

1 **Adaptations in the echolocation behavior of fruit-eating bats when orienting under**
2 **challenging conditions.**

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22 Key words: bats, orientation behavior, active sensing, sensory acquisition

23

24 **Summary statement**

25 When echolocating under demanding conditions e.g. noisy, narrow space, or cluttered environments,
26 frugivorous bats adapt their call pattern by increasing the call rate within biosonar groups.

27 **Abstract**

28 For orientation, echolocating bats emit biosonar calls and use echoes arising from call reflections.
29 They often pattern their calls into groups which increases the rate of sensory feedback over time.
30 Insectivorous bats emit call groups at a higher rate when orienting in cluttered compared to uncluttered
31 environments. Frugivorous bats increase the rate of call group emission when they echolocate in noisy
32 environments. Here, calls emitted by conspecifics potentially interfere with the bat's biosonar signals
33 and complicate the echolocation behavior. To minimize the information loss followed by signal
34 interference, bats may profit from a temporally increased sensory acquisition rate, as it is the case for
35 the call groups. In frugivorous bats, it remains unclear if call group emission represents an exclusive
36 adaptation to avoid interference by signals from other bats or if it represents an adaptation that allows
37 to orient under demanding environmental conditions. Here, we compared the emission pattern of the
38 frugivorous bat *Carollia perspicillata* when the bats were flying in noisy versus silent, narrow versus
39 wide or cluttered versus non-cluttered corridors. According to our results, the bats emitted larger call
40 groups and they increased the call rate within the call groups when navigating in narrow, cluttered, or
41 noisy environments. Thus, call group emission represents an adaptive behavior when the bats orient in
42 complex environments.

43

44 **Keywords:** behavioral adaptation, sensory acquisition rate, bats, echolocation, active sensing, spatial
45 orientation

46 Introduction

47 For collision-free locomotion, animals constantly update the location of surrounding obstacles.
48 With increasing obstacle density, successful orientation becomes challenging and some animals
49 increase their sensory acquisition rate (Geva-Sagiv et al., 2015). Electrolocating and echolocating
50 animals utilize self-emitted signals and echoes for orientation (Geva-Sagiv et al., 2015; Hofmann et
51 al., 2013; Kössl et al., 2014; Moss and Surlykke, 2010; Nelson and MacIver, 2006; Neuweiler, 1990).
52 The amount of signals emitted within a certain period represent the sensory acquisition rate. This
53 makes the acquisition rate highly accessible and allows us to answer questions on how bats adapt their
54 sensory acquisition rate when orienting under different conditions. Many behavioral studies showed
55 that bats often pattern their echolocation calls in form of groups (Amichai et al., 2015; Brinklov et al.,
56 2011; Brinklov et al., 2009; Galambos and Griffin, 1942; Grinnell and Griffin, 1958; Kothari et al.,
57 2018a; Luo et al., 2015; Roverud and Grinnell, 1985a; Roverud and Grinnell, 1985b; Wheeler et al.,
58 2016; Wohlgemuth et al., 2016; Figure 1). Results from insectivorous bats led to the hypothesis that
59 the emission of call groups may represent an adaptation to orient in complex environments (Falk et al.,
60 2014; Fawcett et al., 2015; Kothari et al., 2014; Moss et al., 2006; Petrites et al., 2009; Sändig et al.,
61 2014; Surlykke et al., 2009). Frugivorous bats emit more and larger call groups when orienting in the
62 presence than in the absence of acoustic playbacks (Beetz et al., 2018; Luo et al., 2015). Acoustic
63 playbacks potentially interfere with the bat's echolocation system making echolocation highly
64 demanding. Thus, for frugivorous bats, it has been proposed that the call groups may represent an
65 adaptation to avoid signal interference (Beetz et al., 2018). However, it remains unknown if
66 frugivorous bats show similar adaptations when orienting in narrow-spaced or cluttered environments
67 as it has been shown for insectivorous bats. To clarify the role of call group emissions in frugivorous
68 bats, the present study characterizes the call emission pattern of *Carollia perspicillata*, when the bats
69 were flying in narrow versus wide or in cluttered versus non-cluttered corridors. We hypothesized that
70 if *C. perspicillata* shows similar adaptations as it was previously demonstrated with the playback
71 experiments (Beetz et al., 2018), then the adaptations are not exclusive to avoid acoustic interference,
72 but they rather assist echolocation under highly demanding conditions.

73 **Materials and methods**

74

75 **Animals**

76

77 Experiments were conducted in 45 bats of the species *Carollia perspicillata*. The bats were bred and
78 kept in a colony at the Institute for Cell Biology and Neuroscience (Goethe-University Frankfurt). The
79 experiments comply with all current German laws on animal experimentation and they are in
80 accordance with the Declaration of Helsinki. All experimental protocols were approved by the
81 Regierungspräsidium Darmstadt (experimental permit # FU-1126).

82

83 **Flight room**

84 The experiments were performed in a flight room (length: 4 m; width: 1.4 m; height: 2 m). A
85 wall, made out of foam, separated the room into two corridors. At the end of each corridor, a landing
86 platform (20 x 20 cm), made out of metal mesh, was positioned. Behind each metal mesh, one speaker
87 (Neo CD 1.0 Ribbon Tweeter; Fountek Electronics, China) and one ultrasound sensitive microphone
88 (Avisoft Bioacoustics, Germany) were installed. The emission of the echolocation calls was monitored
89 by the microphones which had a sensitivity of 50 mV/Pa and an input-referred self-noise level of 18 dB
90 SPL. Each microphone was connected to a sound acquisition system (one microphone to an
91 UltraSoundGate 116 Hm mobile recording interface and the second microphone to an UltraSoundGate
92 116 Hb mobile recording interface, + Recorder Software, Avisoft Bioacoustics, Germany) for sound
93 digitalization at 333 kHz (16-bit precision). Bats were hand-released at one side of the flight room
94 (starting position in figure 2) and they could freely fly in the flight room. The flight behavior was
95 monitored with a webcam (500 SX, Manhattan, USA) placed above the starting point (frame rate = 30
96 Hz). The trial ended when the bat land on one of the two platforms. Since the ultrasound sensitive
97 microphones were directly behind the landing platforms, the bats directly approached one of the
98 microphones before ending a trial. This allows to record the patterns of the emitted echolocation calls
99 while the bat was approaching the platform.

100

101 **Experiment 1: Influence of high frequency playback on call emission pattern**

102

103 For comparative reasons, in the present report, data from a previously published manuscript (Beetz et
104 al., 2018) are used. The echolocation behavior from eight bats was tested in experiment 1. We
105 investigated the influence of acoustic playbacks containing high frequency echolocation calls on the
106 echolocation behavior by presenting the bats one noisy and one silent corridor (Figure 2A). The call
107 emission pattern emitted by the bat while flying in the noisy corridor (test trial) was compared with the
108 emission pattern as the bat flew under entirely silent conditions (training trial). The playback stimuli
109 represented repetitions of representative echolocation calls emitted by the tested bat during the training
110 trials. The played back echolocation calls were repeated in groups of five, ten, or twenty calls. The call
111 rate within the call groups was 66 Hz and the groups were repeated every 35 ms. Acoustic stimuli
112 were generated with a sampling rate of 384 kHz with an Exasound E18 sound card (ExaSound Audio
113 Design, Canada), and sent to an audio amplifier (Rotel power amplifier, RB-850, USA). The stimuli
114 were played with a sound pressure level of 80-90 dB re 20 μ Pascal (dB SPL). For analysis, a sequence
115 of two seconds from each trial was selected. Two seconds was usually the time window that the bats
116 needed to approach and land on the platform for each corridor. During the approach flight the
117 echolocation calls were intense enough to be easily detected by the microphone behind the platform.
118 This allows use to ensure that we did not miss any echolocation call emitted during this sequence and
119 that the recorded echolocation pattern represents the most “natural” one we could observe under these
120 paradigm settings. In total, 48 sequences, six (three training and three test trials) from each animal
121 were analyzed. Call emission pattern emitted during test and training trials were compared pairwise.
122 Thus, three pairs of “test” and “training” trials were compared for each animal.

123

124 **Experiment 2: Influence of corridor width on call emission pattern**

125

126 The influence of the corridor width on the echolocation behavior was tested in 21 bats. We modified
127 the flight room so that the bats could choose flying in a narrow (0.45 m) or in a wide (0.9 m) corridor
128 (Figure 2B). For each bat, we compared the echolocation behavior when flying in the narrow corridor

129 with the behavior elicited when flying in the wide corridor. Thus, two sequences of the recording, each
130 lasting 2 seconds, was selected for data analysis.

131

132 **Experiment 3: Influence of clutter on call emission pattern**

133

134 The influence of clutter on the echolocation behavior was tested in 16 bats. The “cluttered” corridor
135 was equipped with a cord that formed four diagonal loops hanging from the corridor’s ceiling (Figure
136 2C). The uncluttered corridor was free of cord. As in experiment 2, for each bat, a recording sequence
137 was compared when flying in the cluttered corridor with a sequence recorded while the animal was
138 flying in the uncluttered corridor. Each sequence lasted for 2 seconds.

139

140 **Analysis**

141

142 For data analysis, the call emission time points were manually tagged in the software Avisoft SAS Lab
143 Pro (Avisoft Bioacoustics, Germany). The rest of the analysis, except the statistical analysis, was done
144 in a custom written script in Matlab 2014 (MathWorks, USA). Call groups were defined according to
145 the criterions of (Kothari et al., 2014). A call group needs to be temporally isolated (“island
146 criterion”). A temporal isolation is fulfilled, when the preceding and following call interval of a call
147 group are 20% longer than the call intervals within a call group. The size of a call group, indicated by
148 the number of calls of the call group, is defined by the “stability criterion”. For the fulfillment of the
149 “stability criterion”, the call intervals within the call groups need to be invariant with 5% tolerance.
150 Note that doublets, i.e. call groups containing two calls, can only fulfill the “island criterion”. For
151 defining triplets, quartets, quintets, or sextets, both criteria need to be fulfilled.

152 For statistical analysis, we used the software GraphPad Prism 7 (GraphPad Software, USA; *
153 $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$). Since the echolocation behavior in two conditions (control
154 versus test trials) were compared to each other, statistical tests were either based on non-parametric
155 Wilcoxon signed-rank test (W; in case of non-Gaussian distribution) or on parametric paired t-Tests
156 (in case of Gaussian distribution).

157 **Results**

158

159 We simulated three different scenarios, where the bats had to orient under highly demanding
160 conditions (Figure 2). For the first experiment, we challenged the bats by presenting playbacks
161 consisting of echolocation calls while the bats had to fly and echolocate in a flight room (Figure 2A).
162 The playback stimulus represented a sequence of echolocation calls that was recorded initially from
163 the tested animal. Since the playback stimuli were presented only in one of the two corridors, the bats
164 could choose between a noisy or silent corridor (Figure 2A). The influence of acoustic playback on the
165 echolocation behavior was tested in eight bats. Note that the behavioral results from the playback
166 experiment have recently been published elsewhere (Beetz et al., 2018) and the results are described
167 here only to compare the echolocation behavior across different scenarios. Not only acoustic signals
168 which may interfere with the echolocation system make collision-free echolocation challenging but
169 also the corridor width may affect the echolocation pattern. Thus, in the second experiment, we
170 challenged the bats by narrowing (0.45 m) one and widening (0.9 m) the other corridor (Figure 2B).
171 Under these conditions, 21 one bats were tested. For the third experiment, 16 bats oriented in a flight
172 room that had a cluttered and a non-cluttered corridor (Figure 2C). Here, both corridors were equal in
173 size but both differed by the presence or absence of clutter, represented by cord hanging as loops from
174 the corridor's ceiling. For all experiments, the bats had only two landing positions, one platform at the
175 end of each corridor. Behind the platforms, ultrasound sensitive microphones recorded the bats' call
176 emission patterns. Representative echolocation sequences for each paradigm are presented in figure 3.
177 As it can be noted in figure 3, the bats grouped their echolocation calls while flying in the flight room.
178 This can be seen by looking at the time points of call emission, indicated as black dots on top of each
179 sequence. The call group size and the call rate within the call groups, indicated by a reduced inter-call
180 time interval, increased when the bats oriented in the more challenging corridor. This adaptation
181 occurred irrespective of the nature of the challenge, i.e. during presenting acoustic playbacks (Figure
182 3A), narrowing the corridor (Figure 3B), or enriching the corridor with clutter (Figure 3C). The
183 tendency of grouping the calls was always higher in the challenging (noisy, Figure 3A; narrow, Figure

184 3B; cluttered, Figure 3C) than in the non-challenging corridor (silent, Figure 3A; wide, Figure 3B;
185 non-cluttered corridor, Figure 3C).

186 In the challenging corridor, the bats significantly reduced the minimum call-interval (Figure
187 4A). Note that the amount of reduction in minimum call-interval was comparable for each of the three
188 experiments. This suggests that the reduction in minimum call-interval represents an adaptation to
189 echolocate under demanding conditions rather than representing an exclusive adaptation to avoid only
190 signal interference. The bats reduced the median call-interval only when orienting in the cluttered and
191 narrowed corridor (Figure 4B). Acoustic playbacks had no significant effect on the median call-
192 interval.

193 The relative amount of calls emitted as groups did not vary between the three experiments or
194 between the challenging and non-challenging condition within each experiment (Figure 5A). About
195 two thirds of the calls were emitted in form of call groups irrespective of the task or its complexity. In
196 the challenging corridor, the bats reduced the call-intervals within the call groups resulting into a
197 higher call rate within the call groups (Figure 5B). The extent of call rate increase within the call
198 groups was comparable for each paradigm. This shows again that the call rate increase is not exclusive
199 to avoid jamming. By taking a closer look into the call group size, indicated by the amount of calls per
200 call group (two for doublet, three for triplet, four for quartet, five for quintet, and six for sextet), it
201 becomes clear that the bats emitted significantly more triplets in the noisy than in the silent corridor
202 (Figure 5C). In the cluttered and narrow corridors, the bats emitted significantly more quartets than in
203 the non-cluttered and wide corridors (Figure 5D and 5E). Despite the difference in call group size
204 across the three experiments, it is noteworthy, that the tendencies of emitting larger call groups in
205 challenging than in non-challenging corridors was present in each of the three paradigms.

206

207 **Discussion**

208

209 Animals often orient in habitats that are enriched with many obstacles. Under such conditions, rapid
210 and collision-free movements are quite challenging and some animals increase their sensory
211 acquisition rate (for review see: (Geva-Sagiv et al., 2015)). For example, animals probing their

212 surrounding through olfaction, increase the sniffing rate when exploring novel objects (Kepecs et al.,
213 2007; Welker, 1964; Wesson et al., 2008). Humans increase the sensory acquisition rate by reducing
214 the rate of eye blinks (Bentivoglio et al., 1997; Shin et al., 2015; Shultz et al., 2011). The present
215 results show that frugivorous bats of the species *C. perspicillata* adapt their sensory acquisition rate in
216 a context-dependent manner, when comparing between challenging and non-challenging conditions.
217 When flying in complex environments, e.g. narrow, cluttered, or noisy areas, the bats increase the
218 acquisition rate by reducing the minimum and median call intervals (Figure 4), by decreasing the call
219 intervals within the call groups (Figure 5) and by increasing the call group size (Figure 5). All
220 adaptations were similar independent from the nature of the complex environment. Thus, the described
221 adaptations may allow the bats to orient collision-free in complex habitats, as it has been suggested for
222 insectivorous bats (Falk et al., 2014; Fawcett et al., 2015; Kothari et al., 2014; Kothari et al., 2018a;
223 Moss et al., 2006; Petrites et al., 2009; Sändig et al., 2014; Surlykke et al., 2009).

224 Why do bats pattern echolocation calls into groups when orientation becomes demanding?
225 Why do they not simply increase their call rate without grouping the calls? Although there is no direct
226 evidence from *C. perspicillata* that allows to answer these questions, several scenarios seem possible.
227 i) The bats could use the pattern of the call groups to anticipate the correct echo pattern and to
228 associate the echoes to the corresponding calls (Kothari et al., 2018a; Wohlgemuth et al., 2016). For
229 example, a bat emitting a call quartet expects to perceive four echoes with a comparable time pattern
230 as the call quartet. ii) Attentional phenomena often correlate with oscillations of brain activity in the
231 gamma range (higher than 30 Hz; Gregoriou et al., 2009; Gunduz et al., 2011; Sridharan et al., 2011).
232 These oscillations can be imagined as alternating “up” and “down” states of brain activity where “up”
233 stands for high and “down” for low level of attention. The call rate within the call groups lies in the
234 range of 40-50 Hz which might improve stimulus processing by entraining neural activity in the
235 gamma range. Noteworthy, recent neurophysiological data from flying insectivorous bats
236 demonstrated, that the gamma power increases when the bats emit call groups (Kothari et al., 2018b).
237 iii) Another possibility is that each echolocation call of the call group may be spatially directed
238 towards different orientations. Data from the Egyptian fruit-bat *Rousettus aegyptiacus* (Yovel et al.,
239 2010; Yovel et al., 2011) demonstrate that the bats alternate the focus of their sonar beam from left to

240 right and vice versa. This allows a detailed sampling of the distance to surrounding edges and dynamic
241 flight adjustments to avoid sudden collisions. If other bat species (such as *C. perspicillata*) similarly
242 alternate their sonar beam direction is yet to be investigated.

243 Independent from the reason of patterning echolocation calls into call groups, the behavioral
244 adaptations described in the present study lead to an increased sensory acquisition rate. This allows the
245 bats to gather a detailed representation of the surrounding which might, in turn, help animals to avoid
246 obstacle collision while flying in complex environments.

247

248 **Author Contributions**

249 M.J.B. performed experiments. M.J.B. analyzed data. M.J.B. wrote manuscript. M.J.B., J.C.H and
250 M.K. conceived and directed the study. All authors discussed the results and commented on the
251 manuscript.

252

253 **Competing interests**

254 The authors declare no competing financial interests.

255

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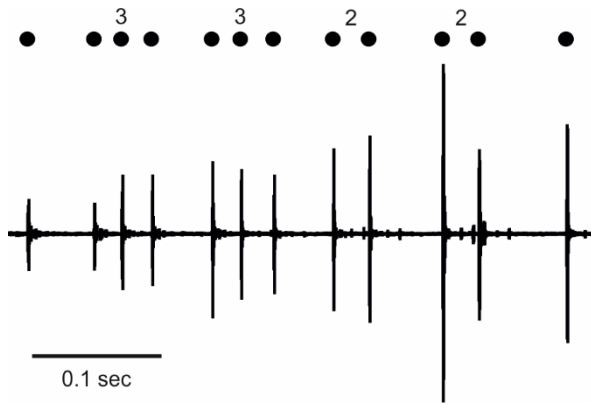
259 **References**

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- 261 **Amichai, E., Blumrosen, G. and Yovel, Y.** (2015). Calling louder and longer: how bats use
262 biosonar under severe acoustic interference from other bats. *Proceedings of the Royal Society B-*
263 *Biological Sciences* **282**.
- 264 **Beetz, M. J., Garcia-Rosales, F., Kössl, M. and Hechavarría, J. C.** (2018). Robustness of
265 cortical and subcortical processing in the presence of natural masking sounds. *Scientific Reports* **8**.
- 266 **Bentivoglio, A. R., Bressman, S. B., Cassetta, E., Carretta, D., Tonali, P. and Albanese, A.**
267 (1997). Analysis of blink rate patterns in normal subjects. *Movement Disorders* **12**, 1028-1034.
- 268 **Brinklov, S., Jakobsen, L., Ratcliffe, J. M., Kalko, E. K. V. and Surlykke, A.** (2011).
269 Echolocation call intensity and directionality in flying short-tailed fruit bats, *Carollia perspicillata*
270 (*Phyllostomidae*). *Journal of the Acoustical Society of America* **129**, 427-435.
- 271 **Brinklov, S., Kalko, E. K. V. and Surlykke, A.** (2009). Intense echolocation calls from two
272 'whispering' bats, *Artibeus jamaicensis* and *Macrophyllum macrophyllum* (*Phyllostomidae*). *Journal of*
273 *Experimental Biology* **212**, 11-20.
- 274 **Falk, B., Jakobsen, L., Surlykke, A. and Moss, C. F.** (2014). Bats coordinate sonar and flight
275 behavior as they forage in open and cluttered environments. *Journal of Experimental Biology* **217**,
276 4356-4364.
- 277 **Fawcett, K., Jacobs, D. S., Surlykke, A. and Ratcliffe, J. M.** (2015). Echolocation in the bat,
278 *Rhinolophus capensis*: the influence of clutter, conspecifics and prey on call design and intensity.
279 *Biology Open* **4**, 693-701.
- 280 **Galambos, R. and Griffin, D. R.** (1942). Obstacle avoidance by flying bats - The cries of bats.
281 *Journal of Experimental Zoology* **89**, 475-490.
- 282 **Geva-Sagiv, M., Las, L., Yovel, Y. and Ulanovsky, N.** (2015). Spatial cognition in bats and rats:
283 from sensory acquisition to multiscale maps and navigation. *Nat Rev Neurosci* **16**, 94-108.
- 284 **Gregoriou, G. G., Gotts, S. J., Zhou, H. H. and Desimone, R.** (2009). High-Frequency, Long-
285 Range Coupling Between Prefrontal and Visual Cortex During Attention. *Science* **324**, 1207-1210.
- 286 **Grinnell, A. D. and Griffin, D. R.** (1958). The Sensitivity of Echolocation in Bats. *Biological*
287 *Bulletin* **114**, 10-22.
- 288 **Gunduz, A., Brunner, P., Daitch, A., Leuthardt, E. C., Ritaccio, A. L., Pesaran, B. and Schalk,**
289 **G.** (2011). Neural correlates of visual-spatial attention in electrocorticographic signals in humans.
290 *Front Hum Neurosci* **5**, 89.
- 291 **Hofmann, V., Sanguinetti-Scheck, J. I., Kunzel, S., Geurten, B., Gomez-Sena, L. and**
292 **Engelmann, J.** (2013). Sensory flow shaped by active sensing: sensorimotor strategies in electric fish.
293 *Journal of Experimental Biology* **216**, 2487-2500.
- 294 **Kepecs, A., Uchida, N. and Mainen, Z. F.** (2007). Rapid and precise control of sniffing during
295 olfactory discrimination in rats. *Journal of Neurophysiology* **98**, 205-213.
- 296 **Kössl, M., Hechavarría, J. C., Voss, C., Macías, S., Mora, E. C. and Vater, M.** (2014). Neural
297 maps for target range in the auditory cortex of echolocating bats. *Current Opinion in Neurobiology*
298 **24**, 68-75.
- 299 **Kothari, N. B., Wohlgemuth, M. J., Hulgard, K., Surlykke, A. and Moss, C. F.** (2014). Timing
300 matters: sonar call groups facilitate target localization in bats. *Frontiers in Physiology* **5**.
- 301 **Kothari, N. B., Wohlgemuth, M. J. and Moss, C. F.** (2018a). Adaptive sonar call timing
302 supports target tracking in echolocating bats. *J Exp Biol* **221**.
- 303 **Kothari, N. B., Wohlgemuth, M. J. and Moss, C. F.** (2018b). Dynamic representation of 3D
304 auditory space in the midbrain of the free-flying echolocating bat. *Elife* **7**.
- 305 **Luo, J. H., Goerlitz, H. R., Brumm, H. and Wiegrebe, L.** (2015). Linking the sender to the
306 receiver: vocal adjustments by bats to maintain signal detection in noise. *Scientific Reports* **5**.
- 307 **Moss, C. F., Bohn, K., Gilkenson, H. and Surlykke, A.** (2006). Active listening for spatial
308 orientation in a complex auditory scene. *Plos Biology* **4**, 615-626.

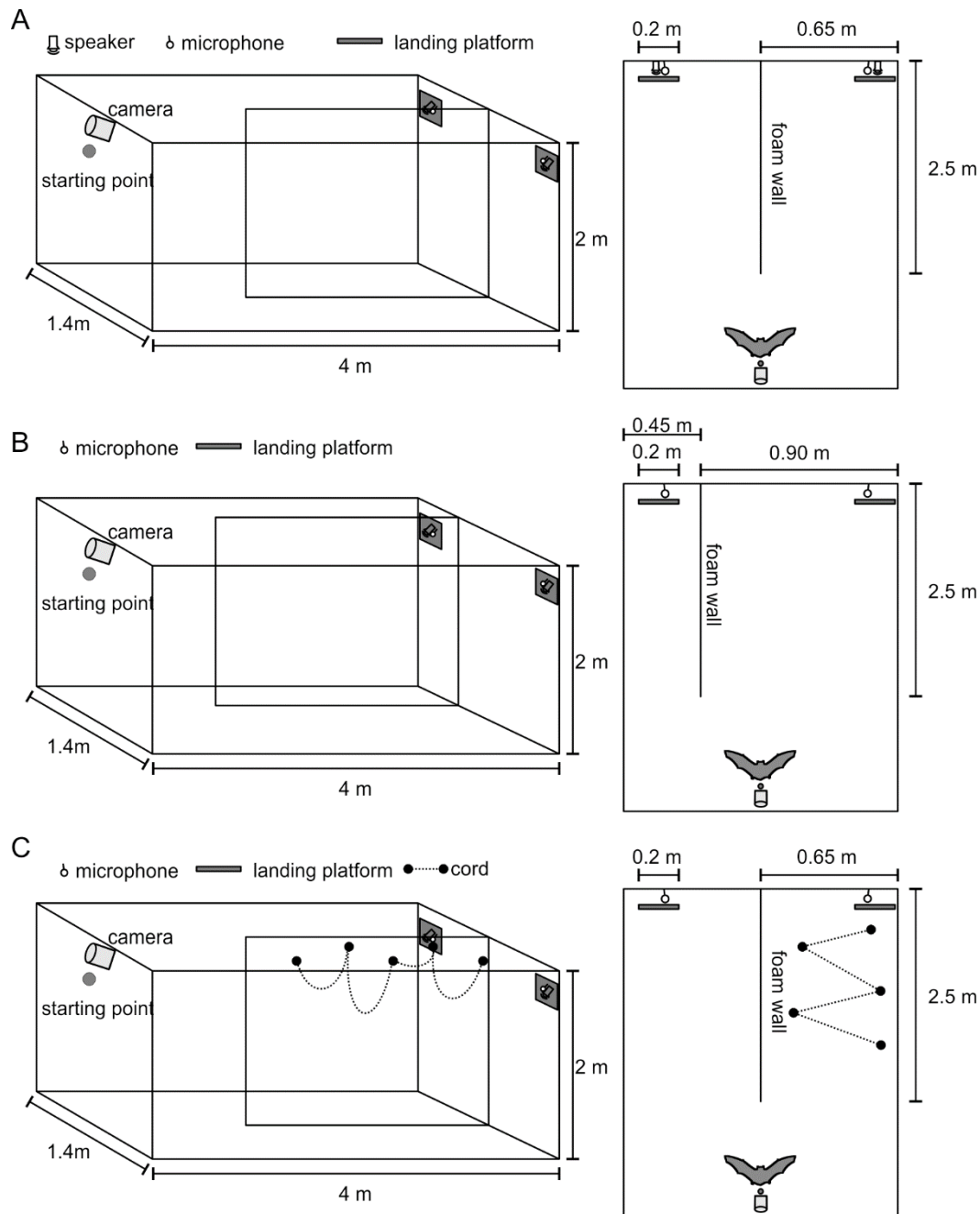
- 309 **Moss, C. F. and Surlykke, A.** (2010). Probing the natural scene by echolocation in bats.
310 *Frontiers in Behavioral Neuroscience* **4**.
- 311 **Nelson, M. E. and MacIver, M. A.** (2006). Sensory acquisition in active sensing systems. *J*
312 *Comp Physiol A* **192**, 573-586.
- 313 **Neuweiler, G.** (1990). Auditory Adaptations for Prey Capture in Echolocating Bats.
314 *Physiological Reviews* **70**, 615-641.
- 315 **Petrites, A. E., Eng, O. S., Mowlds, D. S., Simmons, J. A. and DeLong, C. M.** (2009). Interpulse
316 interval modulation by echolocating big brown bats (*Eptesicus fuscus*) in different densities of
317 obstacle clutter. *J Comp Physiol A* **195**, 603-617.
- 318 **Roverud, R. C. and Grinnell, A. D.** (1985a). Discrimination Performance and Echolocation
319 Signal Integration Requirements for Target Detection and Distance Determination in the CF/FM Bat,
320 *Noctilio-Albiventris*. *J Comp Physiol A* **156**, 447-456.
- 321 **Roverud, R. C. and Grinnell, A. D.** (1985b). Echolocation Sound Features Processed to
322 Provide Distance Information in the CF/FM Bat, *Noctilio-Albiventris* - Evidence for a Gated Time
323 Window Utilizing Both CF and FM Components. *J Comp Physiol A* **156**, 457-469.
- 324 **Sändig, S., Schnitzler, H. U. and Denzinger, A.** (2014). Echolocation behaviour of the big
325 brown bat (*Eptesicus fuscus*) in an obstacle avoidance task of increasing difficulty. *Journal of*
326 *Experimental Biology* **217**, 2876-2884.
- 327 **Shin, Y. S., Chang, W. D., Park, J., Im, C. H., Lee, S. I., Kim, I. Y. and Jang, D. P.** (2015).
328 Correlation between Inter-Blink Interval and Episodic Encoding during Movie Watching. *Plos One* **10**.
- 329 **Shultz, S., Klin, A. and Jones, W.** (2011). Inhibition of eye blinking reveals subjective
330 perceptions of stimulus salience. *Proceedings of the National Academy of Sciences of the United*
331 *States of America* **108**, 21270-21275.
- 332 **Sridharan, D., Boahen, K. and Knudsen, E. I.** (2011). Space coding by gamma oscillations in
333 the barn owl optic tectum. *Journal of Neurophysiology* **105**, 2005-2017.
- 334 **Surlykke, A., Ghose, K. and Moss, C. F.** (2009). Acoustic scanning of natural scenes by
335 echolocation in the big brown bat, *Eptesicus fuscus*. *Journal of Experimental Biology* **212**, 1011-1020.
- 336 **Welker, W. I.** (1964). Analysis of Sniffing of the Albino Rat. *Behaviour* **22**, 223-244.
- 337 **Wesson, D. W., Donahou, T. N., Johnson, M. O. and Wachowiak, M.** (2008). Sniffing
338 behavior of mice during performance in odor-guided tasks. *Chemical Senses* **33**, 581-596.
- 339 **Wheeler, A. R., Fulton, K. A., Gaudette, J. E., Simmons, R. A., Matsuo, I. and Simmons, J. A.**
340 (2016). Echolocating Big Brown Bats, *Eptesicus fuscus*, Modulate Pulse Intervals to Overcome Range
341 Ambiguity in Cluttered Surroundings. *Frontiers in Behavioral Neuroscience* **10**.
- 342 **Wohlgemuth, M. J., Kothari, N. B. and Moss, C. F.** (2016). Action Enhances Acoustic Cues for
343 3-D Target Localization by Echolocating Bats. *Plos Biology* **14**.
- 344 **Yovel, Y., Falk, B., Moss, C. F. and Ulanovsky, N.** (2010). Optimal Localization by Pointing Off
345 Axis. *Science* **327**, 701-704.
- 346 **Yovel, Y., Geva-Sagiv, M. and Ulanovsky, N.** (2011). Click-based echolocation in bats: not so
347 primitive after all. *J Comp Physiol A* **197**, 515-530.

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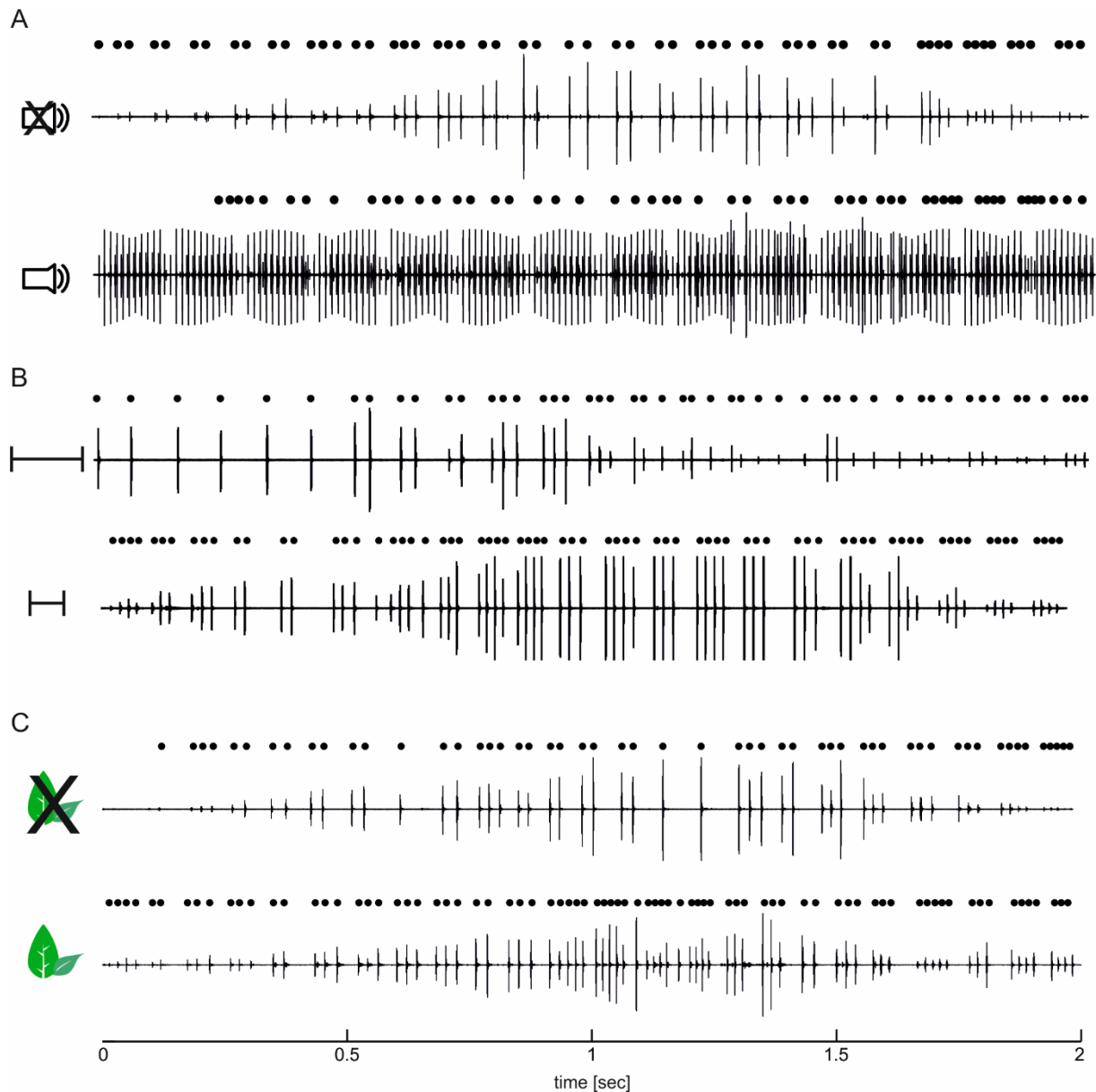
350 **Figure 1: Echolocation sequence of *Carollia perspicillata* showing echolocation calls that are**
351 **patterned into call groups.** The oscillogram shows twelve echolocation calls of *C. perspicillata*.
352 Black dots on top of the oscillogram signal the time points of call emission. The numbers on top of the
353 dots represent the call group size (3 = triplet, 2 = doublet).



354

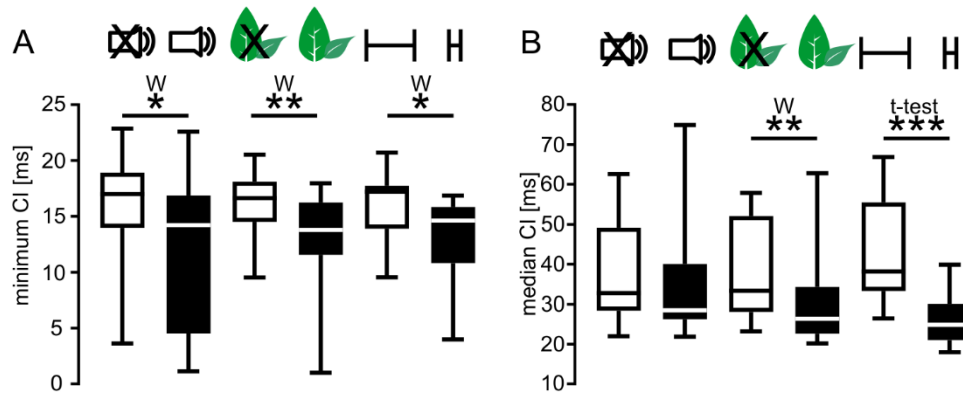
355 **Figure 2: Schematic lateral and top views of the experimental designs for the three experiments.**

356 The experiments were conducted in a 1.4 m x 4 m x 2 m flight room. A moveable wall made of foam
357 separated about two third of the room into two corridors. At the end of each corridor, the bats could
358 land on one landing platform made of metal mesh. The bats were hand released at the starting point
359 and they could freely fly in the flight room. A camera on top of the starting point and two ultrasound
360 sensitive microphones, one positioned behind each landing platform could record the echolocation
361 behavior of the animal. (A) In the first experiment, the speaker behind one of the landing platforms
362 produced an acoustic playback making one corridor noisy (challenging corridor). The second corridor
363 was silent (non-challenging corridor). (B) In the second experiment, the bats could either fly in a
364 narrow (challenging) or in a wide (non-challenging) corridor. (C) In the third experiment, the bats
365 could either fly in a cluttered (challenging) or in a non-cluttered (non-challenging) corridor. Clutter
366 was represented, as loops of cord hanging from the ceiling of the flight room.



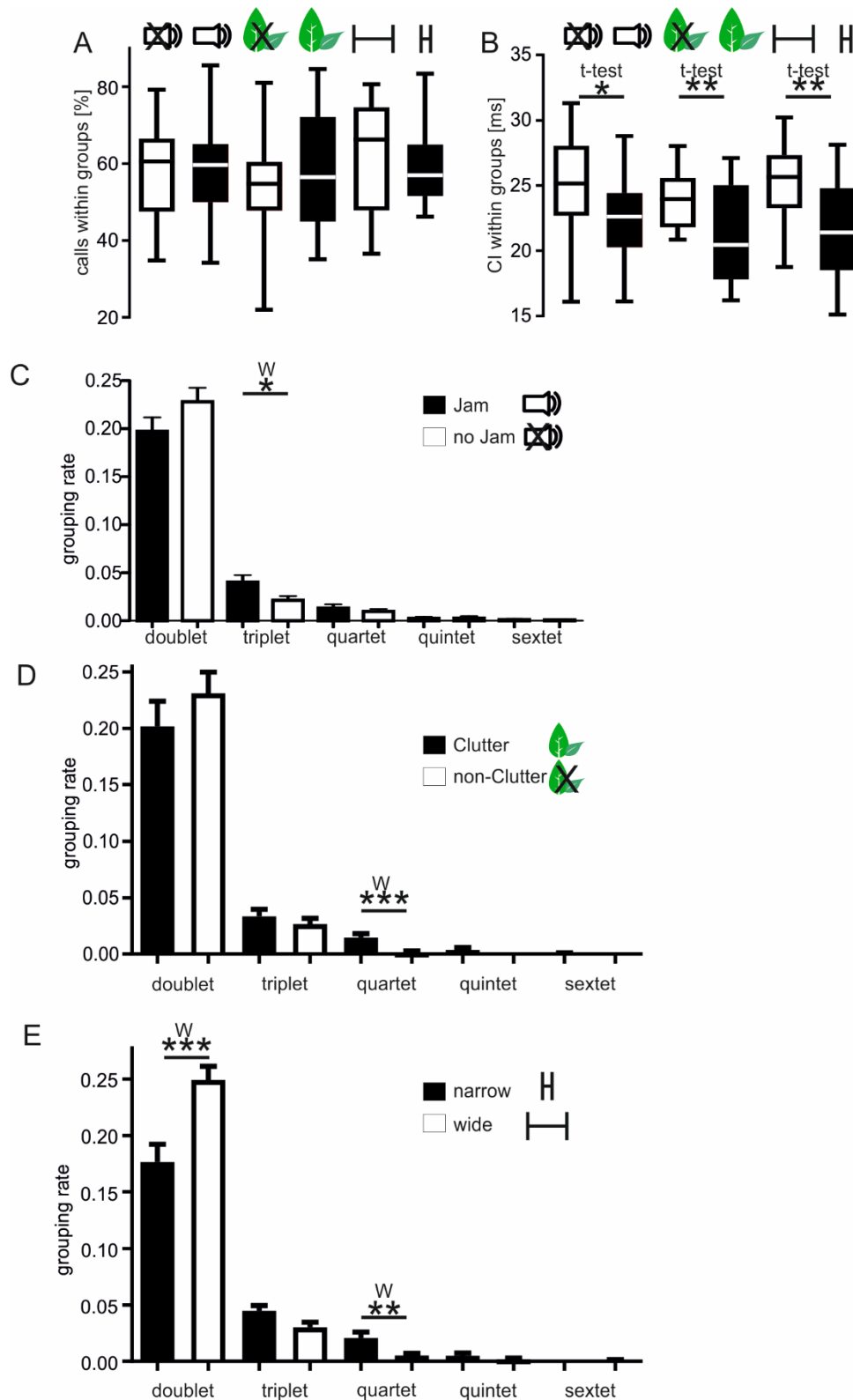
367

368 **Figure 3: Representative echolocation sequences emitted in each corridor for each of the three**
369 **experiments.** Representative oscillograms visualizing the call emission pattern in a silent (upper graph
370 in **A**), noisy (lower graph **A**), wide (upper graph **B**), narrow (lower graph **B**), uncluttered (upper graph
371 **C**), and cluttered corridor (lower graph **C**). The time points of call emission are indicated as dots
372 above each oscillogram. Note that the acoustic playback can also be seen as deflections in the
373 oscillogram in (**A**). Under challenging conditions (lower graphs), the bats emitted more, larger, and
374 more tightly packed call groups than under non-challenging conditions (upper graphs).



375

376 **Figure 4: Boxplots showing the minimum and median call interval (CI) for each experimental**
377 **condition.** The bats decreased the minimum CI, under challenging conditions (black boxplots in A).
378 They decreased the median CI when navigating in cluttered and narrow corridors as (black boxplots in
379 B) as compared to non-cluttered and wide corridors (white boxplots in B). The presence of acoustic
380 playback does not result into a significant decrease of the median CI as compared to the silent
381 conditions. W = Wilcoxon signed rank test; t-Test = paired t-Test. * $p < 0.05$; ** $p < 0.01$; *** $p <$
382 0.0001



383

384 **Figure 5: Parameters of the call groups.** (A) When comparing the different experimental conditions,
 385 the bats did not change the relative amount of calls that were emitted as call groups. (B) When
 386 navigating under challenging conditions (noisy, narrow, or cluttered), the bats decrease the call
 387 intervals within the call groups as compared to the call intervals shown in less demanding conditions
 388 (silent, wide, or non-cluttered). (C-E) Histograms showing the relative amount of doublets, triplets,
 389 quartets, quintets, and sextets. (C) The bats emitted relatively more triplets when navigating in the
 390 noisy than in the silent corridor. (D) The bats emitted relatively more quartets when navigating in the

391 cluttered than in the uncluttered corridor. (E) The bats emitted relatively more quartets and less
392 doublets when navigating in the narrow than in the wide corridor. W = Wilcoxon signed rank test; t-
393 Test = paired t-Test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$

394