and Huber 1982; Atkins and Pollack 1987),
540 shows similarity to the activation of these afferents and as suggested by Boyan (1978)
541 could forward the relevant auditory signal to the motor circuitry, so that even small

542 bilateral auditory differences would accumulate in the behavioral response over time.
543 Exploring this descending circuitry might provide deeper insight into the organization
544 of cricket phonotactic behavior.

545 Our reasoning does not question the crucial role of AN1 in cricket phonotaxis, it
546 rather considers the organization of the auditory and the motor pathway, which underlie
547 the control of phonotactic behavior. In any sensory pathway with a bottleneck, where
548 information is forwarded for processing by a single neuron only (Nolen and Hoy 1984;
549 Ratcliffe et al. 2009), modulating the activity of this neuron will have a substantial
550 effect on sensory processing and resulting motor responses, but this does not reveal the
551 subsequent processing stages.

552

553 **ACKNOWLEDGEMENTS**

554 M. Lv was supported by a stipend from the China Scholarship Council; X. Zhang was
555 funded by the Cambridge Trust, Trinity College and the Department of Zoology. The
556 BBSRC (BB/P022111/1) and the Royal Society provided the equipment for the
557 experiments. We are very grateful to H. Römer/Graz for constructive comments on the
558 manuscript and to G. Harrison and M. Morgan for excellent technical support.

559

560 **REFERENCES**

- 561 **Atkins G, Henley J, Handysides R, Stout J.** Evaluation of the behavioral roles of
562 ascending auditory interneurons in calling song phonotaxis by the female cricket
563 (*Acheta domesticus*). *J Comp Physiol A* 170: 363–372, 1992.
564 doi:10.1007/BF00191425.
- 565 **Atkins G, Pollack GS.** Response properties of prothoracic, interganglionic, sound-
566 activated interneurons in the cricket *Teleogryllus oceanicus*. *J Comp Physiol A*
567 161: 681–693, 1987. doi:10.1007/BF00605009.
- 568 **Ball EE, Oldfield BP, Rudolph KM.** Auditory organ structure, development, and
569 function. In: *Cricket Behavior and Neurobiology*, edited by Huber F, Moore TE,
570 Loher W. Ithaca, NY: Cornell University Press, 1989, p. 391–422.
- 571 **Boyan GS.** Coding of Directional Information by a Descending Interneuron in the
572 Auditory System of the Cricket. *Naturwissenschaften* 65: 212–213, 1978.

573 **Gabel E, Kuntze J, Hennig RM.** Decision making and preferences for acoustic signals
574 in choice situations by female crickets. *J Exp Biol* 218: 2641–2650, 2015.
575 doi:10.1242/jeb.120378.

576 **Givois V, Pollack GS.** Sensory habituation of auditory receptor neurons: implications
577 for sound localization. *J Exp Biol* 203:2529–2537, 2000.

578 **Hedwig B, Poulet JFA.** Complex auditory behaviour emerges from simple reactive
579 steering. *Nature* 430: 781–785, 2004. doi:10.1038/nature02787.

580 **Hedwig B, Poulet JFA.** Mechanisms underlying phonotactic steering in the cricket
581 *Gryllus bimaculatus* (de Geer) revealed with a fast trackball system. *J Exp Biol*
582 208: 915–927, 2005. doi:10.1242/jeb.01452.

583 **Hedwig B, Stumpner A.** Central neural processing of sound signals in insects. In:
584 *Insect Hearing*, edited by Pollack GS, Mason AC, Popper AN, Fay RR. Cham:
585 Springer, 2016, p. 177–214.

586 **Hennig RM.** Ascending auditory interneurons in the cricket *Teleogryllus commodus*
587 (Walker): comparative physiology and direct connections with afferents. *J Comp*
588 *Physiol A* 163: 135–143, 1988. doi:10.1007/BF00612003.

589 **Hennig RM, Franz A, Stumpner A.** Processing of auditory information in insects.
590 *Microsc Res Tech* 63: 351–374, 2004. doi:10.1002/jemt.20052.

591 **Horseman G, Huber F.** Sound localisation in crickets. I. Contralateral inhibition of an
592 ascending auditory interneuron (AN1) in the cricket *Gryllus bimaculatus*. *J Comp*
593 *Physiol A* 175: 389–398, 1994a. doi:10.1007/BF00199247.

594 **Horseman G, Huber F.** Sound localisation in crickets. II. Modelling the role of a
595 simple neural network in the prothoracic ganglion. *J Comp Physiol A* 175: 399–
596 413, 1994b. doi:10.1007/BF00199248.

597 **Hoy RR.** Acoustic communication in crickets: a model system for the study of feature
598 detection. *Fed Proc* 37: 2316–2323, 1978.

599 **Huber F, Thorson J.** Cricket auditory communication. *Sci Am* 253: 46–54, 1985.
600 doi:10.1038/scientificamerican1285-60.

601 **Imaizumi K, Pollack GS.** Central projections of auditory receptor neurons of crickets.
602 *J Comp Neurol* 493: 439–447, 2005. doi:10.1002/cne.20756.

603 **Kleindienst HU, Koch UT, Wohlers DW.** Analysis of the cricket auditory system by
604 acoustic stimulation using a closed sound field. *J Comp Physiol A* 141: 283–296,
605 1981. doi:10.1007/BF00609930.

606 **Knepper M, Hedwig B.** NEUROLAB, a PC-program for the processing of
607 neurobiological data. *Comput Meth Prog Biomed* 52: 75–77, 1997.
608 doi:10.1016/S0169-2607(96)01781-6.

609 **Kostarakos K, Rheinlaender J, Römer H.** Spatial orientation in the bushcricket
610 *Leptophyes punctatissima* (Phaneropterinae; Orthoptera): III. Peripheral
611 directionality and central nervous processing of spatial cues. *J Comp Physiol*
612 *A*;193:1115–1123, 2007. DOI 10.1007/s00359-007-0262-6

613 **Kostarakos K, Hartbauer M, Römer H.** Matched filters, mate choice and the
614 evolution of sexually selected traits. *PloS One* 3: e3005, 2008.
615 doi:10.1371/journal.pone.0003005.

616 **Kostarakos K, Hedwig B.** Calling song recognition in female crickets: temporal tuning
617 of identified brain neurons matches behavior. *J Neurosci* 32: 9601–9612, 2012.
618 doi:10.1523/JNEUROSCI.1170-12.2012.

619 **Kostarakos K, Hedwig B.** Surface electrodes record and label brain neurons in insects.
620 *J Neurophysiol* 118: 2884–2889, 2017. doi:10.1152/jn.00490.2017.

621 **Kostarakos K, Römer H.** Sound transmission and directional hearing in field crickets:
622 neurophysiological studies outdoors. *J Comp Physiol A* 196: 669–681, 2010.
623 doi:10.1007/s00359-010-0557-x.

624 **Lewis B.** Directional cues for auditory localization. In: *Bioacoustics, a comparative*
625 *approach*, edited by Lewis B. London; New York: Academic Press, 1983, p. 233–
626 257.

627 **Michelsen A, Larsen ON.** Pressure difference receiving ears. *Bioinspir Biomim* 3:
628 011001, 2008. doi:10.1088/1748-3182/3/1/011001.

629 **Nolen TG, Hoy RR.** Initiation of behavior by single neurons: the role of behavioral
630 context. *Science* 226: 992–994, 1984. doi:10.1126/science.6505681.

631 **Oldfield BP, Kleindienst HU, Huber F.** Physiology and tonotopic organization of
632 auditory receptors in the cricket *Gryllus bimaculatus* DeGeer. *J Comp Physiol A*
633 159: 457–464, 1986. doi:10.1007/BF00604165.

634 **Pollack GS.** Discrimination of calling song models by the cricket, *Teleogryllus*
635 *oceanicus*: the influence of sound direction on neural encoding of the stimulus
636 temporal pattern and on phonotactic behavior. *J Comp Physiol A* 158: 549–561,
637 1986. doi:10.1007/BF00603799.

638 **Pollack GS.** Who, what, where? Recognition and localization of acoustic signals by
639 insects. *Curr Opin Neurobiol* 10: 763–767, 2000. doi:10.1016/S0959-
640 4388(00)00161-6.

641 **Pollack GS, Plourde N.** Directionality of acoustic orientation in flying crickets. *J*
642 *Comp Physiol A* 146: 207–215, 1982. doi:10.1007/BF00610239.

643 **Poulet, JFA, Hedwig B.** Auditory orientation in crickets: pattern recognition controls
644 reactive steering. *Proc Natl Acad Sci USA* 102: 15665–15669, 2005.
645 doi:10.1073/pnas.0505282102.

646 **Ratcliffe JM, Fullard JH, Arthur BJ, Hoy RR.** Tiger moths and the threat of bats:
647 decision-making based on the activity of a single sensory neuron. *Biol Lett* 5:
648 368–371, 2009. doi:10.1098/rsbl.2009.0079.

649 **Rheinlaender J, Shen JX, Römer H.** Auditory lateralization in bushcrickets: a new
650 dichotic paradigm. *J Comp Physiol A* 192(4): 389–397. 2006.
651 doi.org/10.1007/s00359-005-0078-1.

652 **Robert D.** Sound localisation in Insects. In: *The Senses: A Comprehensive Reference*,
653 edited by Basbaum AI, Kaneko A, Shepherd GM, Westheimer G. San Diego:
654 Academic Press, 2008, p. 725–732.

655 **Ronacher B and Krahe R** Temporal integration vs. parallel processing: coping with
656 the variability of neuronal messages in directional hearing insects *Eurp J*
657 *Neurosci* 12: 2147-2156, 2000. doi.org/10.1046/j.1460-9568.2000.00102.x

658 **Römer H.** Directional hearing: from biophysical binaural cues to directional hearing
659 outdoors. *J Comp Physiol A* 201:87–97, 2015. doi:10.1007/s00359-014-0939-6.

660 **Römer H, Schmidt AKD.** Directional hearing in insects with internally coupled ears.
661 *Biol Cybern* 110: 247–254, 2016. doi:10.1007/s00422-015-0672-4.

662 **Samson AH, Pollack GS.** Encoding of sound localization cues by an identified
663 auditory interneuron: effects of stimulus temporal pattern. *J Neurophysiol* 88:
664 2322–2328, 2002. doi:10.1152/jn.00119.2002.

665 **Schildberger K, Hörner M.** The function of auditory neurons in cricket phonotaxis. I.
666 Influence of hyperpolarization of identified neurons on sound localization. *J*
667 *Comp Physiol A* 163: 621–631, 1988. doi:10.1007/BF00603846.

668 **Schildberger K, Huber F, Wohlers D.** Central auditory pathway: neuronal correlates
669 of phonotactic behavior. In: *Cricket Behavior and Neurobiology*, edited by Huber
670 F, Moore TE, Loher W. Ithaca, NY: Cornell University Press, 1989, p. 423–458.

671 **Schildberger K, Milde JJ, Hörner M.** The function of auditory neurons in cricket
672 phonotaxis. II. Modulation of auditory responses during locomotion. *J Comp*
673 *Physiol A* 163: 633–640, 1988.

674 **Schöneich S, Hedwig B.** Hyperacute directional hearing and phonotactic steering in
675 the cricket (*Gryllus bimaculatus* deGeer). *PLoS One* 5: e15141, 2010.
676 doi:10.1371/journal.pone.0015141.

677 **Schöneich S, Kostarakos K and Hedwig B.** An Auditory Feature Detection Circuit
678 for Sound Pattern Recognition. *Sci Adv* 1(8): e1500325, 2015.
679 doi: 10.1126/sciadv.1500325.

680 **Selverston AI, Kleindienst HU, Huber F.** Synaptic connectivity between cricket
681 auditory interneurons as studied by selective photoinactivation. *J Neurosci* 5:
682 1283–1292, 1985. doi:10.1523/JNEUROSCI.05-05-01283.1985.

683 **Stabel J, Wendler G, Scharstein H.** Cricket phonotaxis: localization depends on
684 recognition of the calling song pattern. *J Comp Physiol A* 165: 165–177, 1989.
685 doi:10.1007/BF00619191.

686 **Stradner J, Römer H.** Reliable coding of small, behaviourally relevant interaural
687 intensity differences in a pair of interneurons of an insect. *Biol Lett* 4: 711–714,
688 2008. doi:10.1098/rsbl.2008.0367.

689 **Thorson J, Weber T, Huber F.** Auditory behavior of the cricket. II. Simplicity of
690 calling-song recognition in *Gryllus*, and anomalous phonotaxis at abnormal
691 carrier frequencies. *J Comp Physiol A* 146: 361–378, 1982.
692 doi:10.1007/BF00612706.

- 693 **Trobe D, Schuster R, Römer H.** Fast and reliable decisions for a dynamic song
694 parameter in field crickets. *J Comp Physiol A* 197: 131–135, 2011. DOI
695 10.1007/s00359-010-0589-2.
- 696 **von Helversen D, Rheinlaender J.** Interaural intensity and time discrimination in an
697 unrestraint grasshopper: a tentative behavioural approach. *J Comp Physiol A* 162:
698 333–340, 1988. doi:10.1007/BF00606121.
- 699 **Wiese K, Eilts-Grimm K.** Functional potential of recurrent lateral inhibition in cricket
700 audition. In: *Acoustic and vibrational communication in insects*, edited by
701 Kalmring K, Elsner N. Berlin, Hamburg: Parey, 1985, p. 33–40.
- 702 **Wohlers DW, Huber F.** Processing of sound signals by six types of neurons in the
703 prothoracic ganglion of the cricket, *Gryllus campestris* L. *J Comp Physiol A* 146:
704 161–173, 1982. doi:10.1007/BF00610234.

705

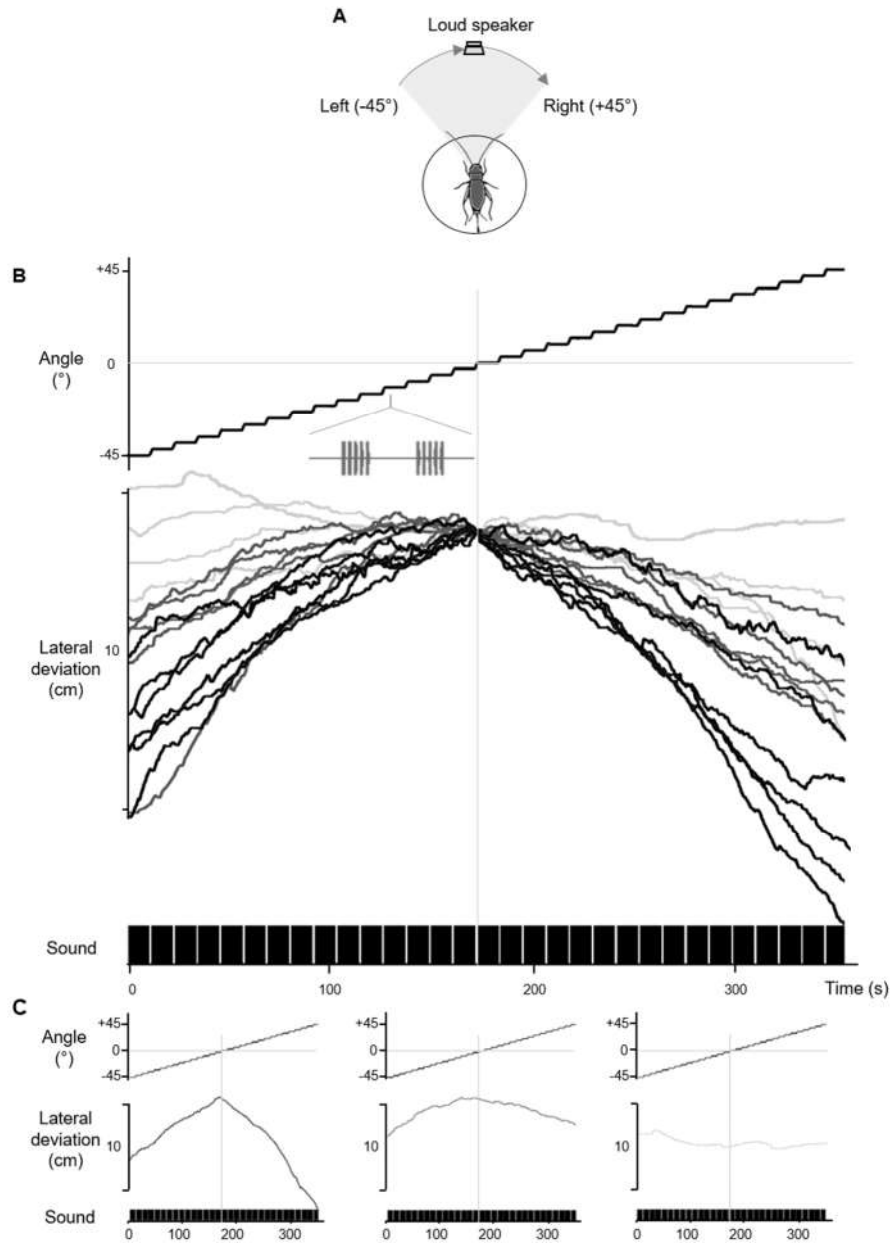
706

707

708

709

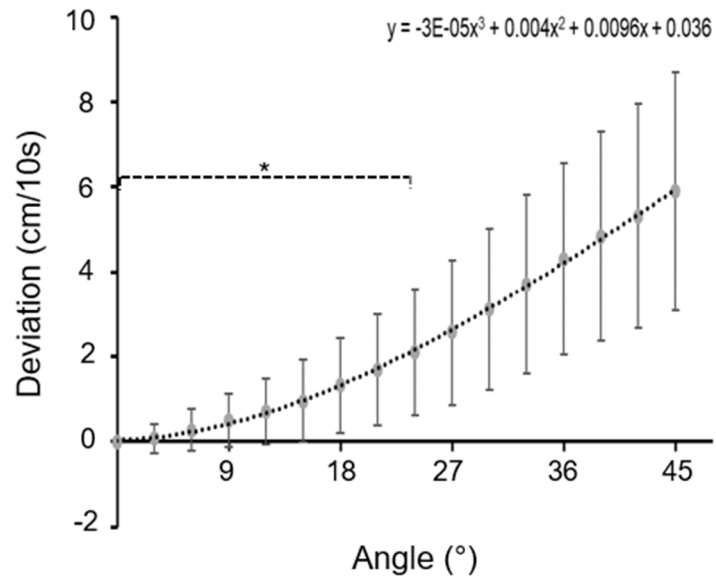
710 **FIGURES**



711
 712
 713
 714
 715
 716
 717
 718
 719
 720
 721
 722
 723

Fig. 1. Phonotactic response of female crickets. *A*: The phonotactic steering response of a female cricket tethered on a trackball is measured, while a speaker presenting calling song is moved from -45° to $+45^{\circ}$ in steps of 3° . *B*: Behavioral characteristic curves to sound stimuli measured for 15 animals. Top trace gives the speaker positions in steps of 3° . Middle trace gives the superimposed steering response of all tested females. The lateral deviation was defined as zero when the speaker was in front of the cricket at 0° , indicated by the vertical grey line. Lower trace indicates the presentation of 10s of calling song at each speaker position. *C*: Three typical examples of very good steering (*left, black*), poor steering (*middle, dark grey*) and an animal that did not show a proper phonotactic response (*right, light grey*).

724



725

726

727 Fig. 2. Phonotactic steering behaviour with the mean lateral deviation pooled for all
728 steering females (N=11), left and right steering behaviour pooled. Dotted line with
729 brackets indicates the smallest angle of 24° with a significant difference when
730 compared to the steering response at 0°. Values are means ± SD.

731

732

733

734

735

736

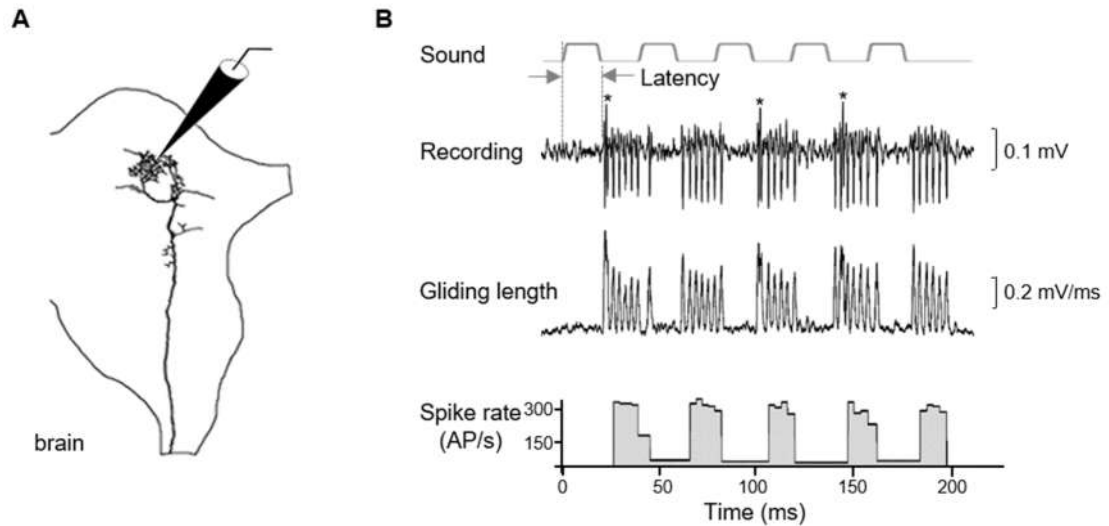
737

738

739

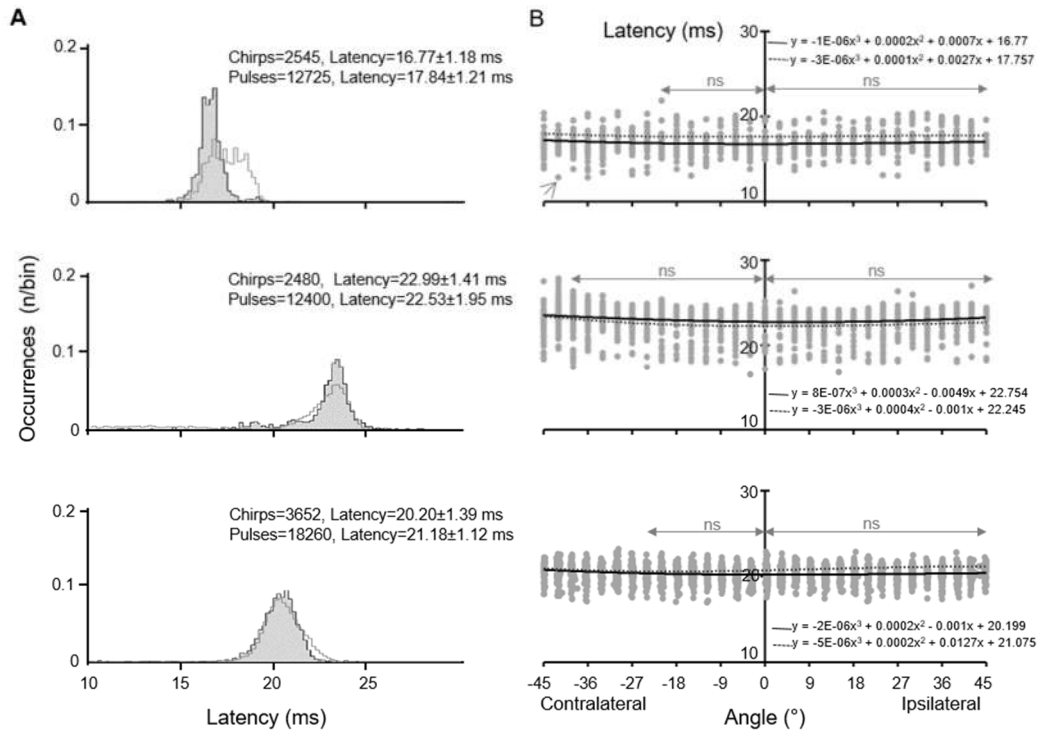
740

741



742
 743
 744
 745
 746
 747
 748
 749
 750
 751
 752
 753
 754
 755
 756
 757
 758
 759
 760
 761
 762

Fig. 3. Surface-electrode recordings of AN1 in the brain. *A*: AN1 activity was recorded with a surface-electrode positioned ventrally at the axonal terminals of AN1 on the brain, as indicated in the diagram. *B*: Recording of AN1 activity in response to a chirp presented at 75 dB SPL; some larger spikes from AN2 (asterisks) are also picked up. A filter algorithm that measures the total voltage change over the time course of a spike (gliding length filter, Knepper and Hedwig, 1997) is applied to increase the signal-to-noise ratio. The instantaneous spike rate of the recording reveals the activity pattern of AN1. Latency of the response is measured from the start of a chirp to the first AN1 action potential (AP).



763

764

765

766 Fig. 4. Latency characteristics of three AN1 recordings. *A*: Frequency distribution of
 767 response latencies to the first pulse of chirps (dark grey shadow) and pulses (light grey
 768 outline) over the complete range of tested angles. Bin width: 0.2 ms *B*: Latencies plotted
 769 over the range of angles tested, each dot indicates the latency in response to a chirp, 80
 770 chirps (top and middle) or 120 chirp (bottom) were presented at each angle. Solid thin
 771 lines with arrowheads indicate the range of angles with no significant difference in
 772 latency when compared with each other. One-way ANOVA with Tukey post-tests:
 773 * $p < 0.05$. Solid bold lines indicate 3rd order polynomial best fit functions for all latency
 774 data to start of chirps, and dotted bold lines indicate 3rd order polynomial best fit
 775 functions for latency data to each pulse over the range of -45° to $+45^\circ$. Histograms are
 776 scaled to one stimulus, i.e. occurrences per bin are divided by the number of stimuli
 777 presented.

778

779

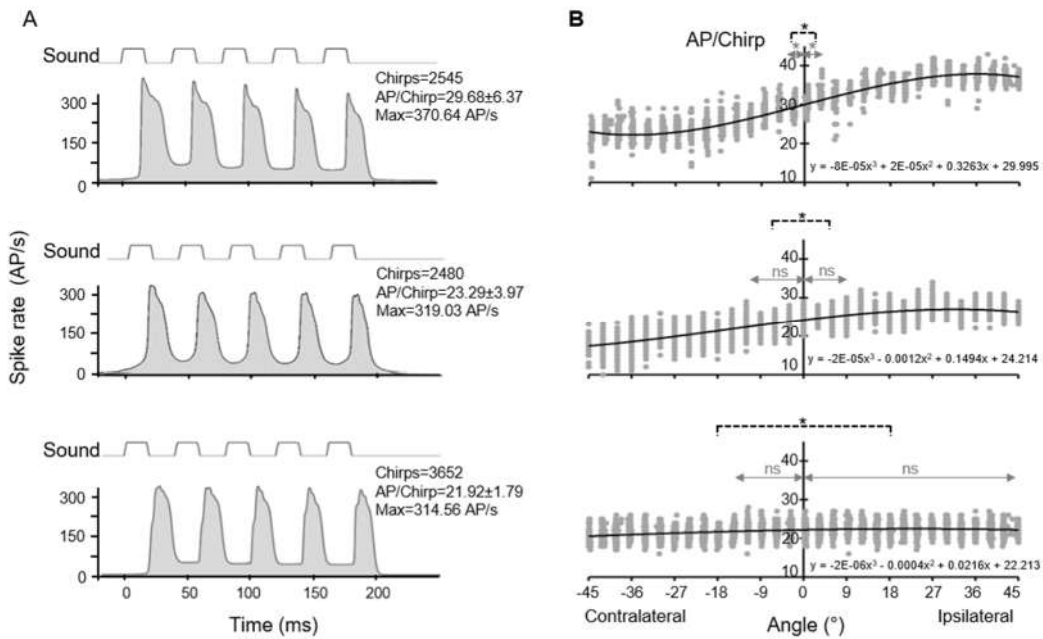
780

781

782

783

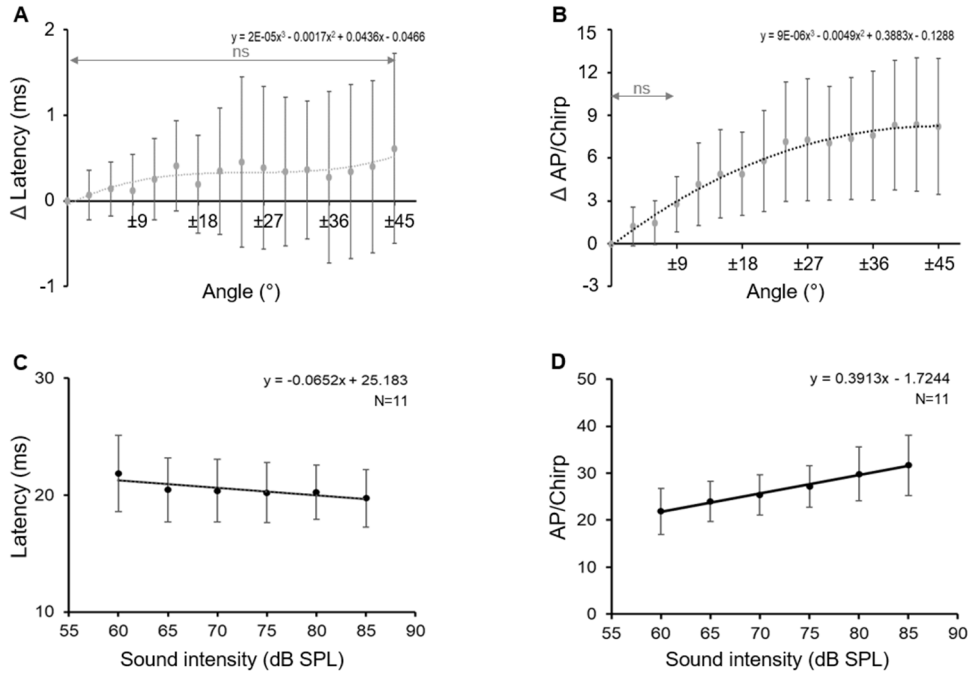
784
 785
 786
 787
 788



789
 790
 791
 792
 793
 794
 795
 796
 797
 798
 799
 800
 801
 802
 803
 804

Fig. 5. Spike response characteristics of the three AN1 recordings in Fig. 4. *A*: Averaged instantaneous spike rate of AN1 in response to the chirps. *B*: Number of APs in response to each chirp (AP/Chirp) plotted for all chirps over the range of angles tested, each dot represents the response to one chirp, 80 chirps (top and middle) or 120 chirp (bottom) were presented at each angle. Dotted lines with brackets indicate the two smallest bilateral angles with a significant difference in AP/Chirp. Solid thin lines with arrowheads indicate the range of angles with no significant difference in AP/Chirp compared with each other; one-way ANOVA with Tukey post-tests: * $p < 0.05$. Solid bold lines indicate 3rd order polynomial best fit functions for all AP/Chirp data points over the range of -45° to $+45^\circ$.

805
806
807



808
809

810 Fig. 6. Response characteristics of AN1. *A, B*: The mean difference of latencies and
811 AP/Chirp for bilaterally corresponding angles based on 20 animals tested. For each
812 angle, the mean difference is shown with standard deviation. Thin lines with
813 arrowheads indicate the range of angles with no significant difference in latency or
814 AP/Chirp when compared to the response at 0°. Dotted lines indicate 3rd order
815 polynomial best fit for all data points over the range of -45° to +45°. *C, D*: Relationships
816 between stimulus intensity tested for frontal position of the speaker from 60 to 85 dB
817 SPL in steps of 5 dB SPL, and response latency (*left*) and AP/Chirp (*right*). Data points
818 show the mean of 11 animals together with standard deviation, solid lines give linear
819 best-fit functions, each sound intensity was tested 60 times.

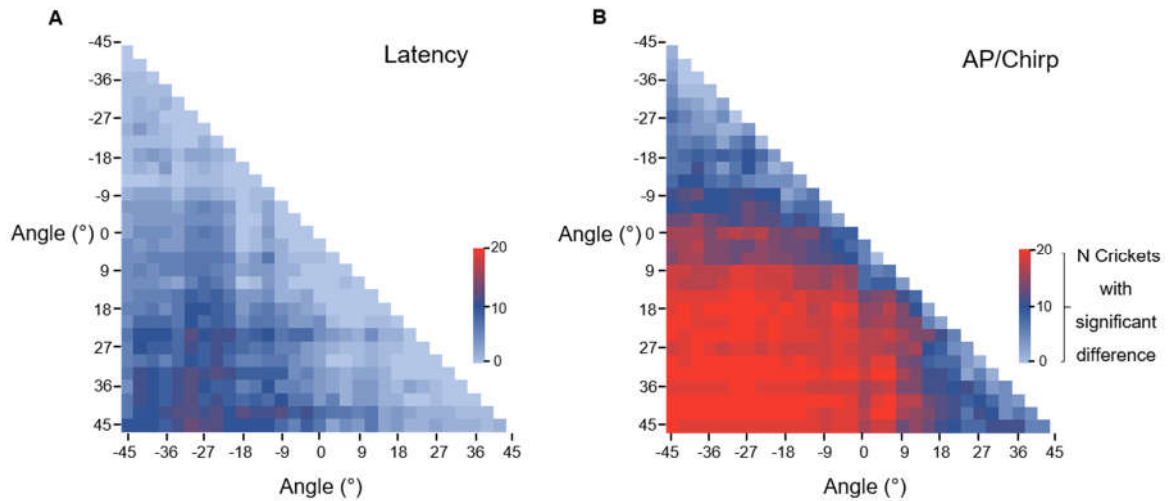
820
821
822
823
824
825

826

827

828

829



830

831 Fig. 7. The number of animals showing significant differences in either latency (*A*) or

832 AP/Chirp (*B*) for any two corresponding angles ($N=20$) as tested with one-way ANOVA

833 with Tukey post-hoc tests: $*p<0.05$ The color code from bright blue to red indicates the

834 number of crickets with a significant difference related to a scale from 0 to 20.

835

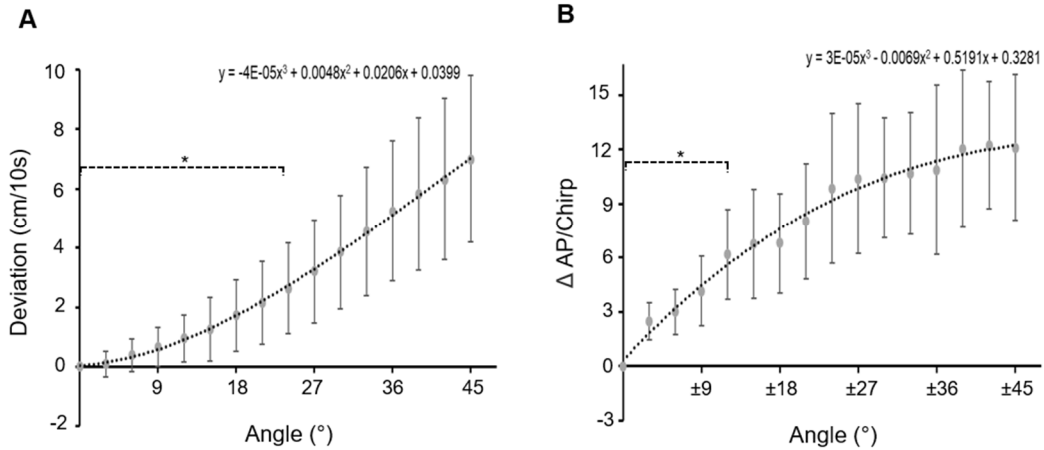
836

837

838

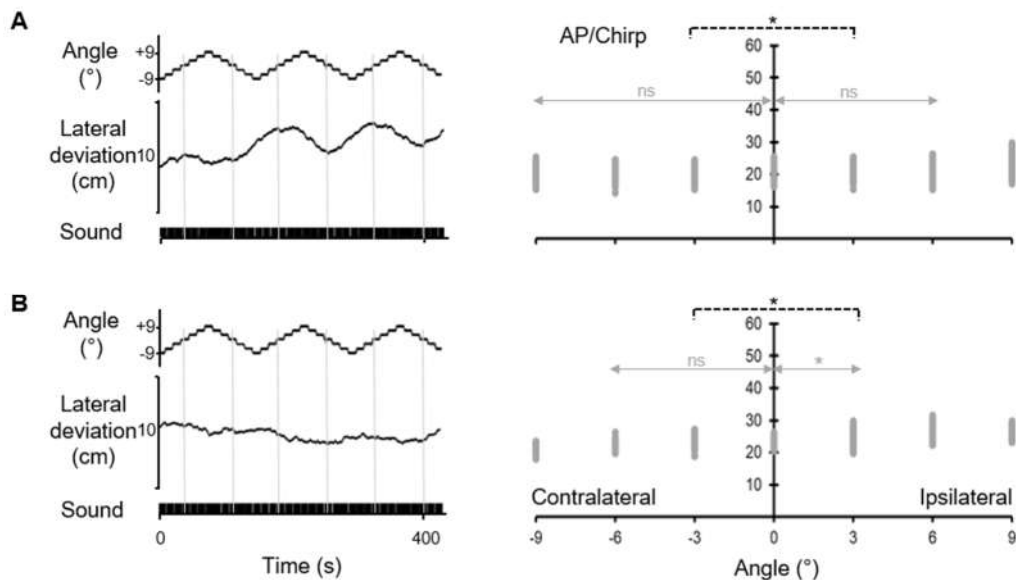
839

840



841
 842 Fig. 8. The mean lateral deviation for bilateral corresponding angles of very good
 843 steering animals (N=6) (A) and the mean difference of the AP/Chirp for responses to
 844 bilaterally corresponding angles of AN1 responses with steep changes (N=7) (B).
 845 Dotted line with brackets indicates the smallest angle with a significant difference when
 846 compared to the steering response at 0°. Values are means ± SD.

847
 848
 849
 850
 851



852
 853
 854 Fig. 9. Phonotactic steering and AN1 activity successively recorded in the same animals.
 855 Females were repeatedly tested over an angular range from -9° to +9°. A: Steering (left)

856 and AN1 activity (*right*) of a female cricket showing very good steering. *B*: Results of
857 an animal that did not show a clear phonotactic response but revealed a similar AN1
858 activity as the previous example. Solid thin lines with arrowheads indicate the range of
859 angles with no significant difference in AP/Chirp when compared with each other.
860 Dotted lines with brackets indicate the smallest angles with a significant bilateral
861 difference. Vertical lines (left diagrams) indicate zero crossing of the speaker.

862

863