

1 Full title: Deviance detection in subthalamic neural population  
2 responses to natural stimuli in bats

3 Short title: Subthalamic deviance detection to natural stimuli in bats

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17 Acknowledgements: We would like to thank Dr. Mirjam Knörnschild for providing us with  
18 some of the vocalisations of *Carollia perspicillata* that were used in this study. The project  
19 was funded by the Deutsche Forschungsgemeinschaft (KO 987/14-1).

20

21

## 22 Abstract

23 Deviance detection describes an increase of neural response strength caused by a stimulus  
24 with a low probability of occurrence. This ubiquitous phenomenon has been reported for  
25 multiple species, from subthalamic areas to auditory cortex. While cortical deviance detection  
26 has been well characterised by a range of studies covering neural activity at population level  
27 (mismatch negativity, MMN) as well as at cellular level (stimulus-specific adaptation, SSA),  
28 subcortical deviance detection has been studied mainly on cellular level in the form of SSA.  
29 Here, we aim to bridge this gap by using noninvasively recorded auditory brainstem responses  
30 (ABRs) to investigate deviance detection at population level in the lower stations of the  
31 auditory system of a hearing specialist: the bat *Carollia perspicillata*. Our present approach  
32 uses behaviourally relevant vocalisation stimuli that are closer to the animals' natural  
33 soundscape than artificial stimuli used in previous studies that focussed on subcortical areas.  
34 We show that deviance detection in ABRs is significantly stronger for echolocation pulses  
35 than for social communication calls or artificial sounds, indicating that subthalamic deviance  
36 detection depends on the behavioural meaning of a stimulus. Additionally, complex physical  
37 sound features like frequency- and amplitude-modulation affected the strength of deviance  
38 detection in the ABR. In summary, our results suggest that at population level, the bat brain  
39 can detect different types of deviants already in the brainstem. This shows that subthalamic  
40 brain structures exhibit more advanced forms of deviance detection than previously known.

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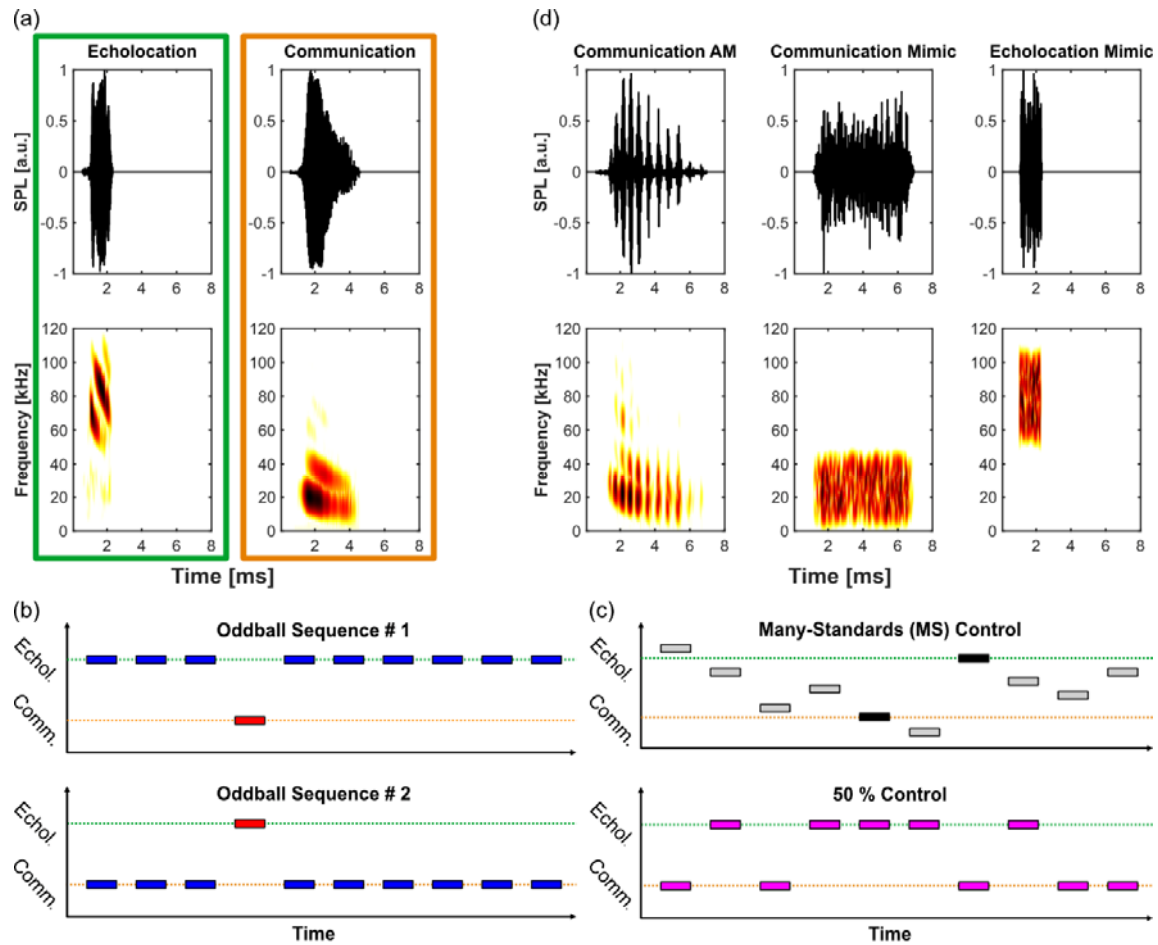
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43 **Keywords:** stimulus-specific adaptation, SSA, mismatch negativity, MMN, ABR, predictive  
44 coding, natural vocalisations, communication, echolocation, acoustic context

## 45 1. Introduction

46 Like all echolocating bats, *Carollia perspicillata* navigates in the dark by emitting  
47 stereotypical acoustic pulses and listening to the echoes reflected off objects in its  
48 environment. In addition, this bat species has a large variety of social communication  
49 calls[1,2], which is a consequence of its social lifestyle, with groups of more than 100  
50 individuals sharing the same roost[3]. This has led to the development of a broad variety of  
51 social communication calls. Echolocation pulses and social communication calls differ from  
52 each other in their carrier frequencies and durations, with echolocation pulses being higher in  
53 frequency and shorter in time (see Fig. 1a for an example echolocation pulse and social  
54 communication call). Those two vocalisation types represent fundamentally different  
55 behaviours (navigation and social communication) and can alternate in rapid succession for  
56 freely behaving bats. This raises a question that has puzzled neuroethologists for years: How  
57 does the bat brain process echolocation and social sounds in a fast and energy-efficient way,  
58 when they occur in the same acoustic stream? A theoretical model that explains how the brain  
59 efficiently deals with the tremendous amount of input it receives is the predictive coding  
60 framework and, in relation to this, the ability of deviance detection[4,5]. According to the  
61 predictive coding theory, the brain is constantly creating predictions about the incoming  
62 stimuli[6]. When the system encounters an unexpected signal, expectations are updated which  
63 is represented by a prediction error component in the electrophysiological response. This  
64 makes the identification of regularities and deviants in the incoming stream of signals (i.e.,  
65 deviance detection) crucial for the predictive coding framework. The present study  
66 investigates deviance detection to naturally occurring sounds – echolocation pulses and social  
67 communication calls – in the bat species *C. perspicillata*. We focussed on studying deviance  
68 detection in subthalamic neural populations of the auditory pathway by combining a  
69 naturalistic oddball stimulation paradigm (Fig. 1b) with noninvasively recorded auditory  
70 brainstem potentials (ABRs). Two experiments were performed: In experiment 1, an

71 echolocation pulse and a social communication call (Fig. 1a) were presented in an oddball  
72 paradigm (Fig. 1b). Additionally, by using two control paradigms (the “Many-Standards“  
73 (MS) and the 50 % control, Fig. 1c), we aimed to shed light on the possible underlying neural  
74 mechanisms responsible for deviance detection, namely deviant enhancement and repetition  
75 suppression of the standard response. These neural mechanisms are affected by both controls  
76 in different ways, allowing a more detailed characterisation of the effects than by only using  
77 an oddball paradigm. In experiment 2, the effect of different acoustic parameters (e.g., carrier  
78 frequency and temporal structure) and the behavioural meaning of the auditory input on  
79 subthalamic deviance detection was evaluated by performing a cross-comparison of the  
80 responses to different stimuli. The stimuli considered ranged from natural vocalisations on  
81 one end, to artificially generated vocalisation-mimics, noise bursts that resemble the  
82 vocalisations in their frequency range and duration but not in their temporal structure, on the  
83 other end (Fig. 1d).



84

85 *Figure 1: Used stimuli and stimulation protocols. (a) Oscillograms (top) and spectrograms (bottom) of an echolocation pulse*  
 86 *(green frame) and a social communication call (orange frame) of *C. perspicillata* that were used as stimuli in this study. The*  
 87 *communication signal is a syllable of a so-called distress call. (b) Schematic representation of the oddball paradigm; blue:*  
 88 *standard, red: deviant. (c) Schematic representation of the two control sequences used; Many-Standards control (top) and 50*  
 89 *% control (bottom). (d) Additional stimuli used for a cross-comparison with the vocalisations in (a) to evaluate the*  
 90 *importance of the frequency-versus-time structure of the stimuli for deviance detection. The amplitude-modulation of the*  
 91 *communication AM call was produced by the animal itself and the call represents another example of a natural distress call*  
 92 *of *C. perspicillata*. Both vocalisation mimics are artificially generated. They resemble the natural vocalisations in their*  
 93 *frequency range and duration but not in their temporal structure.*

94

95        2. Results and Discussion

96        **Deviance detection in broadband filtered ABRs differs between echolocation and social**  
97        **communication sounds**

98        In experiment 1, an echolocation pulse and a social communication call were presented in the  
99        oddball paradigm to investigate differences of deviance detection between both vocalisation  
100        types with the following results: ABRs to the echolocation pulse were significantly larger  
101        when the stimulus was perceived as a low-probability deviant (Fig. 2a, red ABR) than when it  
102        was a high-probability standard (blue ABR). This difference is present across the whole  
103        response, however, most prominently it appears in the last peak of the ABR, a slow wave that  
104        only becomes visible when the responses are broadband filtered between 0.1 and 2500 Hz.  
105        This filtering method is different from the usual narrowband filters between 300-2500 Hz that  
106        are used in many ABR studies and that will be discussed later. The strong effect of deviance  
107        detection in this late part of the response is in line with previous studies that investigated  
108        deviance detection in broadband filtered ABRs with pure tones[7,8]. This confirms that this  
109        slow, most likely inferior colliculus-generated[9] wave plays a key role in ABR-based  
110        deviance detection. It has been proposed that deviance detection is driven by two mechanisms  
111        at the neural level: repetition suppression and deviant enhancement. To disentangle which  
112        mechanism underlies the neural responses, the MS control has been suggested[10] (Fig. 1c,  
113        top). In this control, the target stimuli (Fig. 1a) are pseudo randomly presented together with  
114        multiple other stimuli (here: 8 stimuli; Fig. 1d; Supp. Fig. 1), which makes it impossible for  
115        the brain to detect regularities or deviations in the acoustic input. This results in responses that  
116        are unaffected by repetition suppression and deviant enhancement. A reduction of response  
117        strength to the standard relative to the MS response indicates repetition suppression while a  
118        stronger deviant than MS response is evidence for deviant enhancement. The echolocation  
119        response that was recorded in the MS control was significantly smaller than the deviant

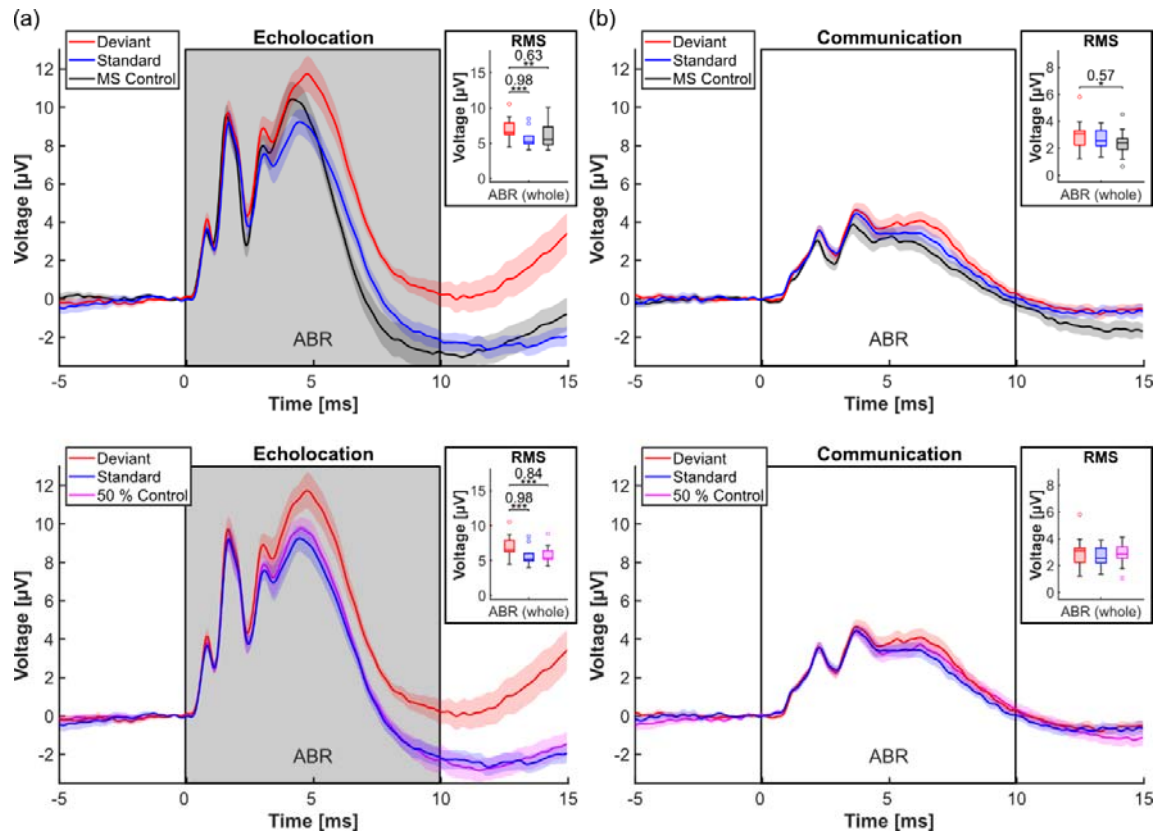
120 response and not significantly different from the standard response (Fig. 2a, top). This  
121 observation shows that the neural mechanism driving deviance detection for echolocation is a  
122 deviant-related enhancement of the response (i.e., a prediction error response in the predictive  
123 coding framework) and not a repetition suppression effect on the standard response. Possibly,  
124 deviant stimuli cause the brainstem neurons to respond more synchronously than standard or  
125 MS stimuli, resulting in larger deviant ABR amplitudes. In line with this hypothesis, former  
126 studies have demonstrated the importance of synchronisation and phase locking of brainstem  
127 neurons for speech[11] and music[12,13] perception in humans. Interestingly, the slow wave  
128 of the MS response has an earlier peak and offset latency compared to the deviant and  
129 standard ABR. This could indicate that additional neural mechanisms become active and  
130 modify the ABRs when the natural acoustic input becomes more complex, as occurs in the  
131 MS control compared to the oddball sequence. To further investigate deviance detection in the  
132 ABR, we used another common control paradigm, the so-called 50 % control. Here, both  
133 target stimuli are presented in a sequence with equal probability of 50 %. The analysis yielded  
134 a similar result to the MS control, that is the deviant response being significantly enlarged and  
135 no difference between control and standard response (Fig. 2a, bottom). The fact that deviant  
136 enhancement and not repetition suppression is driving low-level deviance detection for  
137 echolocation calls is interesting since previous studies have suggested repetition suppression  
138 to be the dominant mechanism causing deviance detection in subcortical nuclei[4,5].  
139 However, those studies used pure tones to stimulate individual neurons instead of measuring  
140 vocalisation-related summed potentials like we did here. It is possible that echolocation pulses  
141 evoke stronger deviant responses and less repetition suppression due to their high behavioural  
142 relevance compared to simple tone pips.

143 As opposed to echolocation, the social communication sounds did not elicit deviance  
144 detection in this experiment (Fig. 2b). While deviant and standard responses were not

145 significantly different from each other, the MS response was attenuated in comparison to the  
146 deviant response (Fig. 2b, top). This is surprising since, as explained above, the MS control is  
147 expected to generate a baseline response that is affected by neither deviant enhancement nor  
148 repetition suppression and hence should be positioned between deviant and standard response.  
149 Likely, the attenuation of the MS communication response is the result of the same  
150 mechanisms that modified the timing of the MS echolocation response. Those mechanisms  
151 seem to get active only when the acoustic input becomes more variable and appear to have  
152 complex, nonlinear effects on ABRs. Interestingly, they are restricted to natural stimuli, as a  
153 previous study by Wetekam et al.[8] in the same species did not find similar effects in the MS  
154 ABRs to pure tones. However, the 50 % control was very similar to the deviant and standard  
155 response, confirming that probability encoding did not affect the ABR size to the social  
156 communication call (Fig. 2b, bottom).

157





158

159 *Figure 2: Deviance detection in broadband filtered ABRs differs between echolocation and social communication sounds (n = 13 animals). (a) Grand averages of ABRs to an echolocation pulse presented as deviant (red) and standard (blue) as well as in the MS control (black, top) and the 50 % control (magenta, bottom). The boxes framing the responses represent the time window taken for RMS calculation, covering the whole ABR response (0-10 ms post stimulus onset). The grey colour of the boxes indicates a significant difference between deviant and standard response. Shaded areas around the ABRs depict the standard error of the mean. The inset on the right shows the RMS values calculated for each animal and condition as an estimation of response strength. (b) As in (a) but the stimulus was a social communication call. The white colour of the boxes framing the responses indicates that there was no significant difference between deviant and standard response.*

167

168 **For echolocation, deviance detection is measurable very early in narrowband filtered**

169 **ABRs**

170 To further characterise the effects of deviance detection on the echolocation response, the data

171 were narrowband filtered (bandpass Butterworth, 300-2500 Hz, 4<sup>th</sup> order) to analyse the fast

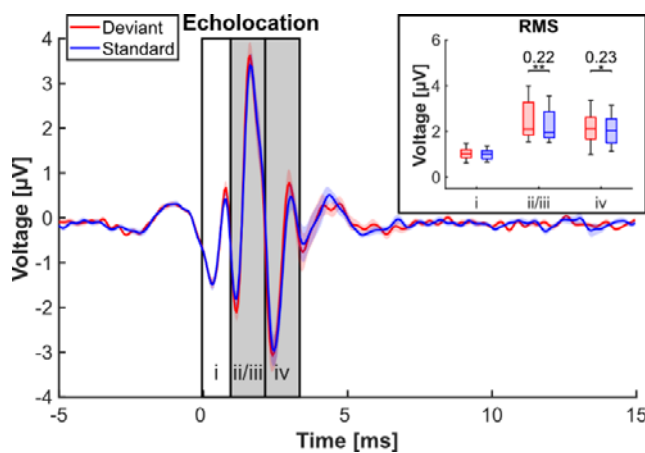
172 ABR components in more detail (Fig 3). ABR wave ii/iii as well as wave iv of the deviant

173 response were significantly larger than the respective components of the standard response.

174 Given that wave ii and iii represent neural activity in the cochlear nucleus and superior olivary

175 complex, respectively[14], this finding strongly supports the hypothesis that auditory

176 probability encoding at population level is happening already below the inferior colliculus, as  
177 it has been suggested in former studies[8,15]. In fact, effects of novelty detection have  
178 recently been described for an even lower auditory structure, the cochlea[16,17]. In these  
179 reports, the authors propose that the medial olivocochlear reflex is responsible for those  
180 effects by suppressing outer hair cell activity, mediated by feedback from the cortex. Since  
181 the ABRs presented here are averaged over many trials, it is possible that similar cortical  
182 feedback mechanisms are responsible for the very early effects seen in our ABR data.



183

184 *Figure 3: For echolocation, deviance detection is measurable very early in narrowband filtered ABRs (n = 13 animals).*  
185 *Grand averages of ABRs to an echolocation pulse presented as deviant (red) and standard (blue), with a social*  
186 *communication call as context. The boxes framing the responses represent the time window taken for RMS calculation,*  
187 *covering the typical ABR peaks i, ii/iii and iv. The colour of the boxes indicates whether a significant difference between*  
188 *deviant and standard response could be measured (grey: yes, white: no). Shaded areas around the graphs depict the*  
189 *standard error of the mean. The inset on the right shows the RMS values calculated for each animal, condition and time*  
190 *window as an estimation of response strength.*

191

## 192 **Behavioural meaning and complex sound features of a stimulus affect deviance detection** 193 **in broadband filtered ABRs**

194 The second experiment of this paper tackles the question of how low-level deviance detection  
195 is affected by individual stimulus parameters and possible behavioural meaning of the stimuli.  
196 Therefore, in addition to the previously used echolocation pulse and social communication  
197 call (Fig. 1a), an *amplitude-modulated* communication call (another distress vocalisation of *C.*  
198 *perspicillata*) and two artificial vocalisation-mimics that resembled the natural vocalisations

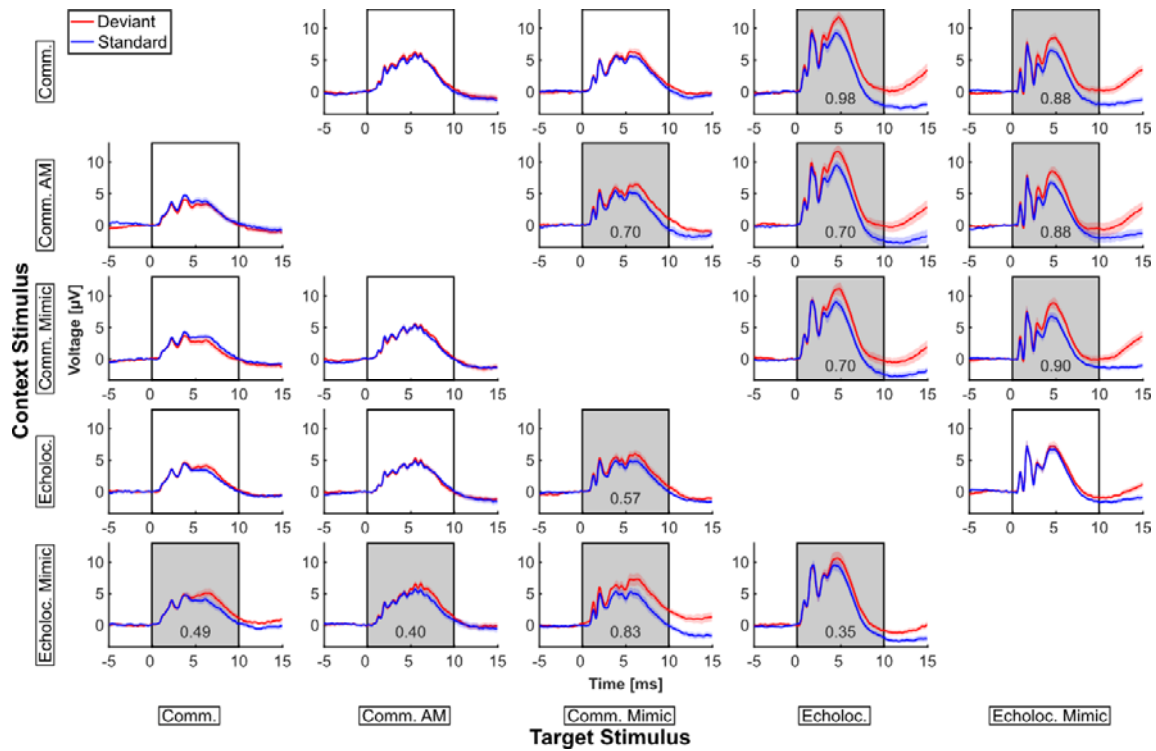
199 in their frequency range and duration but not in their temporal structure (Fig. 1d) served as  
200 stimuli. The aim was to assess the relevance of the frequency-versus-time structure of a signal  
201 for producing deviance detection in broadband filtered ABRs. In addition, it was tested  
202 whether the AM property of a communication call influenced deviance detection, as  
203 amplitude modulation appears in natural communication calls[2,18] and could bear additional  
204 meaning for the animal. To answer these questions, the five different stimuli were presented  
205 to the animals in all possible pairings of the oddball paradigm.

206 When the echolocation pulse or the echolocation mimic served as target stimulus,  
207 significantly larger deviant than standard responses could be measured when any  
208 communication stimulus was the context (Fig. 4, Supp. Fig. 2 for statistics). Interestingly,  
209 deviance detection could also be recorded for the responses to the echolocation pulse when  
210 the echolocation mimic was the context, but not vice versa. This indicates that differences in  
211 auditory input beyond simple frequency deviations – e.g., the frequency modulation of the  
212 echolocation pulse that is absent in the mimic – have a direct influence on subthalamic  
213 deviance detection in the bat brain. The fact that this effect is not present in the echolocation  
214 mimic responses when the echolocation pulse was context supports the claim that the  
215 behavioural meaning of a stimulus plays a key role in low-level population-based deviance  
216 detection. Modulatory effects of the behavioural meaning of a stimulus on the strength of  
217 deviance detection has previously been known for cortical areas[19], but not for the  
218 brainstem. Both natural communication calls – whether amplitude modulated or not – did not  
219 reveal significant deviance detection in any oddball combination except when presented with  
220 the echolocation mimic. This exception could be due to the very different physical properties  
221 of both call-types where the artificial nature of the mimic increases the contrast even further.  
222 On the other hand, as in experiment 1, the natural echolocation pulse as context did not cause  
223 deviance detection in the responses to either of the natural communication calls. Evidence for

224 differences in the processing of novelty detection between echolocation and communication  
225 stimuli in *C. perspicillata* has been reported before[20,21] and is in line with the current data.  
226 A possible reason for this phenomenon is the fact that both natural communication calls used  
227 in this study are distress calls that the animal emits when it is under physical duress[1,2].  
228 Those distress calls might always elicit the strongest possible neural response in the brains of  
229 conspecifics due to the relevance and importance their perception has for the behavioural  
230 response, independent of their probability of occurrence. In contrast, ABRs to the  
231 communication mimic did reveal strong deviance detection with significantly enlarged  
232 deviant responses when the AM communication call, the echolocation pulse or the  
233 echolocation mimic was the context. Only when the unmodulated communication call was the  
234 context, no significant difference between deviant and standard response could be measured.  
235 Together, these results indicate that the AM of the communication stimulus contributed to the  
236 differentiation between the true call and an artificial sound while it did not have a significant  
237 impact on the distinction between two different natural communication calls at subthalamic  
238 level.

239

240



241

242 *Figure 4: Behavioural meaning and complex sound features of a stimulus affect deviance detection in broadband filtered*  
 243 *ABRs ( $n = 13$  animals). All possible pairings of the oddball paradigm. Each column contains the recorded responses to the*  
 244 *target stimulus of the oddball sequence while each row represents one context stimulus (the second stimulus of the oddball*  
 245 *paradigm that served as context for the target stimulus). The stimuli tested were: communication call (Comm.), AM*  
 246 *communication call (Comm. AM), communication-mimic (Comm. Mimic), echolocation pulse (Echoloc.) and echolocation*  
 247 *mimic (Echoloc. Mimic). Response plots like in Fig. 2. The colour of the boxes indicates whether a significant difference*  
 248 *between deviant and standard response could be measured (grey: yes, white: no). If deviant and standard response differed*  
 249 *significantly, Cohen's  $D$  is provided as a measure of effect size (number in the grey boxes).*

250

## 251 Conclusion

252 In this study, noninvasively recorded ABRs revealed that deviance detection responses to  
 253 vocalisations that have different behavioural meanings for bats – navigation and  
 254 communication – are processed in a complex and asymmetric way already at the earliest  
 255 stations of the ascending auditory pathway. In fact, the results show that when considering the  
 256 population response, subthalamic deviance detection is sensitive to physical (carrier  
 257 frequency, FM and AM) as well as abstract stimulus features (behavioural meaning of a  
 258 vocalisation). By this, population-based subthalamic deviance detection showed a higher  
 259 complexity than what has been reported for cellular SSA of neurons in the same brain areas.

### 260 3. Material and Methods

#### 261 **Animals**

262 For the experiments of this study, 13 adult bats (7 males, 6 females) of the species *Carollia*  
263 *perspicillata* from the breeding colony of Goethe University Frankfurt were used. After being  
264 caught for the first time, all animals were held separately from the colony until the end of the  
265 study. Before every recording session, the animal was anaesthetised by a mixture of ketamine  
266 (Ketavet © 10 %, Medistar GmbH Ascheberg, Germany; 7.5 mg per kg bodyweight) and  
267 xylazine (Rompun © 2 %, Bayer HealthCare AG, Mohnheim, Germany; 16.5 mg per kg  
268 bodyweight) and the anaesthesia was maintained by follow-up injections of the same mixture  
269 with reduced volume every 1-1.5 h, for up to 4 h total. A DC-powered heating pad that was  
270 attached to the animal holder was used to maintain the animal's body temperature of 37 °C.  
271 Two consecutive recording sessions in the same animal were at least five days apart. This  
272 study was approved by the Regierungspräsidium Darmstadt (permits: FR/1010 and FR/2007)  
273 and was performed in full compliance with current German laws.

274

#### 275 **Stimulation and recording procedure**

276 Custom written MATLAB (MathWorks Inc., US) scripts were used for stimulation and data  
277 acquisition. The digital stimulus signal was D/A-converted by a 384 kHz Adi-2 Pro soundcard  
278 (RME, Haimhausen, Germany) before it was fed into a HiFi-amplifier (Power Amplifier RB-  
279 1050, Rotel, Hongkong, China) and presented to the animal by a Fountek NeoPro 5i Ribbon  
280 Tweeter (Fountek Electronics Co.,Ltd, Jiaxing, China). The speaker was positioned 15 cm  
281 away from the animal and pointed directly towards the left ear in a 45 ° azimuth angle relative  
282 to the head. To ensure a constant distance and angle between ear and speaker, the animal was  
283 head-fixed by a mouth-holder. All stimuli were natural vocalisations of *C. perspicillata* or  
284 vocalisation mimics with durations between about 2 to 10 ms (Fig 1, Supp. Fig. 1). The social

285 communication call that was used as target tone in experiment 1 (Fig. 1a) is a distress call, a  
286 social vocalisation that is emitted by the animal when under physical duress. Like all calls  
287 used in this study, it was recorded from a freely behaving bat. The echolocation and  
288 communication mimics are noise bursts covering very similar frequency ranges as their  
289 natural counterparts. They also resemble the vocalisations in their durations and rise/fall-  
290 times, with only the temporal structure of the natural and artificial stimuli being  
291 fundamentally different from each other. All stimuli had an intensity of 60 dB SPL and were  
292 presented at a rate of 20 Hz, equivalent to a stimulus-onset asynchrony of 50 ms.

293 The oddball paradigm that was used to study effects of deviance detection in the ABR  
294 consisted of 2 sequences of stimuli. In the first sequence, stimulus 1 was presented as standard  
295 (high probability, 90 %) and stimulus 2 as deviant (low probability, 10 %). The second  
296 sequence was presented consecutively and resembled the first one but with opposite roles of  
297 the stimuli, where now stimulus 1 was the deviant and stimulus 2 the standard (Fig. 1b). In  
298 total, a sequence contained 1000 stimuli (900 standards, 100 deviants). To characterise the  
299 measured deviance detection effects in more detail, two control sequences were used. The  
300 first was the MS control[10], presenting the target stimuli (echolocation pulse and social  
301 communication call) in a pseudo randomly arranged sequence together with eight additional  
302 stimuli, all having a probability of occurrence of 10 %. The other eight stimuli were the two  
303 vocalisation mimics, an AM communication (distress) call (Fig. 1d) and 5 other social  
304 communications of *C. perspicillata* that are related to different behaviours (Supp. Fig. 1). The  
305 MS control is expected to generate responses that are unaffected by any modulatory effects of  
306 probability encoding (repetition suppression or deviant enhancement) since the stimuli are  
307 perceived neither as deviant nor standard. As a second control, the echolocation pulse and  
308 social communication call were presented in another sequence where their probability of

309 occurrence was 50 %, respectively. Like in the oddball paradigm, the sequences of both  
310 controls consisted of 1000 stimuli each.

311 ABRs were differentially recorded by two electrodes – chlorinated silver wires (AG-10T,  
312 diameter: 0.25 mm; uninsulated and chlorinated tip of 3 mm) – that were placed  
313 subcutaneously at the vertex of the animal’s skull and close to the bulla of the left ear. A  
314 ground electrode was clipped to the animal’s right thumb. The recorded responses were  
315 hardware filtered (0.1-3000 Hz, 20 dB/decade roll-offs) and amplified by a factor of 20k by a  
316 Dagan EX1 differential amplifier (Science Products GmbH, Hofheim, Germany) before they  
317 were A/D-converted by the soundcard and sent to the computer. Blocks of 20 consecutive  
318 points of the input signal were averaged in order to down-sample the signal to 19.2 kHz.

319

## 320 **Data processing and statistical evaluation**

321 All processing and statistical evaluation of the data was conducted in MATLAB. The  
322 recorded ABRs were bandpass filtered by a Butterworth filter (4<sup>th</sup> order) in two different  
323 ways, dependent on the analysis. For the broadband filtered responses, low- and high-cut  
324 frequencies of 0.1 Hz and 2500 Hz, respectively, were used, which did remove high  
325 frequency noise from the signal but left the ABRs otherwise almost unchanged. On the other  
326 hand, the narrowband filter removed frequencies below 300 Hz and above 2500 Hz,  
327 abolishing all slow components of the response and allowing a more detailed inspection of the  
328 fast ABR waves i-iv. Before averaging, each trial was baseline corrected by calculating the  
329 mean voltage in a time window 1 ms pre stimulus onset. This value was subtracted from the  
330 whole trail resulting in a pre-stimulus activity of 0  $\mu$ V. Subsequently, the averaging procedure  
331 was restricted to those deviant responses that followed a standard response and, vice versa,  
332 those standard responses that preceded a deviant response. This method allows to use the  
333 same number of trials to calculate the deviant and standard average of each animal (here



334 between 89 and 92 trials) and, at the same time, maximises the effects of deviance detection  
335 in the responses[8]. In the case of the 50 % control, the same number of trials that was used to  
336 calculate the deviant and standard average for a given animal was used to randomly choose  
337 trials out of the 500 available responses to each stimulus. The MS average was calculated  
338 based on all responses to a given stimulus in the MS sequence (between 85 and 110 trials;  
339 mean difference to oddball responses: + 2.4 trials). All responses were corrected for the  
340 sound-travelling delay caused by the distance between speaker and ear. In each graph, the  
341 time point of 0 ms represents the moment when the sound reached the bat's ear.

342 To evaluate the response strength of each ABR, time windows were defined within which the  
343 response's RMS value was calculated. This has been done successfully in the same species  
344 before[8,22]. For all broadband filtered responses, this time window spanned from 0 ms to 10  
345 ms, covering the whole ABR with all its fast and slow components. The detailed wave-by-  
346 wave analysis of the narrowband filtered responses was done using three consecutive time  
347 windows, each containing a different component of the ABR. Those windows had borders of  
348 0 ms – 1 ms (wave i), 1 ms – 2.2 ms (wave ii/iii) and 2.2 ms – 3.4 ms (wave iv) which are  
349 similar to previously reported ABR-wave latencies of other bat species[23–25].

350 To compare response strengths between conditions with each other, paired one-tailed t-tests  
351 (deviant vs. standard responses) and repeated measure ANOVAs (deviant vs. standard vs.  
352 control responses) with subsequent Bonferroni-corrected post-hoc tests were used to evaluate  
353 differences between the calculated RMS values. Additionally, the effect size measure Cohen's  
354 D was calculated for all significant comparisons which allows an estimation of strength of the  
355 measured deviance detection effects[26].

356

357

358 Acknowledgements

359 We would like to thank Dr. Mirjam Knörnschild for providing us with some of the  
360 vocalisations of *Carollia perspicillata* that were used in this study. The study was funded by  
361 the Deutsche Forschungsgemeinschaft (KO 987/14-1).

362

363 Author contributions

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365 Palomares, Manfred Kössl

366 Experiments, analysis, and original draft of the manuscript: Johannes Wetekam

367 Review and editing of the original draft: Johannes Wetekam, Julio Hechavarría, Luciana

368 López-Jury, Eugenia González-Palomares, Manfred Kössl

369

370 Conflicts of interest

371 The authors declare no competing interests.

372

373 Abbreviations

374 ABR: Auditory brainstem response

375 AM: Amplitude modulation

376 ANOVA: Analysis of variance

377 FM: Frequency modulation

378 MMN: Mismatch negativity

379 MS: Many-standards

380 RMS: Root Mean Square

381 SSA: Stimulus-specific adaptation

382

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