



# 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

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

<p><b>Response to Reviewers:</b></p>	<p>Response to Editor We list the complete Editor comments, followed by our responses (responses proceeded by ****).</p> <p>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.</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 "relatedness" on the x-axis **** We write "relatedness" instead of "relatedness" 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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# **Hissing like a snake: bird hisses are similar to snake hisses and prompt similar anxiety behavior in a mammalian model**

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## Abstract

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 (*Cyanistes caeruleus*) - 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 (*Mus musculus domesticus*); 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.

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

## **Significance Statement**

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

## Introduction

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

## 118 **Material and methods**

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

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

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

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

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

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

## **Behavioral experiment**

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

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

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

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

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

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

## Results

### Acoustic similarity between blue tit and snake hisses

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

### Mice anxiety reaction to blue tit and snake hisses

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

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

## Discussion

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

### Bird hiss: a case a Batesian mimicry or not?

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

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

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

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



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

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

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

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

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

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## **Compliance with ethical standards**

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

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

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

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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

## FIGURE CAPTIONS

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

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

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



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.

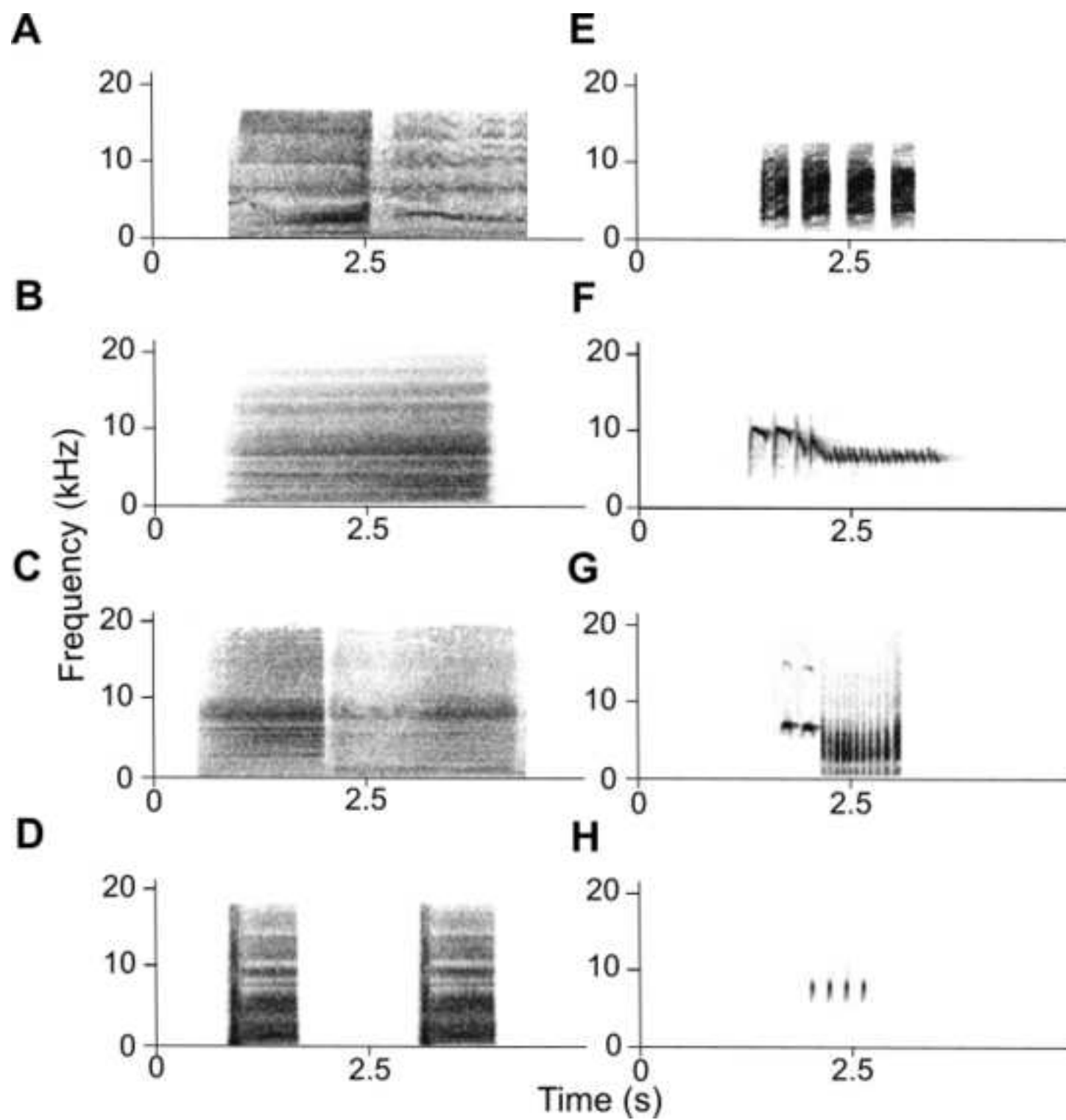


Figure 2

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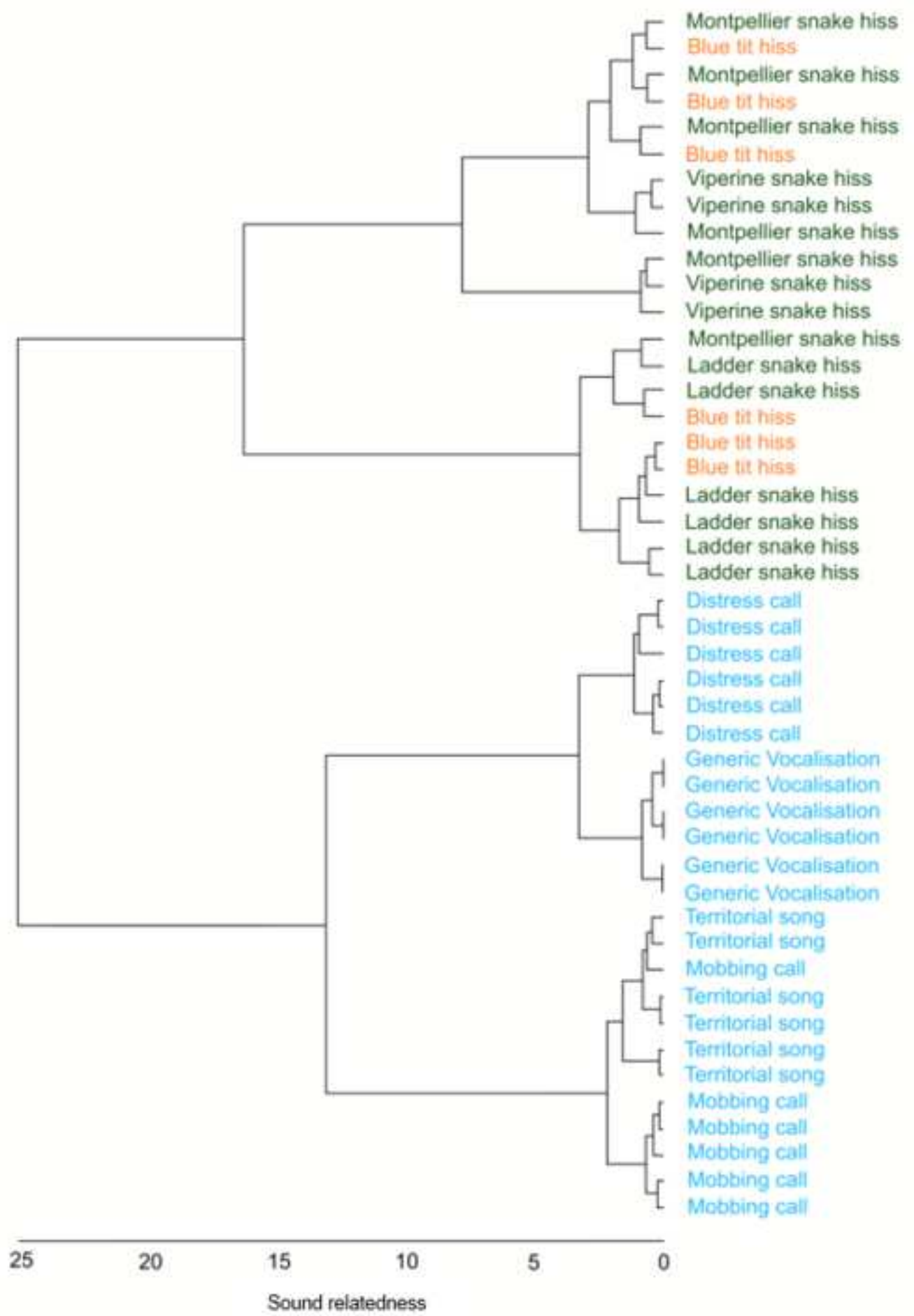
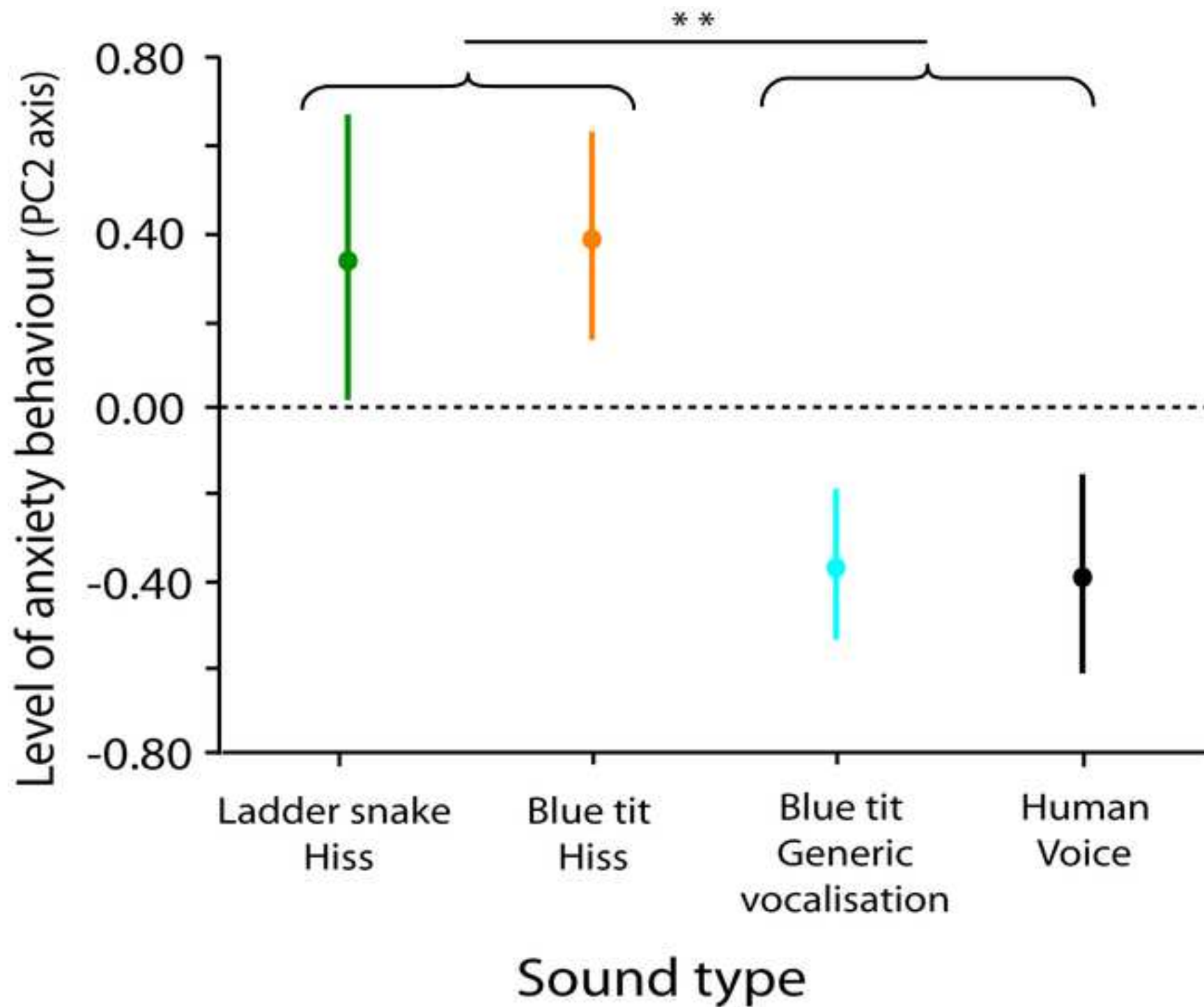



Figure 3

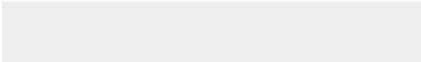










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