



## Hissing like a snake: bird hisses are similar to snake hisses and prompt similar anxiety behavior in a mammalian model

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# Behavioral Ecology and Sociobiology

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--Manuscript Draft--

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<b>Abstract:</b>	<p>Batesian mimicry refers to a harmless species protecting itself from predators by mimicking a harmful species. A case of acoustic Batesian mimicry has been proposed in the naturalist literature: it is suspected that birds called like a snake when disturbed in their cavities to deter mammalian predators or repel competitors. To evaluate this hypothesis, we first test the assumption that the hissing sound produced by adult females of a wild cavity-nesting species - the blue tit (<i>Cyanistes caeruleus</i>) - is acoustically similar to the hisses of three wild sympatric snake species. Then, we tested one prediction of this hypothesis which is that the receiver of the signal should react similarly to the snake and bird hisses. To do so, we used, hiss-naïve individuals, without any past experience with predators: the house mouse (<i>Mus musculus domesticus</i>); representing a model of a possible nest competitor. We quantified mouse responses to blue tit and snake hisses and two non-hiss sounds (other blue tit vocalisations and human voices). Our results show that snake hisses and blue tit hisses are structurally more similar to each other than to other blue tit vocalizations, and that both hisses provoke comparable levels of anxiety behavior in mice. Taken together, these results are compatible with the hypothesis that blue tits have evolved to mimic the sound of snakes, i.e., the Batesian mimicry hypothesis. We also note however that our results also agree with another hypothesis, suggesting that mechanisms underlying the production and perception of hisses are conserved across vertebrates. Further research is needed to disentangle these two hypotheses.</p>	

<b>Response to Reviewers:</b>	<p><b>Response to Editor</b>      We list the complete Editor comments, followed by our responses (responses proceeded by ****).</p> <p><b>EDITOR COMMENTS:</b></p> <p>My comments:      see Word file corrected with track change. Please check the corrections, and if agree, accept them and resubmit a clean version.</p> <p>**** We checked the corrections, and accepted all of them.</p> <p>line 221: Give details of what was nested in what      **** We provided details (lines 220-222).</p> <p>- Add sample size or df to the statistics in lines 237-238      **** We have added the sample size (lines 236-237).</p> <p>line 253: add df      **** We have added the df (lines 251).</p> <p>- Acknowledgments: you may wish to thank the reviewers      **** We now write: "We thank the editor and reviewers for constructive comments on an earlier version of this manuscript" (lines 336-337).</p> <p>- Ethical approval: Although, statement on ethical approval for using mice in the study is provided, clarification is required whether the same was obtained for other animals (blue tit and snake) AND state which guidelines for using animals are followed (e.g. All applicable international, national, and/or institutional guidelines for the use of animals were followed.)      **** We added clarification for other animals (blue tit and snake) (lines 343-357).</p> <p>line 537: write ** and then change 0.05 into 0.01?      **** Done (line 494).</p> <p>- Fig. 2: write "relatedness" instead of "relatdness" on the x-axis      **** We write "relatedness" instead of "relatdness" on the x-axis.</p> <p>- ESM: Please include in each file the following information: article title, journal name, author names, affiliation and e-mail address of the corresponding author.      **** Done.</p>
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2   **prompt similar anxiety behavior in a mammalian model**

3

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14

15    **Abstract**

16    Batesian mimicry refers to a harmless species protecting itself from predators by mimicking a  
17    harmful species. A case of acoustic Batesian mimicry has been proposed in the naturalist  
18    literature: it is suspected that birds called like a snake when disturbed in their cavities to deter  
19    mammalian predators or repel competitors. To evaluate this hypothesis, we first test the  
20    assumption that the hissing sound produced by adult females of a wild cavity-nesting species -  
21    the blue tit (*Cyanistes caeruleus*) - is acoustically similar to the hisses of three wild sympatric  
22    snake species. Then, we tested one prediction of this hypothesis which is that the receiver of  
23    the signal should react similarly to the snake and bird hisses. To do so, we used, hiss-naïve  
24    individuals, without any past experience with predators: the house mouse (*Mus musculus*  
25    *domesticus*); representing a model of a possible nest competitor. We quantified mouse  
26    responses to blue tit and snake hisses and two non-hiss sounds (other blue tit vocalisations  
27    and human voices). Our results show that snake hisses and blue tit hisses are structurally more  
28    similar to each other than to other blue tit vocalizations, and that both hisses provoke  
29    comparable levels of anxiety behavior in mice. Taken together, these results are compatible  
30    with the hypothesis that blue tits have evolved to mimic the sound of snakes, i.e., the Batesian  
31    mimicry hypothesis. We also note however that our results also agree with another  
32    hypothesis, suggesting that mechanisms underlying the production and perception of hisses  
33    are conserved across vertebrates. Further research is needed to disentangle these two  
34    hypotheses.

35    **Keywords** Anti-predator strategy · Blue tit · Hissing call · Mimicry · Nest defense

36

37 **Significance Statement**

38 Mimicry is a fascinating illustration of the principles of evolution in communication. In the  
39 case of Batesian mimicry, species evolve to resemble other species as a mean of deterring  
40 harmful receivers. While visual mimicry has been thoroughly investigated across a wide range  
41 of species, vocal mimicry remains less studied. In the present study, we compared the acoustic  
42 similarity of the hissing sound produced by female blue tits, a cavity-nesting species, to the  
43 hisses of three snake species. Then, we exposed mice, a model of a possible cavity  
44 competitor, to bird and snake hisses. We showed that snake and blue tit hisses are acoustically  
45 similar and provoke comparable anxiety behaviors in mice. These results are compatible with  
46 the hypothesis that blue tits utilise an innate fear response to hisses in mammals, a result that  
47 may explain why blue tit hisses have been linked to increased survival by other authors.  
48 Furthermore, the results also suggest a conserved function of, and response to, hiss  
49 vocalizations across vertebrates.

50

51    **Introduction**

52

53    Mimicry occurs when species evolve to resemble other species in ways that benefit the mimic  
54    (e.g. warning or deceiving predators). It represents a fascinating illustration of the principles  
55    of evolution making intuitively understandable how different (sometimes phylogenetically  
56    distant) species could share similar traits (Joron and Mallet 1998; Darst and Cummings 2006).  
57    Visual mimicry has been well demonstrated across a wide range of species (animals: Norman  
58    et al. 2001; Stanger-Hall and Lloyd 2015; and plants: Dafni and Ivri 1981). For example,  
59    cuckoos lay eggs that closely resemble those of their hosts (Brooke and Davies 1988;  
60    Stoddard and Stevens 2010). Vocal mimicry has also been studied (Dalziell et al. 2015;  
61    Dalziell and Welbergen 2016), although to a much lesser degree. In birds, different functional  
62    explanations for vocal mimicry have been suggested, which fall into two categories:  
63    intraspecific communication (sexual context or social affiliation) and interspecific  
64    communication (avoidance of threats or competitors) (Kelley et al. 2008). In the second case,  
65    vocal mimicry is known to occur during predator-prey interactions (Dalziell et al. 2015) and  
66    such heterospecific mimicry can take on two main functional forms. First, heterospecific  
67    mimetic alarm calls can provide protection against predators by attracting individuals of other  
68    species (Chu 2001; Goodale et al. 2014) or by signalling the presence of top predators (Igic et  
69    al. 2015). For instance, greater racket-tailed drongos (*Dicurus paradiseus*) attract the aid of  
70    heterospecifics during mobbing by the mimicking mobbing calls of heterospecifics (Goodale  
71    et al. 2014). Secondly, birds might also mimic predators vocally in order to deter other  
72    predators or competitors (Sibley 1955; Krams et al. 2014; Amaral et al. 2017) that  
73    significantly reduce fledgling numbers (Martin 1993). Also called Batesian mimicry (Bates  
74    1862; Maynard Smith and Harper 2003), it corresponds to a particular form of deception in  
75    which the copying species co-opts a signal used by the copied species. For instance, it has

76 been suggested that *Neomorphus* ground-cuckoos mimic peccary tooth clacking to deter  
77 predators such as mustelids or small felids (Amaral et al. 2017). Because of the clear selective  
78 advantage to mimic other species to deter competitors and predators (Kelley et al. 2008), ones  
79 may question to what extent this behavior is frequent in nature.

80 Cavity birds emit a type of vocalization - a hiss - that has been hypothesized to mimic  
81 a snake hiss and therefore represent a case of Batesian mimicry (Sibley 1955; Rowe et al.  
82 1986; Magrath et al. 2010). The hiss-like vocalisation is produced by at least 15 cavity-  
83 nesting bird species including the burrowing owl (*Athene cunicularia*) and the great tit (*Parus*  
84 *major*) (Pickens 1928; Sibley 1955; Rowe et al. 1986; Owings et al. 2002; Magrath et al.  
85 2010). In the great tit, the hiss has been associated with enhanced adult overwinter survival  
86 and thus appears important for fitness (Krams et al. 2014). Hissing is suggested to deter  
87 predator and heterospecific competitors and possibly conspecific competitors that try to  
88 occupy the same nesting cavity, but at this stage only experiments directed to heterospecific  
89 audiences have been conducted. In agreement with Batesian mimicry, bird hissing sounds  
90 have been suggested to deter mammals under playback experiments. The tits' hisses have  
91 been shown to deter: (i) feral cats (Krams et al. 2014), (ii) woodpeckers (*Dendrocopos major*,  
92 Koosa and Tilgar 2016), and (iii) yellow-necked mice (*Apodemus flavicollis*, Zub et al. 2017).  
93 To date, however, none of these studies have used a control (i.e. another sound), and thus has  
94 not been possible to surmise whether the hiss is better than any other type of sounds in  
95 deterring predators and competitors. Additionally, so far acoustic similarities between snake  
96 and bird hisses have never been quantified.

97 Here, we focus on the hisses of the blue tit (*Cyanistes caeruleus*) (Jourdain 1929;  
98 Hinde 1952; Sibley 1955; Zub et al. 2017). About 70% of tits produce hisses when trying to  
99 deter an unknown intruder approaching the nest cavity, which could be a potential predator or  
100 competitor (Krams et al. 2014). We first tested whether that the blue tit hiss is more

101 acoustically similar to the snake hiss than to other tit sounds. To measure acoustic similarity  
102 between tit hisses and snake hisses, we recorded blue tit hisses and compared the acoustic  
103 similarity of the hissing sound produced by an incubating female blue tit, non-hissing blue tit  
104 vocalizations, and the hisses of three snake species living in the same habitat. Secondly, we  
105 asked whether a naïve mammal species representing a model of a possible cavity competitor  
106 reacts similarly to the snake and blue tit hiss, and whether this response varies under playback  
107 of the control sounds (the generic vocalisation of a female blue tit and a familiar human  
108 voice). To measure mammalian response to the playback treatments, we used the house  
109 mouse (*Mus musculus domesticus*). This species was used as a naïve mammalian model  
110 representing a rodent that could prey upon blue tit eggs or compete with blue tits for nest sites  
111 (Cramp and Perrins 1993). Although it would have been interesting to have a natural predator  
112 or competitor of tits, like garden dormouse (*Eliomys quercinus*) and edible dormouse (*Glis*  
113 *glis*), the advantage of using house mice is that they are easy to keep in captivity, naïve to  
114 snake and tit vocalizations, and because rodent responses to predators are often considered to  
115 be innate and shared across species (Berton et al. 1998). Any responses should represent the  
116 sensory system and behavioral responses of a potential cavity competitor.

117

## 118 **Material and methods**

119

### 120 **Sound recording and acoustic analysis**

121

122 We compared the acoustic structure of hisses produced by adult female nesting blue tits with  
123 hisses produced by three snake species (Fig. 1): the ladder snake (*Rhinechis scalaris*), the  
124 viperine snake (*Natrix maura*) and the Montpellier snake (*Malpolon monspessulanus*). These  
125 three snake species co-occur with blue tits in the Mediterranean basin and are known to prey

126 upon small animals like rodents (Capizzi et al. 1995; Pleguezuelos et al. 2007). We also  
127 included four other blue tit vocalisations in our analysis: nestling distress calls, male territorial  
128 songs, mobbing calls (mobbing calls produced in response to a predator, see Dutour et al.  
129 2017 for more details), and female generic vocalisations (vocalisations produced while in nest  
130 boxes; details about these recordings can be found in Gorissen and Eens 2005) (Fig. 1).

131 For each vocalization type, we recorded or collected good quality (22.05 and 44.1  
132 kHz) recordings. Generic vocalization, distress calls, blue tit hisses and snake hisses were  
133 recorded at 1 to 3 meters from the focal individual. Mobbing calls were recorded at 3 to 6  
134 meters. Blue tit hisses, snake hisses, and nestling distress calls were recorded using a  
135 MARANTZ PMD660 digital or a MARANTZ PMD222 recorder connected to a Sennheiser  
136 ME66-K6 microphone. In order to standardize the collection of blue tit hisses that are  
137 produced by females without seeing the intruder (suggesting a generalized response to  
138 intruders), the blue tit hiss was provoked by slowly inserting the end of a stick in the nest  
139 entrance and, if no hiss occurred by gently scraping, on the nest box door. Male blue tit songs  
140 were acquired from the xeno-canto online database ([www.xeno-canto.org](http://www.xeno-canto.org)). Generic female  
141 vocalisations were recorded using a Sony MD MZ-R700 recorder connected to an EMC-2005  
142 Electret Tie Clip Microphone. The snake hiss is usually produced as a warning signal.  
143 However, for the purposes of this study, the snake hisses were collected while experts were  
144 gently manipulating individuals after capture. For each of the eight vocalization types, we  
145 used recordings from three individuals. For each individual, we used two recordings, except  
146 in the case of one ladder snake and one viperine snake, for which we only had one recording  
147 each. Consequently, we analysed 46 vocalisations in total.

148 To properly characterise signal structure and variability, six acoustic parameters were  
149 measured on each call that reflected key temporal and frequency-related features: signal  
150 duration of the full call (seconds); peak frequency (the frequency with the highest energy

151 content, kHz); mean frequency (also known as centre frequency, the frequency that divides  
152 the selection into two frequency intervals of equal energy, kHz), maximum frequency (also  
153 known as high frequency, the highest frequency of the call, kHz); minimum frequency (also  
154 known as low frequency, the lowest frequency of the call, kHz); frequency bandwidth (kHz  
155 [threshold -10dB]). Recordings were in 16-bit WAV format and analysed on Avisoft  
156 SASLab© software (Fast Fourier Transformation (FFT) length of 1024 samples). We  
157 excluded mean frequency from the statistical analysis because it was strongly correlated with  
158 peak frequency ( $r = 0.77$ ,  $P < 0.001$ ); we also excluded minimum and maximum frequency  
159 because they were both strongly correlated with frequency bandwidth ( $r = -0.89$ ;  $r = 0.77$ ,  $P <$   
160  $0.001$ ). To obtain a dendrogram of signal similarity, we carried out a cluster analysis  
161 (hierarchical clustering using Ward's method, UPGMA, Euclidean distances) that included  
162 signal duration, peak frequency and frequency bandwidth. The function *dendro.gp* was used  
163 to choose the number of groups to be retained. The scale of a variable can have a large impact  
164 on how influential it is in the clustering process; however, all our acoustic measurements were  
165 already standardised to avoid this issue (function *scale*). Next, we performed non-parametric  
166 t-tests for signal duration, peak frequency and frequency bandwidth between the blue tit and  
167 snake hisses.

168

## 169 **Behavioral experiment**

170

171 To test whether blue tit hisses are a functional signal in inducing the same anxiety behaviors  
172 as snake hisses, we quantified the responses of a laboratory house mouse species ( $n = 16$  per  
173 sound type). Using naïve individuals excludes the possibility of any past predator exposure or  
174 captivity-related stress. Furthermore, it allowed us to specifically test whether there is an  
175 innate fear of hisses. The mice (8–10 weeks old) were housed in groups of 13 at CECEMA

176 (Centre d'Elevage et de Conditionnement Expérimental des Modèles Animaux, Montpellier,  
177 France) in accordance with legislative requirements and kept under controlled temperature  
178 (21°C), humidity (50%), and light/dark (12h:12h) conditions, and provided with ad libitum  
179 access to water and food.

180 We exposed mouse subjects to four acoustic treatments: the blue tit hiss, the ladder  
181 snake hiss (an arboreal species known to depredate bird clutches and chicks; Pleguezuelos et  
182 al. 2007), the generic vocalisation of a female blue tit and a familiar human voice (i.e. sounds  
183 associated with human caregivers of the animals). We broadcast the acoustic stimuli using a  
184 computer connected to a portable field speaker (SME-AFS; Saul Mineroff Electronics) placed  
185 1 metre from the centre of the arena. The arena belonged to the CECEMA and reflects a  
186 standard system to evaluate behavioral anxiety in neurosciences (Célérier et al 2004;  
187 Chauveau et al. 2008; Pierard et al. 2017). It is a square box (40 cm in length/width; 32 cm in  
188 height) with opaque acrylic glass walls. We used Audacity software to standardize the peak  
189 amplitude of the stimuli to 75 dB at 1 m using a sound level meter (Bioblock Scientific  
190 50517) placed in the center of the arena. This amplitude level is equivalent to that naturally  
191 produced by snakes (Young et al. 1999), similar to the natural range intensity of blue tit  
192 vocalisations (Doutrelant et al. 1999), and within the perception range of mice. The sounds  
193 (mean sound duration  $\pm$  SD:  $0.69 \pm 0.35$  seconds) were broadcasted once every 30 seconds  
194 over a 5-minute period. To reduce pseudoreplication, we created three playback samples from  
195 three individuals for each sound type (Hurlbert 1984; Kroosdma 1989; Kroosdma 1990). Each  
196 exemplar was randomly played to an individual mouse and no mouse was tested twice. A total  
197 of 16 mice were tested for each sound type.

198 We conducted all tests during the light phase of the light/dark cycle (between 8:00am  
199 and 4:00pm) in a soundproofed air-conditioned room. The mice spent at least 12 hours in the  
200 room before the tests began to allow for habituation. At the beginning of each test, we placed

201 a mouse in a small wire cage (15 cm in diameter) in the centre of the arena for 2 minutes.  
202 After this habituation period, we simultaneously broadcast the acoustic stimuli and removed  
203 the cage lid, allowing the mouse to move freely within the main enclosure for the 5-minute  
204 test period. Experiments were filmed with a video camera (Watec WAT- 660D). The arena  
205 was cleaned between tests with alcohol and water to eliminate any odours. We measured five  
206 responses reflective of behavioral anxiety: time spent in the main arena, number of escape  
207 attempts, number of head-raising events (correlated with vigilance), time spent grooming, and  
208 time spent immobile (Nosek et al. 2008; Kindermann et al. 2009). The observer (LL) was  
209 blind to the type of sound broadcasted to the mice; the videos were analysed without sound  
210 and the observer could not hear the playback happening. We arcsine-transformed the  
211 percentage/proportion data to avoid correlations between the mean and the variance. We first  
212 analysed the data using a Principal Component Analysis performed with R software (R Core  
213 Team 2016). PC1 does not appear to be a biologically meaningful axis: the time spent in the  
214 main arena and the number of escape attempts both contributed negatively, suggesting that  
215 positive values are associated with an animal trying less to escape but spending less time in  
216 the main area. Behavioral variables associated with PC2 are, by contrast biologically  
217 meaningful, with positive values on PC2 indicating an animal trying to escape more and  
218 spending less time in the main area. To test whether hiss sounds and non-hiss sounds elicited  
219 different levels of anxiety behavior, we compared PC2 using a nested analysis of variance  
220 (nested ANOVA; with the two types of emitters acoustic productions nested within hiss vs.  
221 non hiss sounds, respectively blue tit and ladder snake hisses vs. the generic vocalisation of a  
222 female blue tit and a familiar human voice). Alpha levels were set to 0.05.

223

224 **Data availability** The datasets generated are available from the corresponding author on  
225 reasonable request.

226

227 **Results**

228

229 **Acoustic similarity between blue tit and snake hisses**

230

231 In our cluster analysis two principal groups were retained, indicating a clustering of the blue  
232 tit and snake hisses, and a separate clustering of the other blue tit vocalisations (Fig. 2). These  
233 results indicate that blue tit hisses are more acoustically similar to the different snake hisses  
234 than they are to any of the other blue tit vocalisations (Fig. 2). Analysis of peak frequency and  
235 bandwidth showed no statistical differences between blue tit and snake hisses ( $t = -0.21$ ;  $P =$   
236 0.83 and  $t = 0.96$ ;  $P = 0.35$ ;  $n = 22$ ), although we did detect a difference in signal duration ( $t =$   
237 2.44;  $P = 0.02$ ;  $n = 22$ ) (Acoustic parameters mean of the hiss and non-hiss sounds are  
238 available in supplementary material Table A1).

239

240 **Mice anxiety reaction to blue tit and snake hisses**

241

242 The first three principal components (PCs) explained 80.13% of the total variation in the data  
243 (Table 1). However, we considered only PC2 to be biologically meaningful; it explained  
244 variation associated with features reflective of pronounced anxiety (number of escape  
245 attempts, number of head-raising events, and time spent immobile) as well as a feature  
246 reflective of low anxiety (time spent in the main arena) (Table 1). We found that playback of  
247 hiss sounds provoked more anxiety than playback of non-hiss sounds (nested ANOVA:  $F_{1,60}$   
248 = 8.74;  $P = 0.004$ ; Fig. 3). There were no such differences found along PC1 ( $F_{1,60} = 0.46$ ;  $P =$   
249 0.50) or PC3 ( $F_{1,60} = 0.032$ ;  $P = 0.86$ ). The results indicate that mice could therefore

250 discriminate between hiss and non-hiss sounds. Moreover, the responses to playback of blue  
251 tit and ladder snake hisses were not significantly different ( $F_{2,60} = 0.008$ ;  $P = 0.99$ ).

252

253 **Discussion**

254

255 We found that blue tit hisses are more acoustically similar to snake hisses than to any other  
256 blue tit vocalisations, and that mice show similarly strong anxiety behaviors to snake and blue  
257 tits hiss compared to a control. These results are in agreement with the one assumption and  
258 one prediction of the hypothesis of Batesian mimicry - i.e. an acoustic resemblance and the  
259 similar and strong response of potential receivers to the mimic and the predator sounds.  
260 However, these results could also be explained by a widespread conservatism in the  
261 production of basic sounds efficient to deter predators or competitors across vertebrates

262

263 **Bird hiss: a case a Batesian mimicry or not?**

264 A vocalisation is mimetic if its similarity with the species it mimics changes the receiver's  
265 behavior as well as the mimic's fitness (Dalziell et al. 2015). Here, the results we obtained  
266 confirm, for the first time, the assumption that blue tit and snake hisses are acoustically close  
267 and that blue tit hisses are more similar to the different snake hisses than they are to any of the  
268 other blue tit acoustic signals. This result can be explained by the acoustic structures of the  
269 studied vocalizations: there are atonal signals without clear frequency structures (the hisses)  
270 and tonal signals with clear harmonic and frequency modulated elements (the other blue tit  
271 vocalisations). Moreover, in agreement with a key prediction of the Batesian mimicry  
272 hypothesis, and in an experiment using for the first time controls (generic vocalisations of  
273 blue tit and human voices), we found that hisses produced by snakes and blue tits both  
274 provoke the same and similarly strong anxiety behaviors in house mice. As we used a lab

275 model receiver that is unfamiliar with tits or the tits' usual predators, our results suggest mice  
276 are anxious in response to unknown hisses. We used only three distinct playback samples  
277 from three individuals for each sound type, however it is unlikely that our results are  
278 confounded by pseudoreplication as response strength did not differ between playbacks.

279 Together with previous results using feral cats, woodpeckers, and yellow-necked mice  
280 as study species (Krams et al. 2014; Koosa and Tilgar 2016; Zub et al. 2017), and the  
281 responses of humans to this sound (personal observation), this study suggests that the altered  
282 behavior of receivers associated with hisses is shared across diverse species. Additionally, our  
283 results show that this response can be innate (our mice have never been confronted with a  
284 snake hiss). Hisses might, thus, be an effective anti-predator response; a result that is also  
285 corroborated by the fact that hissing females survived better than silent females (Krams et al.  
286 2014). Along with previous studies (e.g. Sibley 1955; Zub et al. 2017), this suggests that blue  
287 tit hisses deter other predators.

288 The reason behind a prey hissing, however, may have nothing to do with mimicry of a  
289 specific type of predator (e.g. snakes in this study) that hiss. It may simply reflect the  
290 production and reaction to a sound that is widely used in fearful or adversarial situations. If  
291 so, this would resonate with theories of shared emotional systems across bird and mammal  
292 species, as described for emotional vocalizations in Morton's Motivation-Structural Rules  
293 (Morton 1977). According to Morton (1977), certain types of sounds (tonal, higher frequency)  
294 tend to attract conspecifics whereas harsh atonal sounds (e.g. hisses, growls) tend to repel.  
295 The hiss is a widespread sound, used in adversarial situations by invertebrates (e.g. hissing  
296 cockroach *Gromphadorhina portentosa*, Nelson and Fraser 1980) and also vertebrates  
297 including mammals (e.g. rats, felids, opossums). Recent work on the evolution of emotional  
298 vocalizations is uncovering evidence for these fundamental structure-function relationships.  
299 Across vertebrates, caregivers respond similarly to the distress calls of infants (Lingle and

300 Riede 2014). Additionally, vertebrates recognize the emotional characteristics (level of  
301 arousal, positive versus negative balance) of vocalizations produced by animals that have  
302 been separated for 100's of millions of years (i.e. divergence times on phylogeny) (Filippi et  
303 al. 2017). These findings suggest that mechanisms underlying the production of basic sounds,  
304 and the processing (perception) of these sounds are conserved across vertebrates. Thus, a tit's  
305 reliance on a hiss to deter intruders, and the response of mice to these sounds, may reflect the  
306 conservation of these structure-function principles, and not necessarily convergence with the  
307 tits' specifically mimicking the vocalization of a particular category of predator (snakes).  
308 Maybe only a thorough investigation on which species hiss or not and the distribution of  
309 hissing in relation to body size or cavity way of life may help. For instance if only cavity birds  
310 hiss (i.e. if birds breeding outside cavities do not hiss) it might be an argument in favor of the  
311 Batesian hypothesis.

312

### 313 **How is hissing acquired and do tits hiss in the same way to all predators?**

314 An additional more proximal next step will be to determine whether mimic hissing is learned  
315 or innate. If it is learned, at what developmental stage does learning occur in birds? Field  
316 observations indicate that nestlings of a Paridae species, the black-capped chickadee (*Poecile*  
317 *atriplicis*), emit hisses at about 12 days post-hatching (Odum 1942). A recent study has  
318 suggested that mothers begin calling to their young when the latter are in their eggs (Mariette  
319 and Buchanan 2016). Therefore, it would be interesting to test whether nestlings exposed to  
320 maternal hissing are more likely to produce hisses themselves and are thus better protected  
321 against predators and/or competitors.

322 Our results also raise many other questions. Does the spatial distribution of hissing  
323 vary with the composition of the predator community? Do tits hiss in areas without snake  
324 species and do tits hiss in the same way to any type of predators or conspecifics? Finally, it

325 will also be important to study any acoustic or visual signals utilised by birds (e.g. snake-like  
326 gaping mouth and slow side-to-side movement while producing the hiss; see Pickens 1928;  
327 Sibley 1955; S1 Video), which likely play an important role in this defence strategy. Indeed,  
328 although communication is often studied from a unimodal perspective, it is often multimodal  
329 because the use of several sensory channels improves communication efficiency (Gomez et al.  
330 2011).

331

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337 earlier version of this manuscript.

338

### 339 **Compliance with ethical standards**

340

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343

344 **Conflict of interest** The authors declare that they have no conflict of interest.

345

346 **Ethical approval** All study protocols involving mice were approved authorization certificate  
347 for animal experimentation (A34-402) from the Direction Départementale de la Protection des  
348 Populations de l'Hérault to Aurélie Célérier, University of Montpellier - CECEMA.  
349 Regarding bleu tit acoustic productions, even if non-invasive (no necessity to handle

350 individuals) the data were collected under permits given by the Hérault local government  
351 office and the Regional Direction of Environment (DREAL) committee to our research  
352 program (permit 2006-01-2014), to our research institute (permit B34-172-204 11) and to  
353 ourselves (permit 3467). Regarding the snake acoustic productions, even if non-invasive, the  
354 sounds were collected with specialists on two field sites where research programs including  
355 Capture Mark Recapture on snakes are conducted; under the permit given by the Hérault local  
356 government office and the DREAL committee (number: 2015-12-17-01415) for the Mejean  
357 reserve and with the reserve ranger for the Tour du Valat Nature Reserve.

358

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485

486 **Table 1** Proportion of variance explained and variable contributions. PC: Principal

487 Component

Behavioral responses	PC 1	PC 2	PC 3
Individual proportion of variance explained	0.397	0.214	0.19
Cumulative proportion of variance explained	0.397	0.611	0.801
Time spent in main arena	-0.502	-0.399	-0.009
Number of escape attempts	-0.518	0.371	0.134
Number of head-raising events	-0.169	0.68	-0.601
Time spent grooming	0.606	-0.056	-0.389
Time spent immobile	0.289	0.487	0.685

488

489

490

## FIGURE CAPTIONS

491

492 **Fig. 1** Spectrograms of snake hissing and blue tit vocalisations. a, ladder snake hiss, b,  
493 viperine snake hiss, c, Montpellier snake hiss, d, female blue tit hiss, e, blue tit nestling  
494 distress call, f, blue tit male territorial song, g, blue tit mobbing call, and h, blue tit female  
495 generic vocalisation produced in nest boxes. These spectrograms were generated in SasLab  
496 Pro Avisoft, Germany

497

498 **Fig. 2** Cluster dendrogram of acoustic results. The snake hisses are in green, the blue tit hisses  
499 are in yellow, and the other blue tit vocalisations are in blue

500

501 **Fig. 3** Levels of anxiety behavior in house mice in response to four sound types (PC2 axis).  
502 Means  $\pm$  SE are indicated ( $n = 16$  mice per group each; \*\*  $P < 0.01$ , nested ANOVA).  
503 Positive values are indicative of anxiety related behaviors (i.e. spending less time in the arena  
504 and more time being immobile, attempting to escape more frequently, and more head-raising  
505 events). Mean PC1 score  $\pm$  SE: ladder snake hiss  $0.194 \pm 0.344$ , blue tit hiss  $-0.020 \pm 0.437$ ,  
506 blue tit generic vocalisation  $-0.047 \pm 0.367$ , human voice  $-0.199 \pm 0.295$ . Mean PC3 score  $\pm$   
507 SE: ladder snake hiss  $0.422 \pm 0.270$ , blue tit hiss  $-0.224 \pm 0.210$ , blue tit generic vocalisation -  
508  $0.150 \pm 0.232$ , human voice  $0.106 \pm 0.239$

509



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Dear Editor,

We wish to thank you for the helpful comments you provided on our manuscript. We have taken all the last corrections into account (please find detailed answers below).

Sincerely yours,

Mylène Dutour and co-authors.

---

**Response to Editor**

We list the complete Editor comments in italics, followed by our responses in plain text in blue (responses proceeded by \*\*\*\*).

**EDITOR COMMENTS:**

*My comments:*

*see Word file corrected with track change. Please check the corrections, and if agree, accept them and resubmit a clean version.*

\*\*\*\* We checked the corrections, and accepted all of them.

*line 221: Give details of what was nested in what*

\*\*\*\* We provided details (lines 220-222).

- *Add sample size or df to the statistics in lines 237-238*

\*\*\*\* We have added the sample size (lines 236-237).

*line 253: add df*

\*\*\*\* We have added the df (lines 251).

- *Acknowledgments: you may wish to thank the reviewers*

\*\*\*\* We now write: "We thank the editor and reviewers for constructive comments on an earlier version of this manuscript" (lines 336-337).

- *Ethical approval: Although, statement on ethical approval for using mice in the study is provided, clarification is required whether the same was obtained for other animals (blue tit and snake) AND state which guidelines for using animals are followed (e.g. All applicable international, national, and/or institutional guidelines for the use of animals were followed.)*

\*\*\*\* We added clarification for other animals (blue tit and snake) (lines 343-357).

*line 537: write \*\* and then change 0.05 into 0.01?*

\*\*\*\* Done (line 494).

- *Fig. 2: write "relatedness" instead of "relatdness" on the x-axis*

\*\*\*\* We write "relatedness" instead of "relatdness" on the x-axis.

- *ESM: Please include in each file the following information: article title, journal name, author names, affiliation and e-mail address of the corresponding author.*

\*\*\*\* Done.

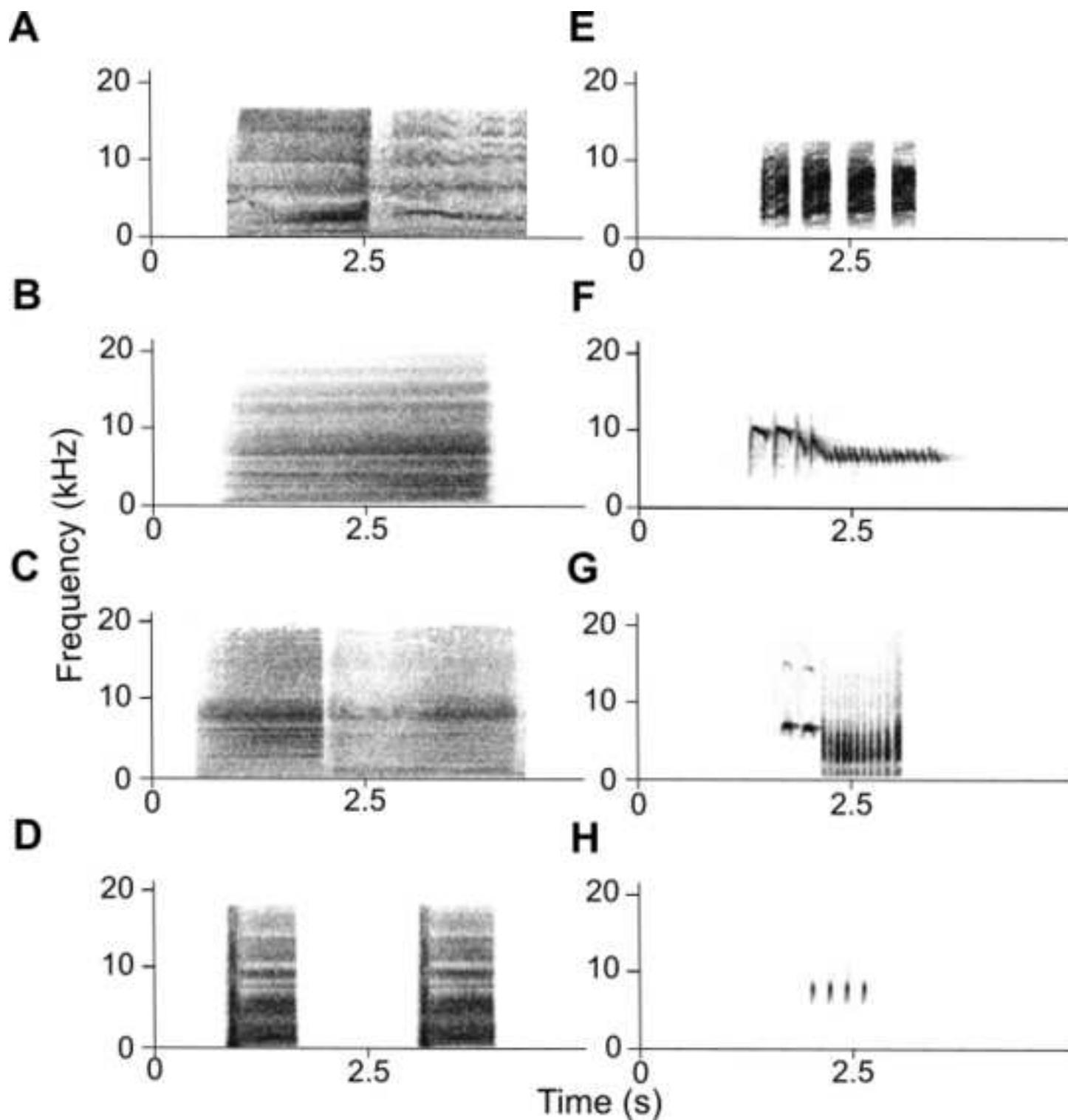
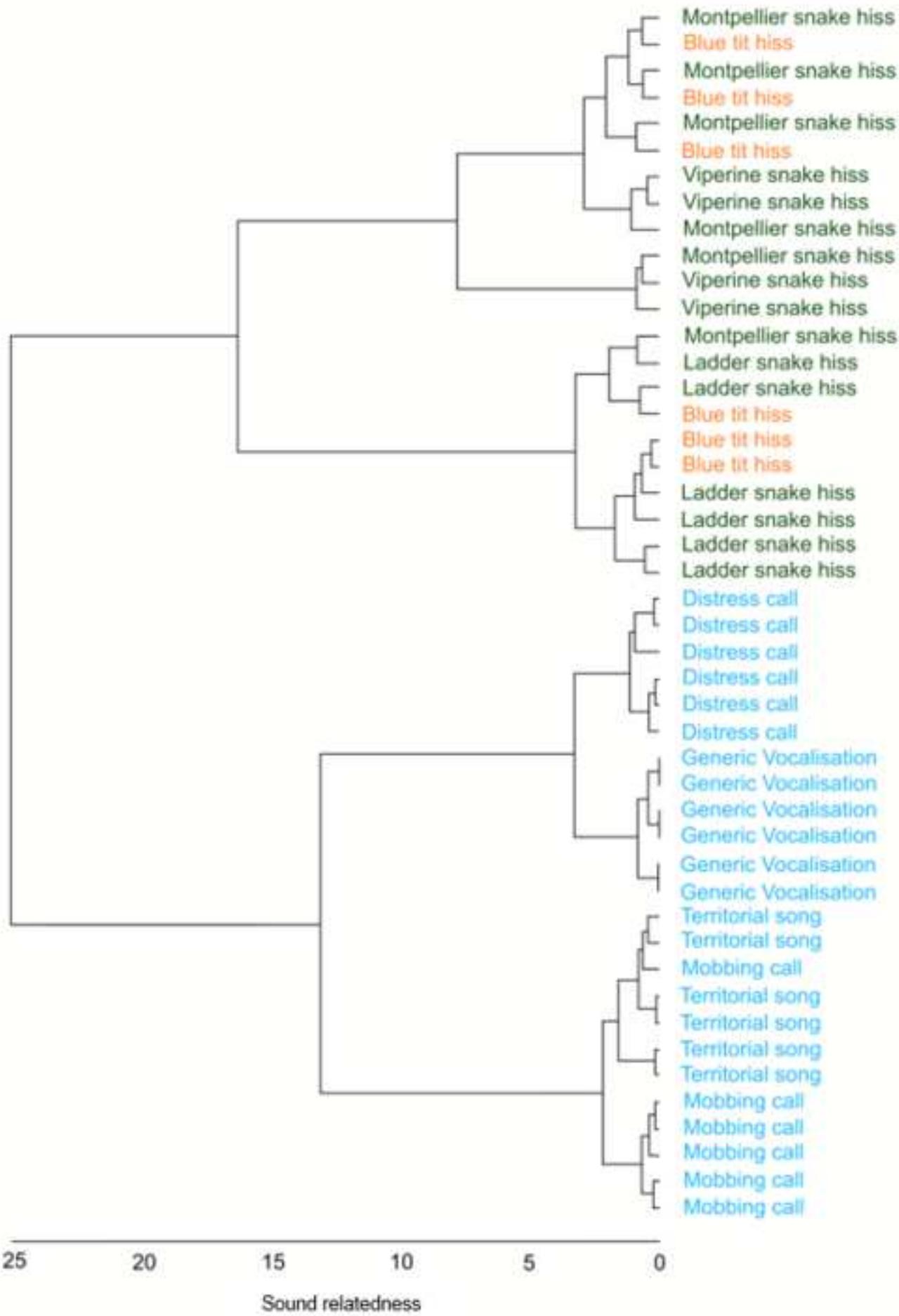
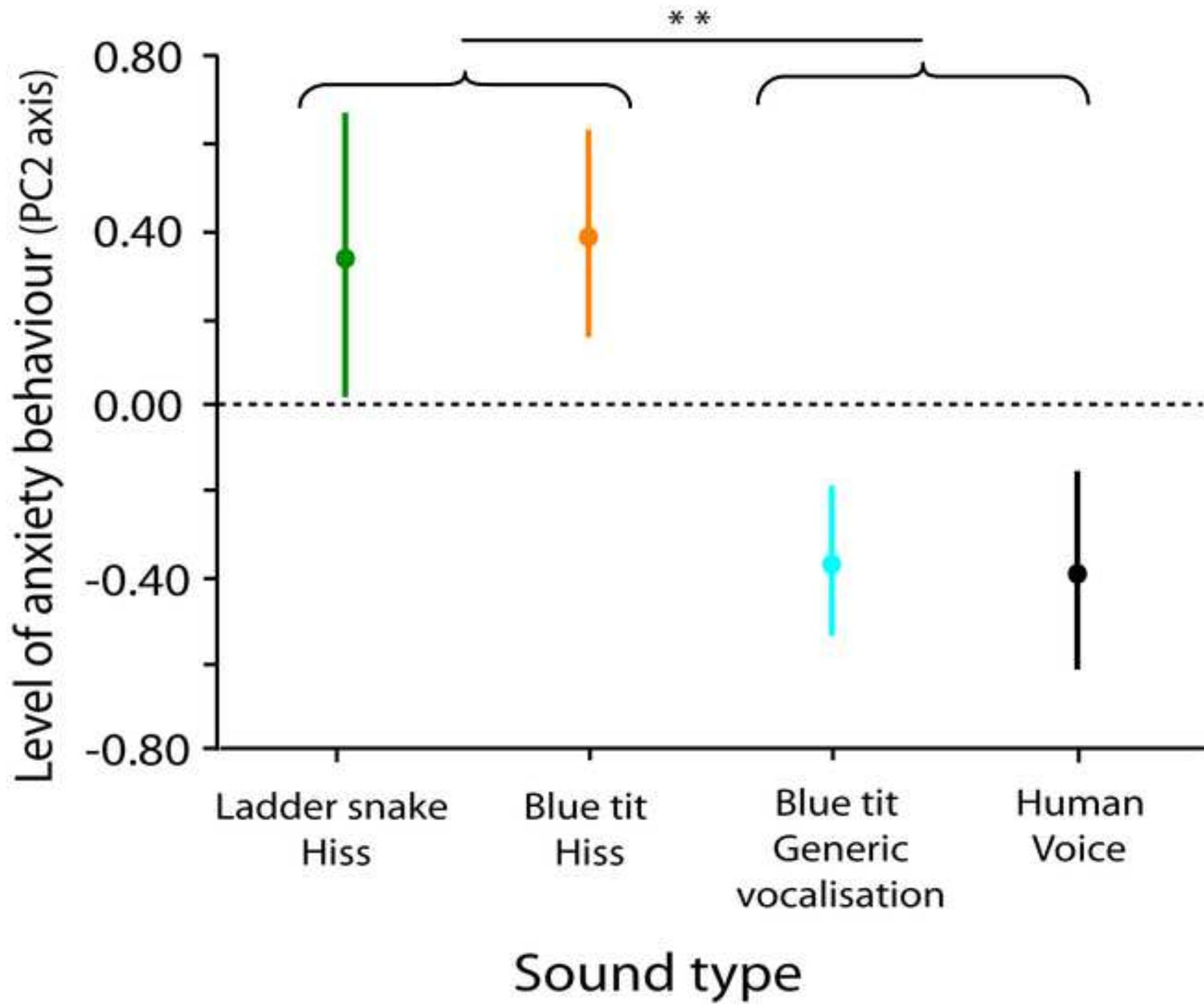


Figure 2

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