1   **Title: Bats dynamically change echolocation parameters in response to acoustic**

2   **playback**

3   **Running Title: Adaptations in response to acoustic playback**

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5   M. Jerome Beetz (*)[1,2], Manfred Kössl[1], Julio C. Hechavarría[1]

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9   **Affiliations**

10   [1]Institut für Zellbiologie und Neurowissenschaft, Goethe-Universität, Frankfurt/M., Germany

11   [2] current address: Zoology II Emmy-Noether Animal Navigation Group, Biocenter, University of

12   Würzburg, Germany

13

14   * Corresponding author

15   *Mailing address*:

16   M. Jerome Beetz

17   Email: jerome.beetz@uni-wuerzburg.de

18   Zoology II, University of Wuerzburg, Am Hubland, 97074, Wuerzburg, Germany, Tel.: +49

19   9313184528

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21   **Keywords** *echolocation, active sensing, bioacoustics, signal interference, spatial orientation,*

22   *Jamming Avoidance Response*

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25 **Summary statement**

26 The frugivorous bat *Carollia perspicillata* dynamically switch between different adaptations when

27 echolocating in acoustically contaminated environments.

28 **Abstract**

29   Animals extract behaviorally relevant signals from "noisy" environments. To investigate signal

30 extraction, echolocating provides a rich system testbed. For orientation, bats broadcast calls and assign

31 each echo to the corresponding call. When orienting in acoustically enriched environments or when

32 approaching targets, bats change their spectro-temporal call design. Thus, to assess call adjustments

33 that are exclusively meant to facilitate signal extraction in "noisy" environments, it is necessary to

34 control for distance-dependent call changes. By swinging bats in a pendulum, we tested the influence

35 of acoustic playback on the echolocation behavior of *Carollia perspicillata*. This paradigm evokes

36 reproducible orientation behavior and allows a precise definition of the influence of the acoustic

37 context. Our results show that bats dynamically switch between different adaptations to cope with

38 sound-based navigation in acoustically contaminated environments. These dynamics of echolocation

39 behavior may explain the large variety of adaptations that have been reported in the bat literature.

40

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Introduction

42

43 For orientation, echolocating bats emit biosonar calls and listen to their echoes arising from reflections

44 of surrounding objects (Kössl et al., 2014; Moss and Surlykke, 2010; Simmons, 2012). Spectro-

45 temporal parameters of echoes inform the animals about the position and identity of objects nearby

46 (Wohlgemuth et al., 2016b). To gain spatial information, bats must assign incoming echoes to their

47 corresponding calls (Corcoran and Moss, 2017; Suga et al., 1983; Ulanovsky et al., 2004). Call-echo

48 assignments become challenging, however, when biosonar signals from many bats are overlapping

49 (Corcoran and Moss, 2017; Levin et al., 2013; Parsons et al., 2003; Ulanovsky and Moss, 2008).

50 Under these circumstances, bats demonstrate a large repertoire of behavioral adaptations that are

51 thought to represent behavioral strategies to improve signal extraction. These adaptations range from

52 spectro-temporal changes in call design, to changes in call emission patterns (Adams et al., 2017;

53 Amichai et al., 2015; Cvikel et al., 2015; Gillam and McCracken, 2007; Gillam et al., 2007;

54 Habersetzer, 1981; Hage et al., 2013; Hiryu et al., 2010; Ibanez et al., 2004; Jarvis et al., 2013; Luo et

55 al., 2015; Miller and Degn, 1981; Obrist, 1995; Ratcliffe et al., 2004; Roverud and Grinnell, 1985a;

56 Roverud and Grinnell, 1985b; Simmons et al., 1979; Simmons et al., 1978; Takahashi et al., 2014;

57 Tressler and Smotherman, 2009; Ulanovsky et al., 2004).

58 Our current understanding of why bats show such a large variety of adaptations when

59 echolocating under "noisy" conditions is sparse. For example, it remains unknown whether the

60 adaptations observed are individualistic and/or depend on the environmental context in which bats

61 vocalize (i.e., the distance between the bat and the nearest target).

62 In this study, we tested the hypothesis that individual bats rely on different combinations of

63 behavioral adaptations to overcome noise and that they can switch adaptation strategies at any

64 timepoint during echolocation. To test this hypothesis and to gain a clearer understanding of

65 echolocation behavior in "noisy" environments, individual bats of the species *Carollia perspicillata*

66 were attached on the mass of a swinging pendulum (Figure 1A). The pendulum offers a behavioral

67 paradigm whereby bats could actively echolocate in controlled scenarios, which could be replicated

68 over several trials (Beetz et al., 2016b; Beetz et al., 2017; Henson et al., 1982; Macias et al., 2016). In

69   our experiments, during forward swings – which mimicked a bat closing in on a target– the animals

70   were acoustically stimulated with patterned echolocation calls broadcast from a speaker, which

71   travelled with and pointing towards the animal (test trial). The call design and emission pattern of test

72   trials were then compared to those recorded during control trials in which bats were swung in the

73   absence of playback stimuli. During test trials, we examined whether bats would change different

74   echolocation parameters, including call duration, call level, call frequency composition, and call

75   emission pattern.

76

Materials and Methods

Animals

Experiments were conducted on 10 bats (5 females and 5 males) of the species *Carollia perspicillata*. The bats were bred and kept in a colony at the Institute for Cell Biology and Neuroscience (Goethe-University Frankfurt). The experiments complied with all current German laws on animal experimentation and in accordance with the Declaration of Helsinki. All experimental protocols were approved by the Regierungspräsidium Darmstadt (experimental permit # #FU-1126).

Pendulum paradigm and audio recordings

For controlling behavioral context, bats were positioned on the mass of a pendulum and were repetitively swung towards an acrylic glass wall (50 × 150 cm, Figure 1A) (Beetz et al., 2016b; Beetz et al., 2017; Henson et al., 1982; Macias et al., 2016). During the swing, the bats emitted echolocation sequences that were recorded, together with their echoes, by an ultrasound sensitive microphone (CM16/CMPA, Avisoft Bioacoustics, Germany). The microphone had a sensitivity of 50 mV/Pa and an input-referred self-noise level of 18 dB SPL, as reported by the manufacturer. The frequency response curve was flat (± 3 dB, as specified by the manufacturer) in the range from 30-130 kHz. The microphone travelled with the mass of the pendulum, which was medially positioned above the bat's head. The membrane of the microphone was adjusted as closely as possible to the bat's ears (~ 4 cm). The microphone was connected to a sound acquisition system (Ultra Sound Gate 116Hm mobile recording interface, + Recorder Software, Avisoft Bioacoustics, Germany). To test the influence of acoustic interference on echolocation behavior, bats were swung in the pendulum while they were acoustically stimulated with a playback stimulus (see below). We compared the echolocation behavior recorded in the absence of playback stimuli (control trials) with the one shown in the presence of playback (test trials). Our reasoning was that because the behavioral context was invariant during control and test trials, except for the occurrence of the playback stimulus, we could correlate adaptations in the echolocation behavior with the presence/absence of the playback.

Initially, the bats were tested in a control trial followed by test trials where an echolocation call recorded during the forward swing of the control trial was selected to construct an individual-specific

105   playback stimulus. The playback stimulus consisted of an echolocation call that was presented as

106   quartets with a call interval of 25 ms and the quartets were repeated with an inter-quartet interval

107   between 130 and 150 ms. The intensity of the playback stimulus was adjusted to rms values (of single

108   calls) between 80 and 90 dB SPL for all animals. We reasoned that using an echolocation call of the

109   tested animal, as a playback stimulus, would be the most effective way of achieving acoustic jamming.

110   The latter is supported by the fact that subtle inter-individual differences in call design could be

111   detected by the animals, which reduces signal interference (Yovel et al., 2009). During test trials, the

112   playback stimulus was presented from an ultrasound speaker (MK 103.1 Microtech Gefell

113   Microphone Capsule used as speaker) that was flat in the range from 5 to 120 kHz (mean level in

114   calibration curve 84 ± 3 dB SPL, the speaker's protection cap was replaced with a self-made cap to

115   prevent energy loss at high frequencies). The speaker was placed pointing towards the bat's head at a

116   distance of 20 cm. The short distance between speaker and animal and the relatively tight fixation of

117   the bat's head prevented situations in which the bat could reduce acoustic interference via motor

118   responses like head "waggling" (Wohlgemuth et al., 2016a). Thus, the bats had to rely mostly on

119   changes in call design or emission pattern to minimize signal interference. Eight out of ten bats were

120   tested on two consecutive days, but with different, day-specific, playback stimuli. The latter controlled

121   for changes of the call design that may occur across days might bias our analysis. An overview of the

122   call parameters used for constructing playback stimuli is shown in Table 1.

123

124   **Analyzed echolocation parameters**

125   Since the time pattern of the playback stimuli was kept constant, we could discriminate between

126   biosonar signals emitted by the bat and the playback stimuli. The call emissions were manually tagged

127   in the software Avisoft SAS Lab Pro (Avisoft Bioacoustics, Germany). To characterize the

128   echolocation calls, different call parameters were measured in Avisoft SAS Lab Pro. The present study

129   focused on call level, call duration, peak frequency at different call time points (start, end, maximum

130   amplitude, and mean), bandwidth 5 (BW5), BW10, and sweep rate (Figure 1B). Regarding the call

131   spectra, we considered the peak frequencies (frequencies with the maximum energy at particular time

132   points of the call or on average of a call), because peak frequencies were likely to be the most salient

6

133 spectral information of the echo that would suffer least from reflective attenuation. BW5 and BW10

134 represents frequency ranges at 5 and 10 dB below the mean peak frequency (Figure 1B). The sweep

135 rate was calculated by subtracting the initial peak frequency from the terminal peak frequency and by

136 dividing by the call duration.

137   The call emission pattern was characterized by measuring the call intervals and the tendency

138 of grouping the calls. Analysis of the call groups was conducted using custom-written scripts in

139 Matlab 2014 (MathWorks, USA). Call groups were defined according to two criteria (Beetz et al.,

140 2018; Kothari et al., 2014). An "island criterion" defined call groups that were isolated in time. An

141 isolation was fulfilled as soon as the preceding and following call intervals of a call group were 20%

142 longer than the call intervals within call groups. If the "island criterion" was fulfilled, a second

143 criterion, the so called "stability criterion", defined the size of the call groups indicated by the number

144 of calls belonging to a group. The stability criterion was fulfilled if the call intervals within call groups

145 were stable with a 5% tolerance. Next, we calculated a strobe index for each animal and each

146 condition (control and test trial). The strobe index represented the relative amount of calls that were

147 emitted as groups.

148

149 **Statistics**

150   For statistical analysis, we used the software GraphPad Prism 7 (GraphPad Software, USA; *

151 $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$; **** $p < 0.0001$). For analyzing distance-dependent changes of

152 the echolocation behavior in the pendulum, non-parametric Kruskal-Wallis tests and a Dunn`s

153 multiple comparison post hoc tests were computed. For analyzing individual specific call adaptations

154 in response to acoustic playback, control and test trials were directly compared from each animal by

155 performing non-parametric Mann Whitney (in case of non-Gaussian distribution according to

156 D´Agostino & Pearson normality test; alpha = 0.05) or parametric t-Tests (in case of Gaussian

157 distribution according to D´Agostino & Pearson normality test; alpha = 0.05). For a comparison of the

158 echolocation behavior between subsequent trials, non-parametric Kruskal-Wallis tests and Dunn`s

159 multiple comparison post hoc tests (in case of non-Gaussian distribution according to D´Agostino &

160 Pearson normality test; alpha = 0.05) or ordinary one-way ANOVA and Tukey´s multiple comparison

161   post hoc tests (in case of Gaussian distribution according to D´Agostino & Pearson normality test;

162   alpha = 0.05) were computed.

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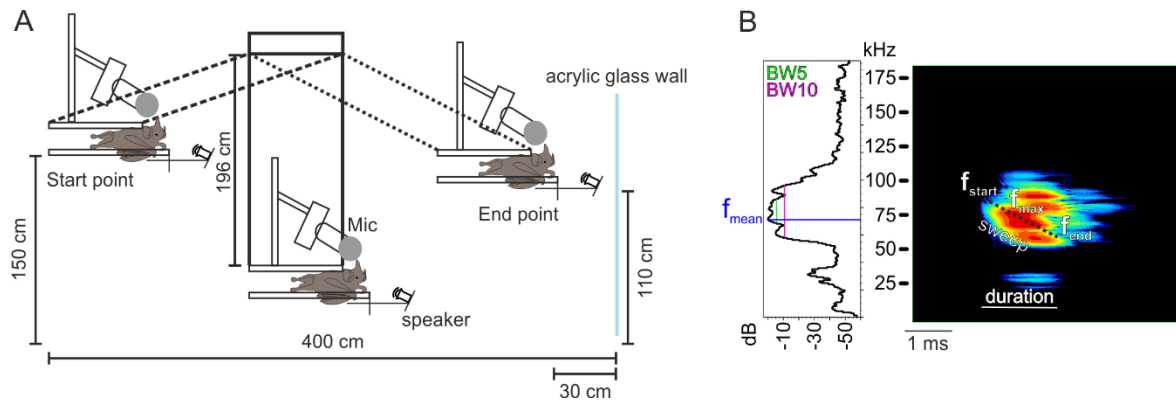
**Fig. 1 Behavioral paradigm and representative echolocation call**
(A) Schematic side view of the pendulum paradigm. The bat was positioned on the mass of a pendulum, which was swung towards an acrylic glass wall. During the swing, the bat emitted echolocation calls that were recorded together with the echoes by an ultrasound microphone (Mic). For test trials, the bat was stimulated with playback echolocation sequences that were composed of a previously recorded echolocation call of the tested bat. The playback stimuli were emitted with a speaker that was pointing towards the bat's head. Microphone and speaker were travelling with the bat and had a constant distance to the bat's head throughout the experiments. (B) Power spectrum (left) and spectrogram (right) of a representative echolocation call recorded with the pendulum paradigm. Different call parameters were measured to characterize spectro-temporal call properties. Spectral parameters that were measured included initial (f_{start}), centre (f_{centre}), terminal (f_{end}), mean (f_{mean}), maximum amplitude (f_{max}) peak frequency, and bandwidths at five (BW5) and ten dB (BW10) below the f_{mean}. Call duration represents one of the temporal echolocation parameters that was considered in the analysis. The sweep rate represents the difference of f_{end} and f_{start} ($f_{end} - f_{start}$) divided by the call duration.

Results

Pendulum paradigm mimics a natural approach flight

When swinging bats on the mass of a pendulum, they often emit echolocation calls (Beetz et al., 2016a; Henson et al., 1982; Macias et al., 2016). Thus, a pendulum paradigm allows to describe echolocation behavior under controlled conditions. This is important to test the influence of acoustic playbacks on echolocation behavior, independent from changes in the echolocation behavior due to target distances.

First, we quantified if pendulum-forward swings evoked consistent distance-dependent adjustments of the echolocation behavior in *C. perspicillata*. Based on 32 forward swings, each recorded from a different individual, we found that the bats shortened their call duration and inter-call intervals with decreasing target distance (Figure 2A-2B). In addition, with decreasing target distance, the bats increased their call intensity, starting peak frequency and peak frequency at the call's maximum energy (peak freq max; Figure 2C-2E). Since the distance-dependent adjustments in call duration and call interval are comparable in the pendulum (laboratory condition) as in freely-flying bats (Thies et al., 1998), we concluded that a forward swing in the pendulum mimics a bat zooming in on a target in natural conditions.

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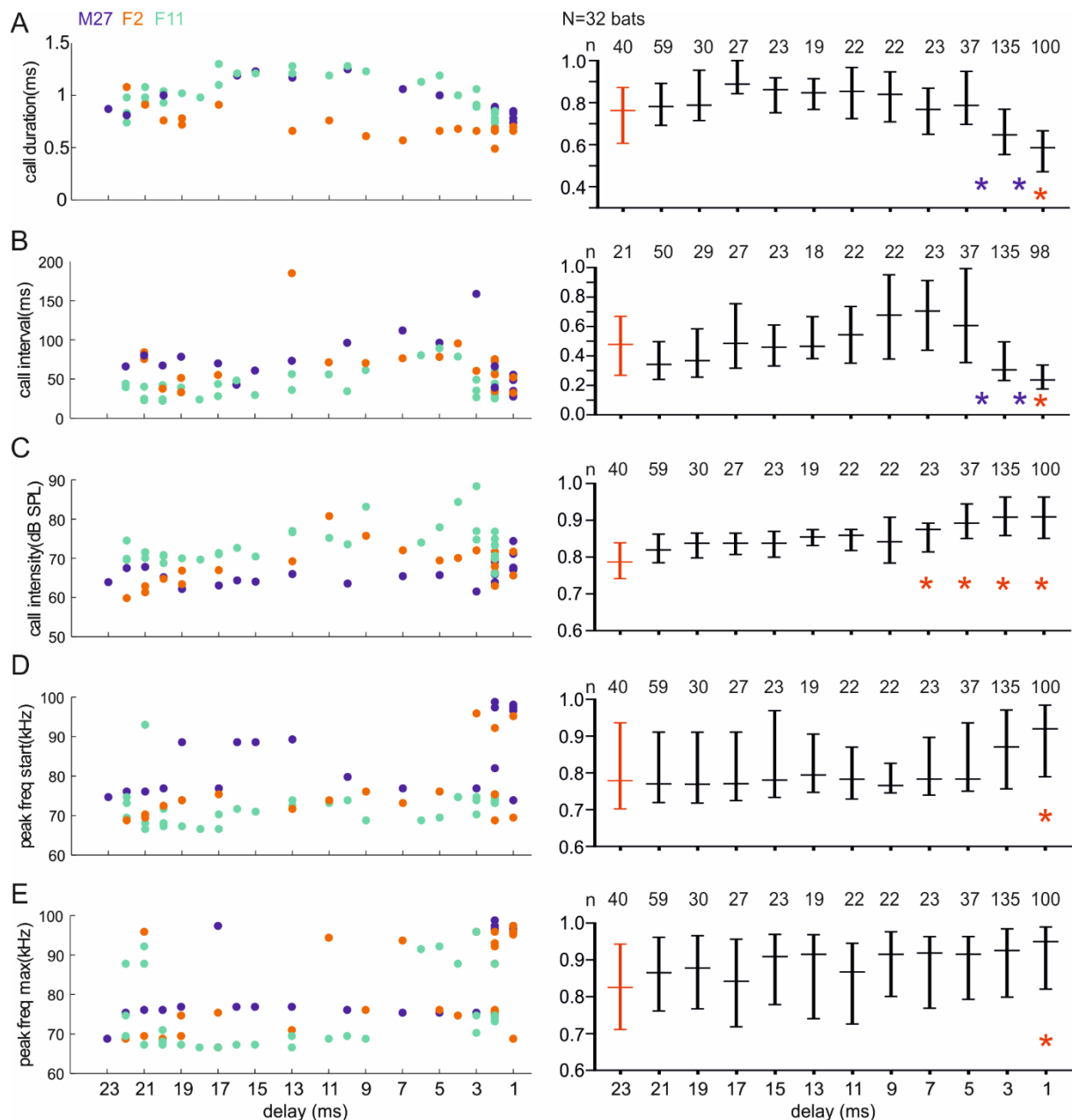
Fig. 2 Distance-dependent changes of echolocation parameters during pendulum-forward swings
C. perspicillata reduces call durations (**A**) and call-intervals (**B**) with shorter distances to an object. Call intensities (**C**), initial peak frequency (**D**), and maximum peak frequency (**E**) slightly increase with shorter distance to an object. Subfigures on the left represent examples from three animals (M27, F2, F11) and subfigures on the right represent the median and the interquartile range from data of 32 bats in which each trial was normalized to its maximum value. Blue stars indicate significant differences ($p < 0.05$) between subsequent echolocation calls. Red stars indicate significant differences ($p < 0.05$) with calls emitted at echo delays between 23-22 ms (red data point). Kruskal-Wallis test + Dunn`s multiple comparison post hoc test.

Next, we tested the inter-swing variability in the echolocation behavior of nine bats (five females, four males, Supplementary Table 1). Call duration, intensity, starting peak frequency, sweep rate, terminal peak frequency, peak frequency at call's maximum energy, mean peak frequency, bandwidth 5, and bandwidth 10 did not vary over subsequent swings (Supplementary Table 1; $p > 0.05$) indicating that one forward swing reliably represents the echolocation behavior of an individual

209   bat. Only four animals (female/F9, F10, F11, and male/M12) increased their call intervals across

210   subsequent trials (Mann-Whitney test for F9 and F10; Kruskal-Wallis test for F11 and M12; p <

211   0.005), which may indicate that the bats habituated to the pendulum and therefore decreased the call

212   rate.

213

214   **Individual bats change different call parameters in response to acoustic playback**

215   To quantify adaptations of the echolocation behavior in response to acoustic playback, bats were

216   swung in the pendulum while presenting an echolocation sequence (playback stimulus). The sequence

217   was presented through a speaker attached to the pendulum mass, pointing towards the animal's head

218   (Figure 1A, test trials). One echolocation call from each tested bat served as building block for the

219   playback stimulus (see methods for details). Thus, for each animal and experimental day, a new

220   "individualized" playback stimulus was constructed (for stimulus details see methods and Table 1). In

221   total, the echolocation behavior in the presence of playback stimuli was characterized in ten bats (5

222   females and 5 males). Echolocation behavior in the presence of playback was compared with the

223   behavior recorded during an initial control trial in which no acoustic stimulus was played back to the

224   animals. To minimize habituation to the pendulum paradigm, we decided to have only one control trial

225   per session (per animal and day). As previously described, call parameters from subsequent control

226   trials do not vary across swings (except for call intervals, Supple Table 1). Thus, one control trial is

227   enough to characterize the bat's echolocation behavior in the absence of playback stimuli. Since bats

228   adjust their call design and emission pattern with the target distance (Figure 2), we pooled the calls

229   into two groups, namely "long delay calls" and "short delay calls". Echolocation calls that were

230   broadcasted as the bat was farther than 1 m away from the acrylic glass wall were defined as "long

231   delay calls". Here, the echoes are delayed by more than 6 ms from the calls. Accordingly, echolocation

232   calls that were emitted when the bat was closer than 1 m from the acrylic glass wall were defined as

233   "short delay calls" (echo delays equal to or shorter than 6 ms).

234

235

236 Table 1 Call parameters of the playback stimuli. Each animal was stimulated with one of its own echolocation calls to ensure
237 a high probability of acoustic interference. Since some call parameters change across days within an individual, a new
238 jamming stimulus was generated each day. BW = bandwidth; p f = peak frequency

animal (day)	call duration [ms]	intensity rms [dB]	p f start [kHz]	p f end [kHz]	p f centre [kHz]	p f max [kHz]	p f mean [kHz]	BW5 [kHz]	BW10 [kHz]	sweep rate [kHz/ms]
f8 (1)	1,57	80,13	68,8	68,1	67,3	68,1	68,1	4,3	20,5	-0,446
f8 (2)	1,62	83,77	82	82	82,7	82	82	9,5	12,4	0,000
f9 (1)	1,94	87,02	61,5	82	82	82	82,7	27	30	10,567
f9 (2)	1,64	78,8	80,5	90,8	87,1	87,1	87,1	11,7	27,8	6,280
f10 (1)	1,85	85,86	68,1	85,6	82,7	86,4	85,6	16,8	28,5	9,459
f11 (1)	2,34	84,9	74,7	76,9	82	82	82	21,2	29,2	0,940
f11 (2)	2	78,13	69,5	79,8	85,6	86,4	86,4	7,3	13,1	5,150
f12 (1)	1,96	85,6	74,7	79,1	79,1	85,6	79,1	19	24,1	2,245
f12 (2)	2,15	80,25	80,5	82	82,7	87,1	82,7	10,2	27	0,698
m9 (1)	2,04	87,08	79,1	84,2	82,7	71	71	33,6	38,8	2,500
m9 (2)	2,3	83,95	78,3	82,7	82,7	82,7	82,7	5,1	22,7	1,913
m10 (1)	2,21	86,72	71	80,5	80,5	81,2	80,5	5,8	10,2	4,299
m10 (2)	1,62	82,25	67,3	84,9	82	87,1	82,7	9,5	32,9	10,864
m11 (1)	1,68	81,53	70,3	81,2	82	82	82,7	5,1	10,9	6,488
m12 (1)	1,4	83,57	79,1	66,6	61,5	79,1	67,3	23,4	24,9	-8,929
m12 (2)	1,53	77,23	79,8	89,3	87,8	87,8	87,8	4,3	27,8	6,209
m13 (1)	2,64	84,92	72,5	82	82,7	85,6	82,7	7,3	11,7	3,598
m13 (2)	2	76,35	67,3	81,2	80,5	82	80,5	10,9	33,6	6,950

239

240   In the presence of the playback stimulus, each individual bat demonstrated different

241 combinations of adaptations (Table 2 and Supple Table 2). Four bats (F11, F12, M9, M12) increased

242 the tendency of grouping their calls into call packs (exemplarily shown for F11 in Figure 3A; test trial,

243 see also population data in Figure 3E). Six bats (F8, F10, F11, M10, M12, and M13) varied their call

244 intervals. However, only a reduction of the call interval (observed in two bats, F11, M13) could be

245 interpreted as an adaptation in response to the playback stimulus. Increased call intervals may be

246 interpreted as habituation to the pendulum paradigm (see also Supple Table 1). Three bats (F8, M9,

247 M13) increased and another three bats decreased (F9, M11, M12) call intensity during the test trials

248 (Table 2; example in Figure 3B). Five bats changed their call duration, two shortened (F8, M11), two

249 lengthened (M9, M10) and one shortened their "short delay calls" and lengthened their "long delay

250 calls" (F9, Figure 3B; Table 2; Supple Table 2). The adaptation in call duration of F9 indicated that

12

251   some bats differentially adapt "long delay calls" and "short delay calls" in response to playbacks.

252   Changes in call spectra were sometimes prominent (Figure 3C and 3D) but also varied across animals

253   (Table 2; Supplement Table 2). Calls shown in figure 3C and 3D were recorded as the bat had

254   approximately the same distance to the target (~2 m). Seven out of eight bats that changed their calls'

255   sweep rate decreased it when in the presence of playback. This indicated that the call frequency

256   changed more slowly during the test compared to the control trials (Table 2; Supple Table 2). Changes

257   in the sweep rate could be caused by changes of the call's frequency range or by changes in call

258   duration. Because lowering the sweep rate was not associated with lengthening the call, the sweep rate

259   was mainly affected by changes in the frequency range. Seven animals (70%) changed either the BW5

260   or BW10 of the calls in the test trials. These changes could either be a BW decrease (shown by 40% of

261   the bats tested; F8, F10, M11, M13) or an increase (shown by 30%; F9, F12, M9). Detailed data from

262   three animals (A: F8; B: F9; C: M9) are plotted as boxplots in figure 4A-4C. For reasons of

263   visualization, only call parameters that differed between the test and the control trials are plotted. Data

264   from the remaining animals are presented in Supple figure 1. In conclusion, each animal adapted at

265   least one call parameter in response to the playback stimuli. M11 was the only individual that did not

266   change their call design (short delay calls) during the test trials. Overall, the bats changed different

267   combinations of their call parameters, indicating that there was no common rule as to how to adapt to
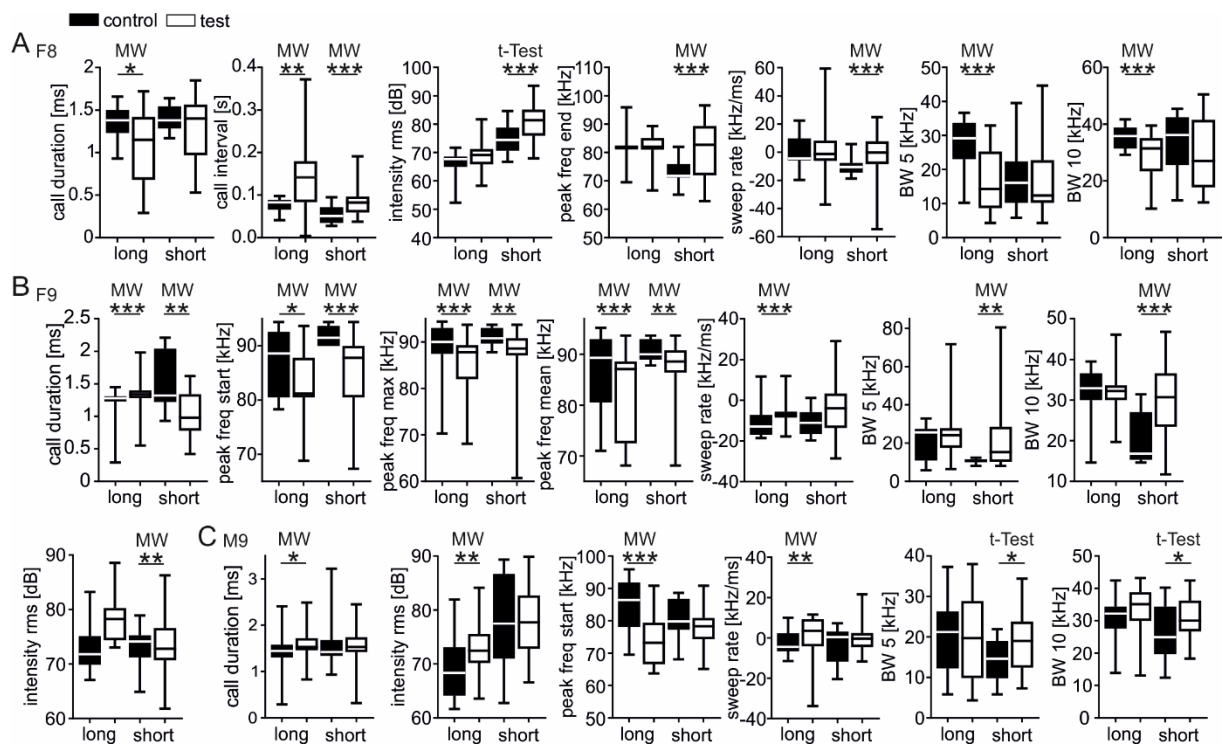
268   the playback stimuli.

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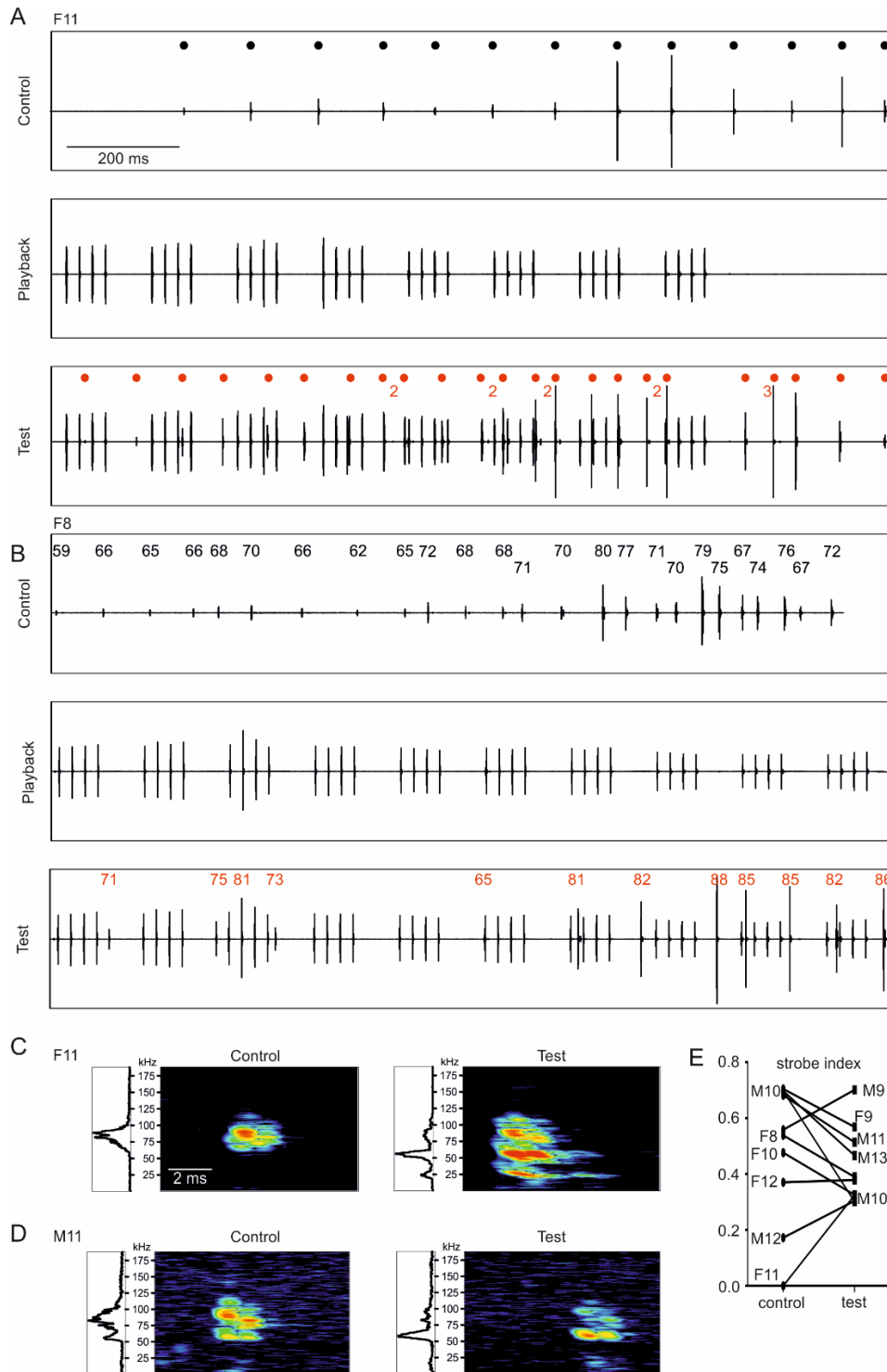
Fig. 3 Examples of echolocation adaptation behaviors in response to playback stimuli
(**A**) Oscillograms of one control trial (top), the playback stimulus (middle), and one test trial (bottom) from female 11 (F11). Time points of call emissions are indicated by black or red dots above each oscillogram. During the control trial, the bat did not emit echolocation call groups. During the test trial, the bat grouped some calls into doublets (indicated by the number 2) or triplets (indicated by a "3"). Note that the jamming stimulus was recorded in addition to the echolocation calls of the test trial. Thus, oscillogram deflections without a dot represent signals coming from the playback stimulus. (**B**) Oscillogram of one control trial (top), the playback stimulus (middle), and one test trial (bottom) from female 8 (F8). In comparison to the calls emitted during the control trials, the call intensity was increased during the test trials. Numbers above each emitted call indicate the call intensity. (**C-D**) Power spectra (left) and spectrograms (right) of representative calls emitted during the control and test trial for two individuals (F11, M11). To exclude distance-dependent changes in the call design, all four calls were recorded as the bat was ~2 meters away from the acrylic wall. Both bats decreased the bandwidth and mean peak frequency of their calls during the test trials as compared with the calls recorded during the control trials. (**E**) Tendency of emitting grouped calls (strobe index) under control and test conditions in all bats tested (n=10).

14

283 Table 2 Changes of the call parameters induced by the presence of playback stimuli. + = higher values for test than for control trials (+ = p <
284 0.05; ++ p < 0.01; +++ p < 0.001); - = lower values for test than for control trials (- = p < 0.05; -- p < 0.01; --- p < 0.001); F = female; M =
285 male; l = long delay calls; p f = peak frequency; s = short delay calls

Animal ID	F8		F9		F10		F11		F12		M9		M10		M11		M12		M13	
Delay	l	s	l	s	l	s	l	s	l	s	l	s	l	s	l	s	l	s	l	s
Interval	++	+++			+	++	--						++	+			+		--	
Duration	-		+++	---								+	+		--					
Intensity		+++		---								++				--	---	---		++
P F start			-	---					+			---								
Sweep rate		---	---		--	-		-	+			--	--				-			
P F end		+++			+								---	-	---		--			
P F max			---	--				-					--				++			
P F mean			---	--				--												
BW5	---			++								+								
BW10	---			+++	---				++			+			--				-	

286

Fig. 4 Individual specific call adaptations in response to playback stimuli

288 **Fig. 4 Individual specific call adaptations in response to playback stimuli**
289 (**A-C**) Boxplots (whiskers represent minimum and maximum values) from three individuals (female 8 = 1st row; female 9 =
290 2nd row; male 9 = 3rd row), showing call parameters that bats changed in response to the playback stimulus. Calls recorded
291 under control conditions (absence of playback stimulus) are indicated by black boxplots, while white boxplots represent calls
292 recorded under test conditions (presence of playback stimulus). Echolocation calls that are followed by an echo within 6 ms
293 were grouped into "short delay calls". Echoes following a call by more than 6 ms were grouped into "long delay calls". Note
294 that each bat changed their different call parameters under test conditions. MW = Mann-Whitney test; * p < 0.05; ** p <
295 0.005; *** p < 0.001.
296

Bats vary adaptation strategies across trials and days

298 We were interested in assessing if each individual bat prefers the same combination of adaptations or

299 whether the bats change their strategies across days or even across trials on the same day. However,

300 before characterizing the temporal dynamics of the adaptations, we quantified the variability of the call

301   design across subsequent days under controlled conditions (absence of playback stimulus). We tested

302   ten bats in the absence of playback stimuli for two (F1, M3, M7) or three (F2, F3, F5, F7, M1, M4,

303   M6) subsequent days (Supple Table 3). Although the bats did not vary their call design across

304   subsequent control trials on the same day (Supple Table 1), they dramatically varied their call design

305   across subsequent days (Supple Table 3). Thus, to test if bats change their adaptations in response to

306   the playback stimulus across days, we recorded an initial control trial on each day and compared the

307   echolocation behavior from the day-specific control trial with the one recorded during test trials.

308   Moreover, to perform a trial-by-trial analysis and to gather enough data points for statistical analysis,

309   we pooled data from long and short delay calls. During the test trials, bats emitted slightly fewer calls

310   than during control trials (median n of calls: 16.5 control and 13 test; Mann-Whitney test: $p = 0.036$).

311   By comparing the call parameters from F9 across days (Figure 5; Table 3), it became clear that the

312   adjustments of call duration (Figure 5C), starting (Figure 5B), maximum (Figure 5F), and mean peak

313   frequency (Figure 5H), bandwidth 5 (Figure 5G) and bandwidth 10 (Figure 5I) exclusively occurred

314   on day 1. On day 2, bat F9 mainly changed call intensity (Figure 5A), terminal peak frequency (Figure

315   5D), and sweep rate (Figure 5J). As already mentioned, increments in call interval (Figure 5E) did not

316   necessarily represent an adaptation to reduce acoustic interference; possibly, they represented

317   habituation to the pendulum paradigm across trials/days.

318   We observed that echolocation adaptation strategies not only varied across days, but also

319   across subsequent trials (Table 3). For example, F9 changed the calls' mean peak frequency in three

320   (trial 3, 4, 5) out of five trials at day 1 (Figure 5H). Changes of other call parameters varied less

321   dramatically across trials of the same day. In all trials on day 1, F9 decreased its starting (Figure 5B)

322   and maximum peak frequency (Figure 5F). For detailed data from the remaining nine animals see S2-

323   S10 figure. Overall, we found in 56 out of 67 test trials (83.6%) statistically significant differences

324   between the control and test trials (Table 3). In eleven test trials, the bats did not change any call

325   parameter compared to the control trial.
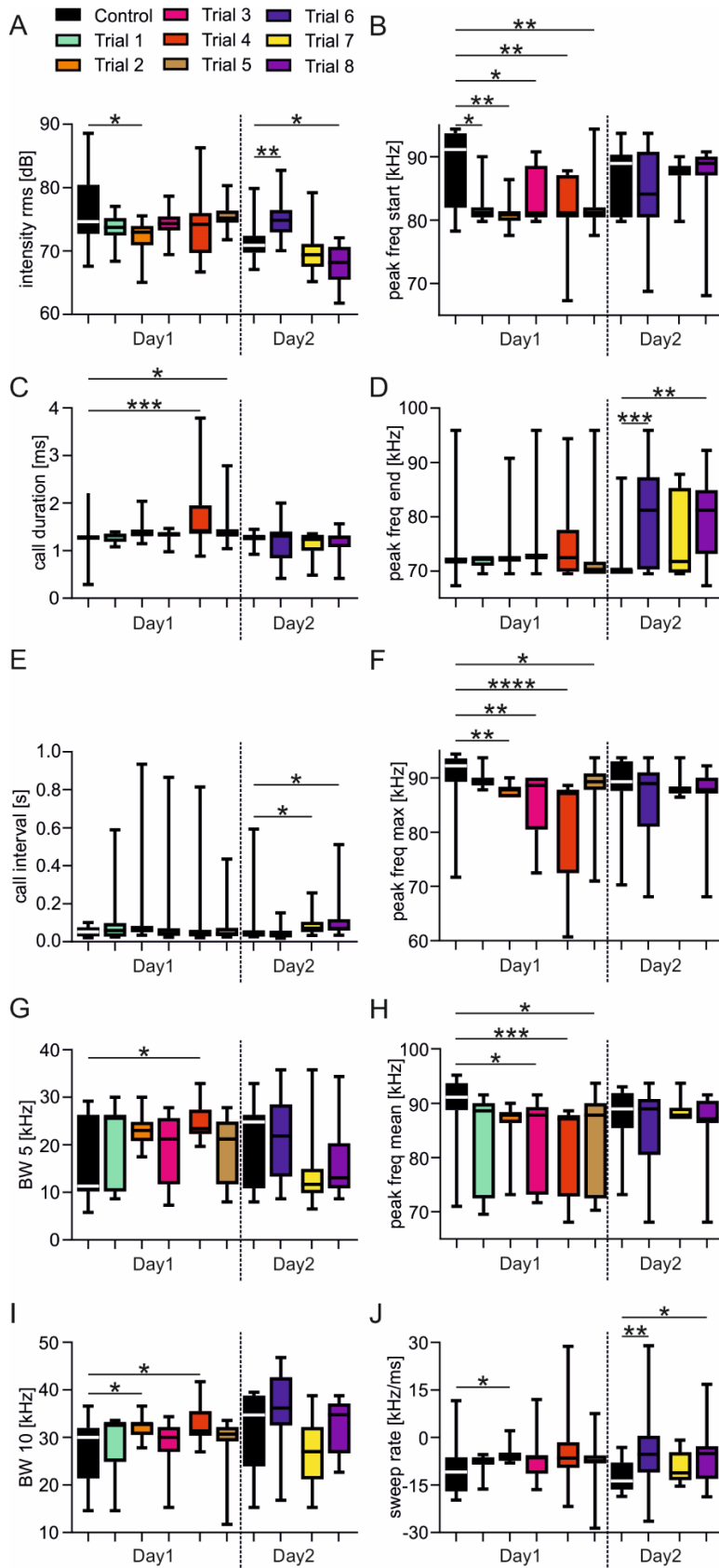
Fig.5 Bats switch adaptation strategies across trials and days

Call parameters are shown as boxplots (whiskers represent minimum and maximum values) for each trial (8 test trials and 2 control trials) across two days (from one bat). For visualization purposes, each trial is color coded and the control trials are shown in black. Note that the bat changes some call parameters only at day 1 (e.g., peak freq start; call duration; peak freq max) and not at day 2. Kruskal-Wallis Test and Dunn's multiple comparison Post hoc test; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$, **** $p < 0.0001$.

17

333 Table 3 Changes of the call parameters across trials. + = higher values for test than for control trials (+ = p < 0.05; ++ p < 0.01; +++ p <
334 0.001); - = lower values for test than for control trials (- = p < 0.05; -- p < 0.01; --- p < 0.001; ----p < 0.0001); F = female; M = male; l = long
335 delay calls; p f = peak frequency; s = short delay calls

Animal ID (calls/trial)	Trial (Day)	Intensity	Duration	Interval	Sweep rate	P F start	P F end	P F max	P F mean	BW5	BW10
F8 (25, 16, 14, 12, 16, 16, 13, 11)	1 (1st)	+	---	+++	-		++			--	---
	2 (1st)							+			
	3 (1st)	++		++						--	----
	4 (2nd)				-		+				
	5 (2nd)										
	6 (2nd)			+							
F9 (28, 15, 10, 15, 17, 23, 18, 34, 16, 16)	1 (1st)					-					
	2 (1st)	-			-	--		--			
	3 (1st)					-		--	-		
	4 (1st)		+++			--		----	---	+	+
	5 (1st)	+				--		-	-		
	6 (2nd)	++			--		+++				
	7 (2nd)			+							
	8 (2nd)	-		+	-		++				
F10 (22, 13, 10, 22, 16, 15, 16)	1 (1st)				--						
	2 (1st)		+		--			--			
	3 (1st)				-						
	4 (1st)				-			-			
	5 (1st)						+				
	6 (1st)			+							
F11 (13, 23, 7, 20, 8, 9, 16, 13, 11, 14, 15, 10, 9, 11)	1 (1st)	+		---				-			
	2 (1st)		-								
	3 (1st)			-			-	--	--		
	4 (1st)			-				-	-		
	5 (2nd)			-						++	
	6 (2nd)										
	7 (2nd)										
	8 (2nd)										
	9 (2nd)										
	10 (2nd)	--									
	11 (2nd)			--							
	12 (2nd)										
F12 (18, 14, 11, 11, 11, 24, 17, 16)	1 (1st)		+								
	2 (1st)			++	-						+
	3 (1st)					-					
	4 (2nd)			----	++++	++	--			++	++
	5 (2nd)			-	+		-			+	+
	6 (2nd)			-	+		-			+	++
M9 (23, 24, 15, 7, 9, 24, 23, 9, 7)	1 (1st)	++	++		-	----					
	2 (1st)			+		-					
	3 (1st)	++++								---	
	4 (1st)				--	----					
	5 (2nd)			--	-	--				+	++
	6 (2nd)									+	+
	7 (2nd)									+	
M10 (17, 7, 14, 18, 12, 12, 7, 7)	1 (1st)						-	--	-		
	2 (1st)						--	----		-	
	3 (2nd)						-				
	4 (2nd)	+++	+++	+							
	5 (2nd)		+	++							
	6 (2nd)		++	+	++	++	----				
M11 (22, 8, 12, 11, 10)	1 (1st)	--					---		--		
	2 (1st)						-				
	3 (1st)								-		
	4 (1st)	---	-								
M12 (20, 10, 7, 11, 13, 11)	1 (1st)						-				
	2 (1st)			++						--	-
	3 (2nd)										
	4 (2nd)				++						
M13 (15, 29, 11, 24, 20, 20, 14, 16, 12, 15)	1 (1st)	++		----	---	+++					
	2 (1st)										
	3 (1st)			-	---	++++					
	4 (2nd)				---	--				--	
	5 (2nd)			-	---	--					
	6 (2nd)										-

7 (2nd)		----	+			+++			-	
8 (2nd)				--	-	+			-	

336

337 Bats dynamically switch adaptation strategies within trials

338 What could have happened during test trials when we could not find an adaptation in the echolocation

339 behavior? For these trials, was the acoustic interference too weak to evoke adaptations? Alternatively,

340 might the bats have dynamically changed their adaptations during trials, so that the adaptation would

341 not be detectable when pooling calls from an entire swing? To assess the latter idea, we compared

342 parameters of each call from the test trial with the same parameters in the call used to construct the

343 playback stimulus. The upper color maps, in figure 6A and 6B, exemplarily show the relative

344 differences between call parameters and the playback parameters for two trials in two different bats

345 (M9 and F12). The calls are ordered along columns in which the leftmost column represents the call

346 with the longest echo-delay and the rightmost column represents the call with the shortest echo-delay.

347 Each line represents the relative difference of a call parameter with respect to a playback parameter.

348 This result was calculated by subtracting the playback parameters from the call parameters and by

349 normalizing the difference against its absolute maximal difference for the entire trial. The darker the

350 red and blue patches are, the more positive and negative were the call parameters in comparison to the

351 playback stimulus. Based on the trial in figure 6A, the bat initially emitted calls with lower starting

352 peak frequencies (peak start) and call intensities than the playback stimulus. At an echo delay of ~3 ms

353 (between the 12th and 13th call, white dashed line in Fig. 6A), the bat abruptly switched the strategy

354 and increased the maximum and mean peak frequency while decreasing the BW of subsequent calls.

355 To visualize abrupt changes better, we calculated the differences of the parameters of subsequent calls

356 and plotted the values in the bottom color maps shown in figure 6A and 6B. We defined an abrupt

357 change when the considered parameter varied by more than 50% between subsequent calls. For

358 example, according to figure 6A, the terminal (peak end), maximum peak frequency (peak max), and

359 sweep rate of call 13, are more than 50% higher than the ones of call 12. This outcome is indicated by

360 red cells at the corresponding column (white dashed line) in the lower color map of figure 6A.
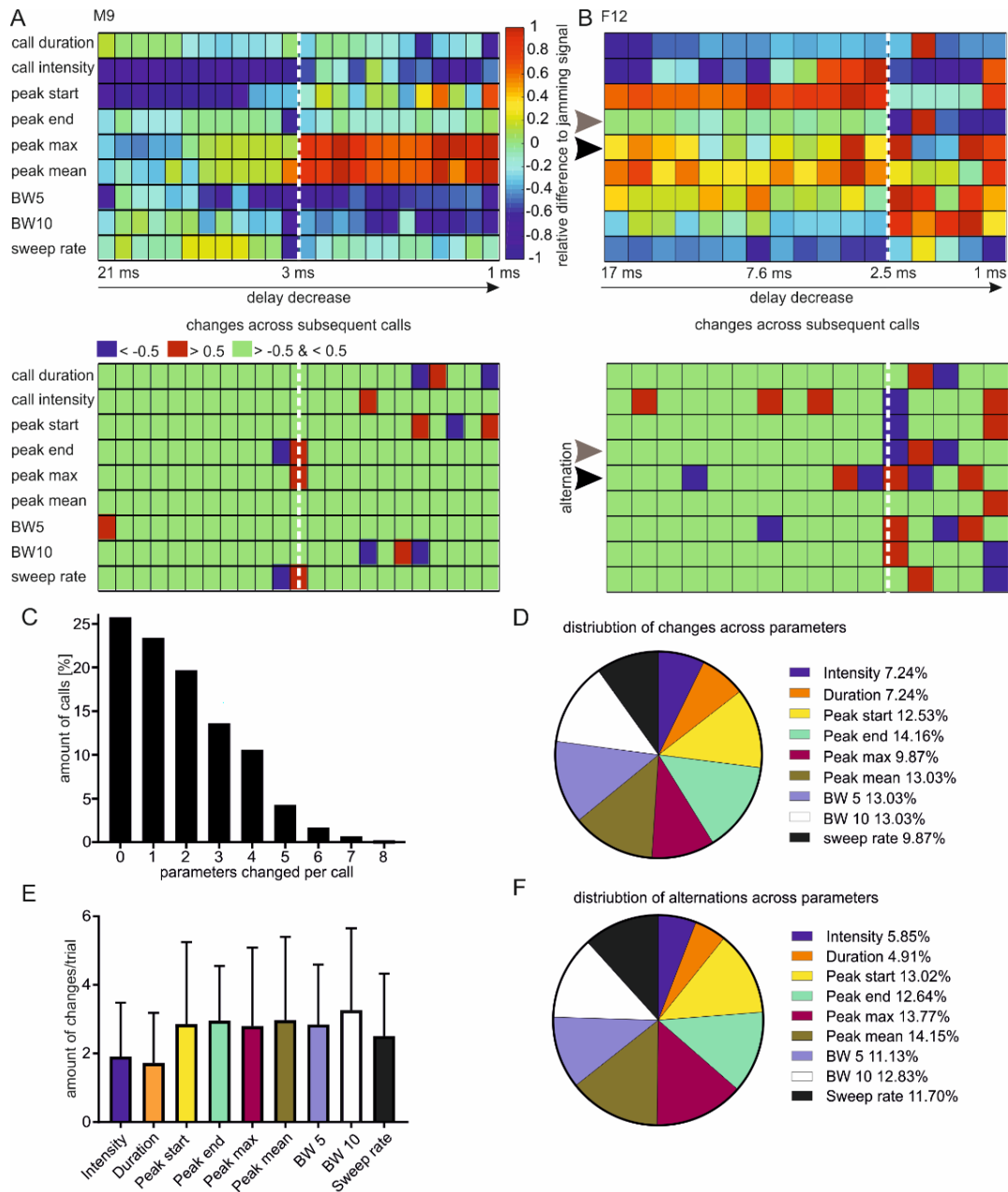
Fig.6 Bats dynamically change echolocation parameters within trials

(**A-B** upper graphs) Color maps from two representative test trials (M9 in (A) and F12 in (B)), illustrating the differences between calls and playback stimuli in a call-wise manner. Along the x-axis, the calls are ordered according to their emission order during the trial. The echo delay value from some call-echo pairs are indicated in the x-axis. Along the y-axis, normalized call parameter differences are color coded. The differences were normalized to their absolute maximum value at the corresponding parameter for the specific trial. The differences of the following call parameters were considered: call duration, call intensity, peak frequency at the beginning, end and maximum of the call, mean peak frequency of the call, bandwidth 5 (BW5), bandwidth 10 (BW10), and sweep rate. In some trials, a clear transition of the adaptation strategies can be detected (white vertical dashed lines). In some cases, the bats alternate call values, as exemplified for F12 for the terminal and maximum peak frequency indicated by a gray and black arrowhead, respectively. (**A-B** lower graphs) Colormaps illustrating abrupt changes of call parameters across subsequent calls. Abrupt changes occurred when a call parameter between two consecutive calls varied by more than 50% (blue and red cells represent reductions or increases in the corresponding call parameter). Changes of the call parameters that are below 50% were not abrupt enough to be defined as a change (green cells). Transitions between adaptation strategies and alternations between call parameter values can be seen more easily in the lower colormaps. (**C**) Histogram showing the level of parameters that are abruptly changed per call for all investigated calls (n = 889). Note that almost 75% of the calls show at least one abrupt change. (**D**) Pie chart illustrating the distribution of abrupt changes over the call parameters. Abrupt changes occur primarily within the call spectrum and less often for the intensity or duration. (**E**) Mean values of the amount of change per trial, plotted against the call parameter. Spectral parameters are shown to vary more often across trials than non-spectral ones (duration and intensity). (**F**) Pie chart representing the relative distribution of alternations across different call parameters.

20

382 Abrupt changes in call design were also visible in other trials, like the one presented in figure

383 6B. Here, abrupt changes mainly occurred at around 2.5-ms echo delay (white dashed line) by

384 decreasing the call intensity, starting (peak start), and terminal frequency (peak end) while the

385 maximum peak frequency (peak max) as well as the call bandwidths (BW5 and BW10) abruptly

386 increased. By considering all calls (889 calls from 69 trials and 10 animals), about three quarters of the

387 calls (74.24%) showed abrupt changes in at least one call parameter (Figure 6C). About half of the

388 calls (50.84%) showed abrupt changes in more than one call parameter. The bats did not focus on a

389 specific call parameter, but rather changed most of their parameters with equal probability (Figure

390 6D). Call intensity and call duration were least (7.24%) abruptly changed within the trials.

391 When taking a closer look on the pattern of call changes over subsequent calls (color maps at

392 the bottom of figure 6B), it became clear that the bats sometimes changed the call parameters in an

393 alternating manner. During the second half of the trial, the bat alternated between high and low

394 terminal (peak end) and maximum peak frequencies (peak max), indicated by gray and black

395 arrowheads, respectively. Before analyzing the alternations in more detail, we questioned how often

396 the bats changed a certain call parameter during the trial. The bar plot in figure 6E shows that the bats

397 changed spectral parameters more often per trial (mean of peak start $= 2.85 \pm 2.39$; mean of peak end

398 $= 2.96 \pm 1.59$; mean of peak max $= 2.8 \pm 2.29$; mean of BW5 $= 2.84 \pm 1.75$; mean of BW10 $= 3.26 \pm$

399 2.39; mean of sweep rate $= 2.51 \pm 1.82$) than the call intensity (mean $= 1.91 \pm 1.57$) and the call

400 duration (mean $= 1.73 \pm 1.46$) ($p < 10^{-5}$ Kruskal-Wallis test). Because spectral parameters varied more

401 often during the trials, alternations occurred with a higher probability in spectral than in non-spectral

402 (call intensity and call duration) parameters (Figure 6F). Across the spectral parameters, the

403 probability of alternations did not differ significantly ($p = 0.91$ Kruskal-Wallis test), indicating that

404 alternations could equally occur in each of the analyzed call parameters.

21

Discussion

The present study characterized adaptation strategies of echolocating bats (fruit-eating bat *C. perspicillata*) in the presence of playback stimuli. These playback stimuli potentially interfered with the bat's biosonar signals, making signal extraction for the bat challenging. In their natural environments, adjustments to echolocation behavior not only occur in the presence of an acoustic interferer, but also when bats approach obstacles or transition between different locales. Thus, it was crucial to test for the influence of acoustic interference under an otherwise invariant behavioral context. The pendulum paradigm, indeed, fulfilled these requirements because the behavioral scenario of an approach flight could be repetitively mimicked (Figure 2).

Our results demonstrate that *C. perspicillata* changes its call parameters and emission pattern when echolocating in the presence of playback stimuli. Interestingly, instead of relying on one adaptation strategy, the bats combined different adaptation strategies (Table 2 and Figure 4). To our surprise, the bats switched between different strategies across (Table 3, Figure 5) and even within trials (Figure 6). This flexibility renders the echolocation behavior, in the presence of acoustic interferers, highly dynamic and unique across different individuals and time points. Utilizing such dynamics, the bats can create unique echolocation streams that may be distinguished from interfering signals.

Coping with signal interference

Signal interference is a challenge with which every animal must cope; they must extract behaviorally relevant signals from a noisy background. The greater the similarity between relevant signals and background, the more challenging signal extraction becomes. To facilitate signal extraction, animals show large repertoires of different behavioral adaptations (Corcoran and Moss, 2017; Ulanovsky and Moss, 2008) like orienting their sensory organs towards relevant signals (Eckmeier et al., 2008; Ganguly and Kleinfeld, 2004; Land, 2015; Ribak et al., 2009; Schroeder et al., 2010; Tarsitano and Andrew, 1999; Towal and Hartmann, 2006). For example, bats increase head waggles and the inter-pinna distance when orienting under challenging conditions (Wohlgemuth et al., 2016a). Potentially, this response improves localization of the echo source (Wohlgemuth et al.,

433   2016a). Additionally, adjustments of the pinna's shape and orientation may increase the directionality

434   of hearing (Gao et al., 2011). In the present study, head waggles were limited by tightly positioning

435   the bats on the platform of the pendulum mass. Moreover, by placing the jamming source close to the

436   animals' head, motor responses would barely facilitate signal extraction under such conditions.

437   For some behaviors - like communication, electrolocation, or echolocation - animals produce

438   behaviorally relevant signals, which allow them to directly control the signal's discriminability from

439   its background. The latter becomes clear when considering the cocktail party problem (Bee and

440   Micheyl, 2008). In a noisy environment, we can focus on our communication partner by carefully

441   listening to him/her and improve the signal-to-noise ratio by increasing our voice intensity (Brumm

442   and Zollinger, 2011; Luo et al., 2015), an adaptation known as the Lombard-effect. Signal extraction

443   may not only be improved by increasing the signal-to-noise ratio but also by limiting the spectral

444   overlap between signal and background. This adaptation has originally been described in

445   electrolocating fish (Bullock et al., 1972; Watanabe and Takeda, 1963). When encountering animals

446   whose signal frequencies overlap with the fish's own signal frequency, the animals shift the signal

447   frequencies away from each other. This behavior has been circumscribed as the Jamming Avoidance

448   Response (JAR) and it reduces the signal interference with signals coming from conspecifics. JAR has

449   also been demonstrated in different bat species (Gillam and McCracken, 2007; Gillam et al., 2007;

450   Habersetzer, 1981; Hage et al., 2013; Ibanez et al., 2004; Miller and Degn, 1981; Ratcliffe et al., 2004;

451   Takahashi et al., 2014; Tressler and Smotherman, 2009; Ulanovsky et al., 2004) and the present study.

452   However, in contrast to weakly electric fish, which try to occupy an individual specific frequency

453   band, bats dynamically adjust their call spectra in various situations. Bats adjust their calls when

454   approaching an obstacle or when transiting between different habitats (Barchi et al., 2013; Falk et al.,

455   2014; Griffin, 1953; Hiryu et al., 2010; Kalko, 1995; Kalko and Schnitzler, 1989; Knowles et al.,

456   2015; Kothari et al., 2014; Petrites et al., 2009; Roverud and Grinnell, 1985a; Schnitzler et al., 1987;

457   Simmons et al., 1978; Surlykke and Moss, 2000; Wheeler et al., 2016). Since frequency adjustments

458   occur frequently and under various conditions, an adaptation that purely depends on a JAR may not be

459   sufficient to orient collision-free in the presence of signal interferers. This hypothesis gains support by

460   recent simulations that have tested for the effectiveness frequency adjustments when navigating in

461   noisy environments (Mazar and Yovel, 2019). Note that some studies reported that bats do not shift

462   their frequency in response to acoustic interference (Götze et al., 2016) or that the frequency shifts are

463   correlated with the object's distance rather with the presence of an acoustic interferer (Cvikel et al.,

464   2015). Because we compared echolocation calls that were emitted roughly at similar object-distances,

465   we can exclude that frequency shifts, present in our study, reflect distance-dependent changes of the

466   call design.

467

468   **Repertoire of behavioral adaptations in response to interfering signals and their possible neural**

469   **correlates**

470   In the present study, most biosonar adjustments occurred in the spectral domain, although others were

471   also detected in the temporal or energy domain (Figure 5D). Each adaptation may facilitate signal

472   extraction in noisy environments. For example, decreasing the call bandwidth may reduce the spectral

473   overlap between the playback stimulus and the biosonar signals. An increase in call bandwidth may

474   recruit neurons that are not sensitive to the playback stimulus and therefore "selectively" process

475   frequencies that are not occupied by the interferers. Bats also increase the signal-to-noise ratio by

476   increasing call intensity (Amichai et al., 2015; Hage et al., 2013; Luo et al., 2015; Simmons, 2017;

477   Simmons et al., 1978; Takahashi et al., 2014; Tressler and Smotherman, 2009). Unexpectedly, in the

478   present study, sometimes the bats decreased their call intensity when they echolocated in the presence

479   of interfering signals. Although this decreases the signal-to-noise ratio, it could still be useful from the

480   perspective of neuronal processing. Many auditory neurons are more sensitive to low rather than to

481   high sound levels (Barone et al., 1996; Hechavarría and Kössl, 2014; Park and Pollak, 1993; Suga and

482   Manabe, 1982; Yang et al., 1992). This attribute makes the neurons highly selective to faint biosonar

483   signals while being insensitive to intense background stimuli. Some studies have reported that bats

484   lengthen their calls when flying in noisy environments (Amichai et al., 2015; Simmons, 2017;

485   Simmons et al., 1979; Simmons et al., 1975; Tressler and Smotherman, 2009). In the present study, we

486   observed that some bats lengthened, and others shortened their calls. Both adaptations putatively

487   minimize acoustic interference. Shortening the calls decreases the chance of a temporal overlap

488   between biosonar signals and the background. Lengthening the calls increases the risk of temporal

24

489   overlap, but it could still be useful if only a small portion of the echo needed to be detected to gain

490   enough spatial information.

491   Furthermore, not only the call design, but also the emission pattern can be adjusted to reduce

492   or even avoid signal interference. Some bat species alternate between two call designs that differ in

493   their frequency spectrum (Obrist, 1995; Roverud and Grinnell, 1985a; Roverud and Grinnell, 1985b).

494   This adaptation allows a higher call rate by emitting a pair of calls before receiving an echo from the

495   first call of the pair (Behr and von Helversen, 2004; Jung et al., 2007). The arising echoes differ in

496   their frequency spectra which makes their discrimination feasible (Hiryu et al., 2010). Alternation of

497   spectral call parameters have also been observed in the present study (Figure 5B, 5F). However, these

498   alternations occurred occasionally and not throughout the entire trial. Thus, the behavioral importance

499   of alternating call parameters in *C. perspicillata* needs to be further assessed.

500   Some bats reduce their call rate (Adams et al., 2017) and temporally even cease to emit calls

501   (Jarvis et al., 2013). This adaptation may be beneficial if the bats eavesdrop on echolocation signals

502   from conspecifics and use the signals for orientation (Barclay, 1982; Chiu et al., 2008; Leonard and

503   Fenton, 1984; Lin and Abaid, 2015). Although, *C. perspicillata* emitted fewer calls during test

504   compared to control trials, the pendulum paradigm was not designed to test for eavesdropping on the

505   playback stimulus.

506   Lastly, some bats increase their rate of grouping calls when orienting in cluttered or noisy

507   environments ((Beetz et al., 2018; Beetz et al., 2019; Luo et al., 2015; Roverud and Grinnell, 1985a)

508   and present study). Grouping calls may improve echolocation performance in different ways. First, a

509   defined periodicity of echo arrivals allows echo identification based on prediction (Petrites et al.,

510   2009; Suga et al., 1983; Wheeler et al., 2016; Wohlgemuth et al., 2016a). Second, grouping the calls

511   could create an information redundancy allowing the bats to rely only on a small portion of the call

512   group (Beetz et al., 2018).

513

514   **Bats show different combinations of adaptations when echolocating in the presence of an**

515   **acoustic interferer**

516   Instead of adjusting just one echolocation parameter, when echolocating in noisy environments, our

517   results indicate that bats have a toolbox of different and combinable adaptations to minimize acoustic

518   interference (Hage et al., 2013; Luo et al., 2015). The dynamics and variability of such strategies are

519   important factors for explaining the high diversity of behavioral adaptations reported in earlier studies.

520   We must keep in mind that the discriminability of a signal from the background is dictated by the

521   difference of the physical parameters between the signal and its background. Essentially, it is

522   unimportant which physical parameter is adjusted, just so the signal has its own physical identity and

523   provides a high level of discriminability from its background. For call-echo assignment, it has been

524   discussed that bats keep an "internal copy" of their broadcasted calls and compare that copy with

525   received echoes (Simmons, 2012). Neural activity occurring before biosonar production in frontal and

526   striatal brain regions could contribute to the formation of such "internal copy" (Weineck et al., 2020).

527   The idea of an "internal copy" is in line with behavioral results showing that correct call-echo

528   assignment is decreased when spectro-temporal properties of the echo are manipulated (Masters and

529   Jacobs, 1989; Masters and Raver, 1996; Masters and Raver, 2000) or when echoes are replaced by

530   noise bursts (Surlykke, 1992). Because of missing behavioral data in *C. perspicillata*, it remains

531   speculative to what extent the echolocation calls need to differ from the playback stimuli so that bats

532   can still extract the signals. When comparing different call parameters against the playback stimuli

533   used in the present study, it becomes clear that some echolocation calls can vastly differ from playback

534   stimuli (S11 Figure). Although having no detection thresholds from *C. perspicillata*, there are some

535   behavioral and electrophysiological results from other bat species that use similar call designs as *C.*

536   *perspicillata* (for example, *Eptesicus fuscus*: (Chiu et al., 2009; von Stebut and Schmidt, 2001);

537   *Tadarida brasilienisis*: (Bartsch and Schmidt, 1993), *Antrozous pallidus* (Fuzessery, 1994)). Based on

538   these studies, we speculate that *C. perspicillata* can extract signals that differ for one of the following

539   parameters by more than 10 dB in intensity, by at least 0.7 ms in duration, by more than 5 kHz in the

540   peak frequency, by more than 12 kHz in bandwidth, and by more than 6 kHz in the sweep rate from

541   playback stimuli. By considering these thresholds, *C. perspicillata* may be able to extract about 94%

542   of the calls from the playback stimuli. Only 5.96% of the calls did not reach our hypothetical detection

543   thresholds for any of the investigated call parameters. Note that the emission pattern could not be

544   considered for a call-by-call analysis. Thus, it is still probable that the remaining 5.96% of the calls'

545   echoes could be detected by anticipation of the echo pattern. This ability could be accomplished by

546   grouping the calls (Figure 3A, (Beetz et al., 2018; Beetz et al., 2019)). In the present study, four out of

547   ten bats increased the tendency of grouping the calls, which increases the stimulus rate (Figure 3E).

548   Neurons of the bat's auditory midbrain and cortex can likely extract relevant echolocation information

549   when the bats face such high call rates (Bartenstein et al., 2014; Beetz et al., 2018; Beetz et al., 2016a;

550   Beetz et al., 2016b; Beetz et al., 2017; Greiter and Firzlaff, 2017; Macias et al., 2018; Sanderson and

551   Simmons, 2005).

558

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