

1 Running head: Noise effects on torpid bats

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4 **Are torpid bats immune to anthropogenic noise?**

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## SUMMARY

1  
2 Anthropogenic noise has a negative impact on a variety of animals. However,  
3 many bat species roost in places with high levels of anthropogenic noise. Here we  
4 test the hypothesis that torpid bats are insensitive to anthropogenic noise. In a  
5 laboratory experiment, we recorded skin temperature ( $T_{sk}$ ) of bats roosting  
6 individually that were subjected to playbacks of different types of noise. We  
7 found that torpid bats with  $T_{sk}$  ~10 °C lower than their active  $T_{sk}$ , responded to  
8 all types of noise by elevating  $T_{sk}$ . Bats responded most strongly to colony and  
9 vegetation noise, and most weakly to traffic noise. The time of day when torpid  
10 bats were exposed to noise had a pronounced effect on responses. Torpid bats  
11 showed increasing responses from morning towards evening, i.e. towards the  
12 onset of the active phase. Skin temperature at the onset of noise exposure ( $T_{sk-start}$ ,  
13 17 ~ 29 °C) was not related to the response. Moreover, we found evidence that  
14 torpid bats rapidly habituated to repeated and prolonged noise exposure.

15 **Keywords:** anthropogenic noise, habituation, *Myotis myotis*, roosting bat, torpor,  
16 traffic noise

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## INTRODUCTION

1  
2 Traffic noise, together with other types of anthropogenic noise, has become both a  
3 biodiversity threat and a potential new selective force affecting the evolutionary  
4 processes of some animal species (Forman and Alexander, 1998; Brumm, 2010;  
5 Halfwerk et al., 2011b). For animals living in the vicinity of roads, traffic noise may  
6 impair their communication (Brumm and Slabbekoorn, 2005), change their vocal  
7 signals (Parks et al., 2011), interfere with predator-prey interactions (Siemers and  
8 Schaub, 2011), or act as a stressor affecting the neuroendocrine system (Rolland et al.,  
9 2012). Consequently, these effects may lead to the reduction of suitable habitats  
10 (Bayne et al., 2008), reduced reproductive success (Francis et al., 2011; Halfwerk et  
11 al., 2011a; Schroeder et al., 2012), decreased population size (Foote et al., 2004;  
12 Reijnen and Foppen, 2006), or altered evolutionary paths (Leonard and Horn, 2005;  
13 Luther and Baptista, 2009; Halfwerk et al., 2011b).

14 Bats, the second largest and ecologically most diverse mammalian order, are both  
15 ecologically and economically important as agents of pest control, pollination and  
16 seed dispersal (Boyles et al., 2011; Kunz et al., 2011). Since bats are highly mobile  
17 and can travel large distances between roosts and foraging grounds, they can be  
18 considerably affected by road systems. Road effects on bats include vehicle mortality  
19 (Kiefer et al., 1995; Lesiński et al., 2011), light pollution (Rydell, 1992; Stone et al.,  
20 2009; Stone et al., 2012), and the barrier and edge effects (Kerth and Melber, 2009;  
21 Abbott et al., 2012; Berthinussen and Altringham, 2012). Moreover, two recent  
22 studies found that bats may avoid foraging areas with loud traffic noise that can  
23 reduce their foraging efficiency (Schaub et al., 2008; Siemers and Schaub, 2011).  
24 Nevertheless, a great number of bat species roost in places exposed to loud  
25 anthropogenic noise (Altringham, 2012).

26 Two aspects of bat biology may minimize the potential adverse effects of  
27 anthropogenic noise on roosting bats. First, many bat species conserve energy by  
28 engaging in torpor, which is characterized by a reduction of metabolic rate, body  
29 temperature ( $T_b$ ) and other physiological processes (Speakman and Thomas, 2003;  
30 Geiser, 2004). Although the central nervous system of hibernators and daily  
31 heterotherms continues functioning at low  $T_b$ , the peripheral and brainstem auditory  
32 systems become less responsive as  $T_b$  declines (Coats, 1965; Rossi and Britt, 1984).  
33 This is true for hibernating bats and it has been corroborated in one  
34 neurophysiological and one behavioural experiment independently (Harrison, 1965;

1 Speakman et al., 1991). Specifically, results from *Myotis lucifugus* suggested that the  
2 frequency above which no responses were elicited decreased continually with  
3 decreasing body temperature (Harrison, 1965). Second, nearly all bats are most  
4 sensitive to frequencies > 10 kHz (Neuweiler, 1990; Pfalzer and Kusch, 2003;  
5 Hoffmann et al., 2008), which is well above the frequencies in anthropogenic sounds  
6 (such as traffic noise) that typically contain the most sound energy. Consequently, the  
7 purpose of our study was to assess whether torpid bats were sensitive to  
8 anthropogenic noise. We experimentally tested this hypothesis with the greater  
9 mouse-eared bat (*Myotis myotis* Borkhausen 1797), a species that is commonly found  
10 to roost in noisy areas such as under bridges (Dietz et al., 2009). Virtually all animal  
11 species show a decrease in behavioural responses to repeated stimulation (Rankin et  
12 al., 2009; Thompson, 2009), therefore we additionally assessed whether torpid bats  
13 became habituated to noise with repeated exposure.

14

## 15 MATERIALS AND METHODS

16

### Study site and animals

17 This study was conducted at the Tabachka Bat Research Station of the Sensory  
18 Ecology Group (MPI Seewiesen), which is run in cooperation with the Directorate of  
19 the Rusenski Lom Nature Park in the district of Ruse, northern Bulgaria. The  
20 experiment was performed between late May and July, 2012. Fifteen male greater  
21 mouse-eared bats (body mass at capture,  $27.8 \pm 2.1$  g) were captured with a harp trap  
22 at the Orlova Chuka cave. One bat managed to remove its temperature logger during  
23 the night before the test and hence only 14 of them were tested in the laboratory.  
24 Except for the individual being tested, other bats were housed together in a cage ( $49 \times$   
25  $35 \times 37$  cm, length  $\times$  width  $\times$  height) at an ambient temperature of approximately  
26  $25^\circ\text{C}$  and a relative humidity of 75 %, and had access to water *ad libitum*. All bats  
27 were fed mealworms (larval instars of *Tenebrio molitor*) *ad libitum* at 22:00 every  
28 evening. Capture, husbandry and behavioural studies were carried out under the  
29 license of the responsible Bulgarian authorities (license no. 465/29.06.2012). All bats  
30 were released in good health to the wild after the experiment.

31

### Acoustic stimuli

32 We created a series of sound files for playback, containing different types of noise:  
33 bird noise, bat colony noise, vegetation noise, traffic-like noise and silence. All  
34 playback files (Fig. 1) were created in Adobe Audition 5.5 and had a sampling rate of

1 250 kHz. With the exception of the traffic noise and the silent sound files, which were  
2 digitally generated, the frequency and amplitude of noise files varied with time. To  
3 standardize variation in the noise recordings we created 14 different playback files for  
4 each noise type (i.e. bird noise, colony noise and vegetation noise) by randomly  
5 arranging sound segments cut from the original recordings. The silent sound (control)  
6 was generated by setting all amplitude values to zero. All files were high-pass filtered  
7 at 1 kHz (digital fast Fourier transform filter, 2048 points, Blackman window) to  
8 remove sounds that were probably not audible to the bats and to avoid damage to the  
9 speakers (Siemers and Schaub, 2011).

10 For the 25 m and 50 m traffic noise, we digitally generated noise that would  
11 correspond to the loudest average 0.5 s sound made by a passing vehicle 25 m and  
12 50 m away on a highway (Siemers and Schaub, 2011). Although the traffic noise was  
13 audible at 100 m distance, our recordings were unsuitable for playback because the  
14 traffic noise was totally masked by the natural background noise and the thermal  
15 noise of the microphone itself. Therefore, to reproduce realistic playback files for  
16 traffic noise at 100 m, we first made recordings at a distance of 7.5 m from the  
17 highway edge (Schaub et al., 2008), which had a high signal-to-noise ratio to serve as  
18 a baseline. We then computed the average power spectral density of 50 passing cars  
19 and 50 passing trucks at speeds of approximately 80 km / h. Based on these values  
20 and the decay of amplitude over distance, we generated the 100 m traffic noise  
21 digitally. For the bird noise, recordings of the vocalizations of four bird species  
22 (*Hirundo rustica*, *Oriolus oriolus*, *Passer domesticus*, and *Sturnus vulgaris*), which  
23 are typically found in our study area were extracted from a CD (Bergmann et al.,  
24 2008). Each bird noise file contained the vocalizations of all four species arranged  
25 randomly. For the bat colony noise, we made recordings between 15:00 and 16:00 in  
26 an afternoon under the large colony from which our bats were collected. This colony  
27 consists primarily of four species, *Myotis myotis*, *Myotis blythii oxignathus*,  
28 *Rhinolophus mehelyi* and *Rhinolophus euryale*. For the vegetation noise, we made  
29 recordings approximately 10 cm from a tree (*Tilia platyphyllos*) on a windy afternoon,  
30 with average and maximum wind speeds of 2.77 m/s and 4.07 m/s, respectively  
31 (Skywatch Atmos Anemometer, Skyview Systems Ltd., Suffolk, UK). Both the  
32 colony noise and the vegetation noise were recorded with a broadband microphone  
33 (UltraSoundGate CM16 / CMPA, Avisoft Bioacoustics, Berlin, Germany) connected  
34 to an ultrasound recording interface (UltraSoundGate 116H, Avisoft Bioacoustics,

1 Berlin, Germany) at a sampling frequency of 250 kHz. The microphone had a flat  
2 frequency response between the frequency ranges of 10 - 200 kHz, but frequencies  
3 below 10 kHz were underrepresented to varying extents.

4 The average playback amplitudes for 100 m, 50 m and 25 m traffic noise, bird  
5 noise, colony noise and vegetation noise were 62, 68, 74, 70, 70, and 50 dB SPL (root  
6 mean square (RMS) with reference to 20  $\mu$ Pa) respectively, which were all measured  
7 1 m in front of the loudspeaker with a calibration microphone (G.R.A.S. 1/8" 40DP  
8 pressure microphone, Holte, Denmark). For the bird noise, the RMS amplitude was  
9 determined for the loudest syllable, while for other types of noise the RMS amplitudes  
10 were determined by measuring the whole five-minute sound file. To ensure that the  
11 bats were subjected to similar amplitudes in each stimulus, we placed the playback  
12 loudspeaker 1 m away from the roosting bat.

### 13 **Experimental setup**

14 We used the skin temperature ( $T_{sk}$ ) as a means to determine whether environmental  
15 noise disturbs torpid bats. Changes in  $T_b$  are reflected by changes in  $T_{sk}$  (Audet and  
16 Thomas, 1996; Barclay et al., 1996). Given an ambient temperature ( $T_a$ ) that is below  
17 the  $T_b$  of torpid animals and a lack of an external heat source, internal heat production  
18 is the only way to raise  $T_b$  (Lyman et al., 1982). In our experiment,  $T_{sk}$  of the bats (and  
19 hence  $T_b$ ) was never below  $T_a$ , which was fairly stable over the course of a day (max.  
20  $\Delta T_a = 2.8$  °C). As a result, an increase in  $T_{sk}$  in response to stimuli must be active and  
21 may be taken as a sign of disturbance.

22 One night before the experiment, a small patch of fur between the scapulae of the  
23 respective bat was removed and a miniature temperature logger (ca.  $18 \times 12.5$  mm<sup>2</sup>,  
24 modified iButton, after (Lovegrove, 2009), Maxim Integrated Products Inc.,  
25 Sunnyvale, CA, U.S.A) was glued to the exposed skin with mastic spirit gum  
26 (COIDRO-AG, Kems, Switzerland). Below the interscapular skin bats have a large  
27 repository of brown adipose tissue (Neuweiler, 2000), which is involved in active heat  
28 production (Withers, 1992). Even if warming up the whole body takes some time, this  
29 spot becomes very warm compared to the surrounding skin, particularly at the  
30 beginning of the warming up process. Thus, measuring changes in  $T_{sk}$  at this spot  
31 allows high accuracy in determining the timing of the start of the warming up process.  
32 The temperature loggers recorded  $T_{sk}$  every 60 seconds to the nearest 0.5 °C. The  
33 loggers were three-point calibrated in a cooling incubator (KB 53, Binder GmbH,

1 Möhringen-Tuttlingen, Germany) at 0, 25 and 50 °C. After the experiment, the logger  
2 was removed with mild spirit gum remover (COIDRO-AG, Kems, Switzerland).

3 Bats were tested individually in a test roost (a cage like those used for husbandry in  
4 the holding room, see above) during their natural roosting time (07:30 - 20:30). One  
5 night before the test, the bat was placed in the test roost that was positioned in a large  
6 sound-absorbing room. Two infrared cameras with infrared light illumination (CCD-  
7 651, Conrad Electronic, Hirschau, Germany) were fixed in two corners inside the  
8 roost. To encourage the bats to roost in a location that would enable clear video  
9 recordings, and for playback amplitude control, one white ceiling light in the room  
10 was switched on and wet towels were used to create a single dark corner and to  
11 increase the humidity. Noise stimuli were presented using an ultrasonic dynamic  
12 speaker (ScanSpeak, Avisoft Bioacoustics, Berlin, Germany) with a frequency range  
13 of 1 - 120 kHz. We triggered the playback only when the bat was completely still for  
14 at least one minute (i.e. no movement of any body parts were observable on the  
15 surveillance monitor). The infrared cameras were synchronized by the surveillance  
16 software DigiProtect (ABUS Security, Affing / OT Mühlhausen, Germany) installed  
17 on a desktop computer. All equipment (playback and video recording devices) was  
18 controlled from a separate observation room.

19 Two types of tests were conducted that differed in the duration of the noise  
20 exposure during each trial. The five-minute experiment was used to assess the  
21 sensitivity of torpid bats to different types of noise and to assess their potential for  
22 habituation. The one-hour experiment was only used to assess the habituation ability.  
23 In the five-minute playback experiment, each individual was singly tested on two  
24 consecutive days. On each day the bat was subjected to a pseudo-randomized  
25 sequence of playbacks of the seven acoustic stimuli of five minute duration each (Fig.  
26 1). Since playbacks were only triggered when the bat was completely still for at least  
27 one minute, sometimes not all seven playbacks could be presented within the time  
28 frame of the first experimental day. These playbacks were presented on the second  
29 day followed by a second presentation of the same noise sequence. Again, sometimes  
30 not all of the playbacks of the second sequence could be presented within the time  
31 frame of the second experimental day. Hence only 12 of the 14 individuals were  
32 subjected to the same stimulus twice in two days. For the other two individuals, only  
33 2 and 3 types of stimuli could be repeated on the second day due to their continuous  
34 active status. For the one-hour playback experiment, 7 bats were subjected to one-

1 hour noise treatment twice and 4 bats once. For these treatments, only the 25 m traffic  
 2 noise and the colony noise were used. The one hour playbacks were conducted after  
 3 finishing the five-minute trials above, either in the morning between 7:30 - 8:30 or in  
 4 the afternoon between 17:00 - 18:00, which correspond with the typical rush hour  
 5 traffic peaks.

### 6 **Data analysis**

7 To quantitatively represent the physiological status of bats in response to noise  
 8 treatments, multiple parameters based on  $T_{sk}$  were defined and extracted (Fig. 2). The  
 9  $T_{sk-start}$  was defined as the  $T_{sk}$  at the onset of each playback, which represented the  
 10 initial physiological status of bats before noise treatments.  $T_{sk-max}$  was the highest  $T_{sk}$   
 11 achieved after each trial in the five-minute playback experiment. In our experiment,  
 12  $T_{sk-max}$  always occurred before the start of the following trial. The interval between  
 13 two trials within a testing day varied from 9 to 258 minutes, with the median of 39  
 14 minutes. When no increase in  $T_{sk}$  occurred within the five-minute playback period  
 15 (Fig. 2A, red-shaded area),  $T_{sk-max}$  was equal to  $T_{sk-start}$ . A trial was categorized as “*No*  
 16 *Response*” if  $T_{sk-max} = T_{sk-start}$  or as “*Response*” when  $T_{sk-max} > T_{sk-start}$ . The proportion  
 17 of responses for an individual bat was the number of *Response* trials for that subject  
 18 divided by the number of total trials that the bat participated in.  $T_{sk-max}$  only represents  
 19 the physiological status of a bat at a single time point, instead of over a period of time,  
 20 and thus may not be a close approximation of the overall response. Also, by our  
 21 definition,  $T_{sk-max}$  could not be used to distinguish between trials in which the bats did  
 22 not change  $T_{sk}$ , or between trials in which the bats lowered the  $T_{sk}$ . To overcome these  
 23 limitations, we also computed  $\Delta T_{sk-sum}$  by summing  $\Delta T_{sk}$  across a certain time period  
 24 after the onset of the playback. The change in  $T_{sk}$  relative to  $T_{sk-start}$  for each sampled  
 25 time point was denoted as the  $\Delta T_{sk}$  for that parameter (Fig. 2A). For example,  $\Delta T_{sk-max}$   
 26  $= T_{sk-max} - T_{sk-start}$ . For the five-minute playback experiment, there was a trade-off  
 27 between data extrapolation and underrepresentation in determining the time period for  
 28 calculating  $\Delta T_{sk-sum}$ . The time, i.e. the minutes after the onset of the playback when  
 29  $T_{sk-max}$  was reached, varied between 2 and 127 minutes in different trials, with the  
 30 median of 7 minutes. We chose a time period of 15 minutes, which balanced the  
 31 number of data extrapolation and underrepresentation, based on our dataset. In other  
 32 words, setting the time period at 15 minutes minimized the number of trials that ended  
 33 before the time limit, and thus required data extrapolation, and also minimized the  
 34 number of trials for which the  $T_{sk-max}$  occurred later than the time. Specifically, in



1 about 6% of the trials (all of which were *No Response* trials), a new trial started  
2 sooner than 15 minutes after the onset of the previous trial. For these trials, we  
3 extrapolated the missing values for the last few data points (between 1 and 6) by  
4 taking the last recorded  $T_{sk}$ . In about 7% of the total trials the  $T_{sk-max}$  occurred more  
5 than 15 minutes after the onset of the playback. For these, we only considered data  
6 points within 15 minutes after the onset of the playback.

7 The  $T_b$  distinguishing torpor from normothermy was calculated following the  
8 equation proposed by Willis (2007) ( $T_{b-onset} - 1$  SE). To be conservative, we used the  
9 minimum values for both the body mass (BM, minimum value = 21.5 g) and the  
10 ambient temperature ( $T_a$ , minimum value = 15 °C) in our calculations. Moreover,  $T_b$   
11 might be as much as 2.6 or 3.3°C higher than the corresponding  $T_{sk}$  (Audet and  
12 Thomas, 1996; Barclay et al., 1996). Taken together, the calculated  $T_{sk}$  differentiating  
13 torpor from normothermy in our experiment was 30 or 29.3°C. Based on a threshold  
14 of 29°C, 137 of 180 trials in this experiment were performed when bats were in  
15 torpor. The analyses for the five-minute experiments were thus limited to these 137  
16 trials.

17 Statistical analysis was performed for the five-minute experiment in SPSS (version  
18 21, IBM Corporation, New York, US). Except for the Pearson Correlation analysis,  
19 all other statistical analyses were conducted with generalized linear mixed models  
20 (GLMMs), and both linear and binary probit link functions were selected according to  
21 the data probability distribution. Initially, we built a GLMM for each of the three  
22 response variables (i.e. the proportion of responses,  $\Delta T_{sk-max}$ , and  $\Delta T_{sk-sum}$ ) by setting  
23 the individual identity as a random effect and by including all the potential  
24 explanatory factors as fixed effect (i.e.  $T_{sk-start}$ , noise type, the time of day for noise  
25 exposure, the order of noise presentation, the first or second time of noise repetition,  
26 and the waiting time between the trials). Each model was then optimized by stepwise  
27 variable removal to achieve the smallest value of the corrected Akaike Information  
28 Criteria (AICc) (Burnham and Anderson, 2002; Burnham et al., 2011). For pair-wise  
29 comparisons, the Sequential Šidák method was used (Holm, 1979).

30

## RESULTS

31 In the five-minute experiment, torpid bats responded to noise by raising their  $T_{sk}$ . The  
32 lowest  $T_{sk-start}$  at which one bat responded to the noise stimuli (bat colony noise and  
33 bird noise) by elevating  $T_{sk}$  within the five-minute noise exposure period was 17.6°C.  
34 For bats with a  $T_{sk-start}$  above 17.6°C, an increase of  $T_{sk}$  was observed for all noise

1 types. There was a clear effect of noise type on roosting bats (Fig. 3; Fig. S1). All  
 2 three parameters showed that colony and vegetation noise had the strongest effects on  
 3 torpid bats, whereas bats showed the weakest responses to traffic noise treatments.  
 4 Specifically, the greatest  $\Delta T_{sk-max}$ , the greatest  $\Delta T_{sk-sum}$ , and the highest proportion of  
 5 responses were all associated with colony and vegetation noise treatments. The effects  
 6 between colony and vegetation noise treatments however, did not differ significantly  
 7 (Sequential Šidák, all  $P > 0.05$ ). On the other hand, the variation of the median values  
 8 of  $T_{sk-start}$  between different noise types was small ( $\pm 1.26^\circ\text{C}$ , Fig. S2) relative to the  
 9 average increase in  $T_{sk}$  for colony and vegetation noise treatments (Fig. 3C).

10 In addition to the effects of different types of noise, the GLMMs indicated that the  
 11 time of day when bats were tested (e.g. 8:00 vs. 20:00) affected the responses  
 12 significantly. From morning towards evening when the nocturnal active phase of bats  
 13 approached, the responses of torpid bats became progressively stronger as indicated  
 14 by greater  $\Delta T_{sk-max}$  (GLMM,  $P < 0.05$ ) and  $\Delta T_{sk-sum}$  (GLMM,  $P < 0.01$ ). However, all  
 15 three parameters consistently suggested that lower  $T_{sk-start}$  did not result in a lower  
 16 proportion of responses, smaller  $\Delta T_{sk-max}$ , or smaller  $\Delta T_{sk-sum}$  (GLMMs, all  $P > 0.05$ ).

17 Based on both the five-minute and one-hour experiments, we found that bats  
 18 rapidly habituated to noise. The proportion of responses in the five-minute experiment  
 19 decreased significantly when bats were exposed to the same noise type a second time  
 20 (Fig. 4A, GLMM,  $P < 0.05$ ). Particularly, habituation to traffic noise was more  
 21 pronounced than to bird, colony, and vegetation noise. Moreover, a profound decline  
 22 of  $T_{sk}$ , averaged for all tested bats, occurred 11 minutes after noise onset and further  
 23 dropped towards the end of the noise exposure (Fig. 4B).

24

25

## DISCUSSION

26 Based on changes in  $T_{sk}$ , we found that (I) traffic noise was less disturbing than  
 27 colony or vegetation noise for torpid bats; (II) the time of day when bats were  
 28 exposed to noise affected their response; (III) an individual's  $T_{sk-start}$  was not related to  
 29 their responsiveness; and (IV) torpid bats shown rapid noise habituation capabilities.  
 30 In this experiment we relied on three parameters (i.e. the proportion of responses, the  
 31  $\Delta T_{sk-max}$  and the  $\Delta T_{sk-sum}$ ) to make inferences, although parameters were not absolutely  
 32 independent from each other (e.g. Fig. S3). We chose these three parameters for two  
 33 reasons. First, despite the fact that in many cases all three parameters gave similar  
 34 results, in two cases they did not (Results II and IV). Second, both the proportion of

1 responses and the  $\Delta T_{\text{sk-max}}$  represent only the physiological status of bats at a single  
2 time point, while the  $\Delta T_{\text{sk-sum}}$  may approximate the overall response more closely.  
3 Thus, by ignoring any single parameter, some information would have certainly been  
4 lost.

### 5 **Noise type and the strength of response**

6 Bats responded differently to different noise stimuli. They responded the least to  
7 traffic noise and the most to the vegetation and colony noise playbacks. Since the  
8 vegetation noise, which had the lowest playback amplitude, impacted the bats most  
9 strongly, we are confident that playback amplitude alone was not a good predictor.  
10 Rather, the frequency with the highest energy might be more important in determining  
11 the responses of the bats. In general, all bats have good hearing sensitivity in the  
12 frequency ranges of both their social and echolocation calls and these frequencies are  
13 usually above 10 kHz (Neuweiler, 1990; Pfalzer and Kusch, 2003; Hoffmann et al.,  
14 2008). Both traffic noise and bird noise stimuli had the most energy below 5 kHz  
15 (Fig. 1), which falls outside of their range of best hearing sensitivity (Schwarz, 2007).  
16 Thus, the different responses of bats to noise stimuli may simply result from the  
17 unequal hearing sensitivity to the different frequency ranges represented in the  
18 different noise types.

19 It is interesting to note that the vegetation noise, although set with 20 dB lower  
20 amplitude and a narrower frequency band, had an effect equal to that of colony noise.  
21 This is in line with the results from the foraging performance of this species (Schaub  
22 et al., 2008). As passive listening foragers, *M. myotis* are extremely sensitive to the  
23 rustling sounds of their prey (Schwarz, 2007). We suggest that the strong response of  
24 bats to vegetation noise may be attributable to the similarity of the frequency range  
25 between the vegetation noise and the rustling sound of their prey. Additionally, our  
26 results may also be explained by the likely deterioration of the hearing sensitivity to  
27 the higher frequencies of the colony noise when bats were in torpor.  
28 Neurophysiological results from *Myotis lucifugus* suggested that the frequency above  
29 which no responses were elicited decreased gradually with decreasing  $T_b$  (Harrison,  
30 1965). Specifically, they found that *M. lucifugus* did not respond to sound frequencies  
31 higher than 40 kHz when the  $T_b$  was lower than 20 °C. If this is also true in our study  
32 species, the higher frequency parts of the colony noise (> 40 kHz) might have affected  
33 the torpid bats less than was indicated by the original spectrogram, due to their  
34 reduced hearing sensitivity to higher frequencies.

## Torpid bats and noise disturbance

1  
2 There has been a long-standing interest in the response of hibernating bats to  
3 non-tactile human disturbances such as noise, light, and environmental temperature  
4 (Ransome, 1971; Speakman et al., 1991; Thomas, 1995; Park et al., 2000; Humphries  
5 et al., 2002; Speakman and Thomas, 2003; Haarsma and de Hullu, 2012). The first  
6 studies in the laboratory showed that hibernating bats were affected little by the  
7 non-tactile disturbances (Speakman et al., 1991). Subsequently, a field study by  
8 Thomas (1995) demonstrated that hibernating bats were indeed sensitive to non-tactile  
9 disturbances. As noted by the author, this difference may have been attributable to  
10 some bats being in shallow torpor or even normothermic status in the field  
11 experiment, which are both states that are seldom exhibited in laboratory studies  
12 (Thomas, 1995; Speakman and Thomas, 2003). However, it is not yet known which  
13 factor(s) (i.e. noise, torch light or temperature change) was directly responsible. Here  
14 we provide empirical evidence that noise disturbance alone can increase  $T_{sk}$  of torpid  
15 bats. Even bats with a  $T_{sk}$  as low as  $\sim 20^{\circ}\text{C}$  responded to all types of noise  
16 disturbances.

17 Moreover, it was surprising that we found that  $T_{sk\text{-start}}$  did not affect the response of  
18 torpid bats to noise disturbance. Several neurophysiological studies have shown that  
19 both the peripheral and brainstem auditory systems become less responsive when  $T_b$   
20 declines (Coats, 1965; Rossi and Britt, 1984). It is known that torpid animals become  
21 more sensitive to stimuli at the end of the torpor bout, when they have higher  $T_b$   
22 (Kristoffersson and Soivio, 1964; Twente and Twente, 1968). At first sight, it appears  
23 that our finding contradicts these well-established views. However, this is not  
24 completely true. In this experiment, differences in the  $T_b$  of the bats at the onset of  
25 noise disturbance, as indicated by  $T_{sk\text{-start}}$ , do not necessarily mean that they were in  
26 different phases of torpor. In other words, bats with a lower  $T_{sk\text{-start}}$  did not necessarily  
27 correspond to the earlier phase of torpor, and vice versa. This is fundamentally  
28 different from the natural situation in which  $T_b$  might be correlated with the phase of  
29 torpor. Nevertheless, we agree that bats with a lower  $T_{sk\text{-start}}$  might have had reduced  
30 hearing sensitivity in this experiment, considering the neurophysiological results  
31 obtained from *M. lucifugus* at a similar  $T_b$  (Harrison, 1965). As indicated by our  
32 results, it seems that a slightly reduced hearing sensitivity may not necessarily  
33 correspond to a weaker physiological or behavioural response. However, it is logical

1 to predict that torpid bats would become totally unresponsive when  $T_b$  is sufficiently  
2 low, such as in hibernation situations (Speakman et al., 1991).

3 Furthermore, we found that bats became more sensitive to noise when their active  
4 phase was approaching (i.e. the dusk), which suggests that the time of day affects the  
5 response of torpid bats to noise disturbance. In general, small nocturnal mammals,  
6 including many bat species, exhibit a strong propensity for torpor in the early  
7 morning, when daily  $T_a$  is lowest (Körtner and Geiser, 2000; Turbill et al., 2008).  
8 Moreover, torpid animals become more sensitive to a variety of external stimulations  
9 as the bout of torpor progresses (Lyman et al., 1982). As a result, tests conducted in  
10 the morning thus are more likely to occur in the early phases of torpor, when torpid  
11 animals are generally less sensitive to disturbances (Kristoffersson and Soivio, 1964;  
12 Twente and Twente, 1968). Turbill et al. (2008) have provided clear evidence that the  
13 time of day affects the critical arousal temperature of torpid bats. The critical arousal  
14 temperature was lower when external heating occurred later in the day. This  
15 phenomenon might constitute an underlying mechanism for the higher sensitivity of  
16 torpid animals in the later afternoon to disturbances.

17 Lastly, we found evidence that torpid bats can rapidly habituate to repeated and to  
18 prolonged noise disturbance. This indicates that a decline in  $T_b$  during torpor does not  
19 inhibit animal's habituation ability. Habituation occurs for virtually all behavioural  
20 responses in virtually all animals (Thompson, 2009). In general, animals show  
21 decreased behavioural responses to repeated stimulations (Rankin et al., 2009). The  
22 underlying processes of behavioural habituation are associated with the central  
23 nervous system (Thompson, 2009). As the central nervous system is among one of the  
24 few processes that continue functioning during torpor, in contrast to a diversity of  
25 suppressed physiological processes at the same time (Carey et al., 2003), it is  
26 reasonable that torpid bats could exhibit habituation to noise in this experiment.  
27 Moreover, it is well known that the weaker the stimulus, the more rapid and/or more  
28 pronounced is the habituation (Rankin et al., 2009; Thompson, 2009). Hence, the  
29 result that habituation is more pronounced to traffic noise than to other noise stimuli  
30 suggests that traffic noise is less disturbing for torpid bats than is colony or vegetation  
31 noise.

32

33

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10

11

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15

16

### LIST OF ABBREVIATIONS

17 °C = degree Celsius

18  $T_a$  = ambient temperature

19  $T_b$  = body temperature

20  $T_{sk}$  = skin temperature

21  $T_{sk-max}$  = highest skin temperature after the onset of the playback

22  $T_{sk-start}$  = skin temperature at the onset of the playback

23  $\Delta T_{sk} = T_{sk} - T_{sk-start}$

24  $\Delta T_{sk-max} = T_{sk-max} - T_{sk-start}$

25  $\Delta T_{sk-sum}$  = the sum of the  $\Delta T_{sk}$  for 15 minutes

26

1 **Figure captions**

2 Figure 1. Spectrograms of acoustic stimuli used in the experiment. The three types of  
3 traffic-like noise correspond to real traffic noise recorded at 100 m, 50 m and 25 m  
4 distance from a highway, respectively.

5

6 Figure 2. (A) Illustration of parameter definitions used for the analysis of skin  
7 temperature. The red-shaded area indicates the five-minute acoustic playback period.  
8  $T_{sk-start}$  is defined as the  $T_{sk}$  at the onset of the playback (arrows below the line).  $T_{sk-max}$   
9 is defined as the maximum  $T_{sk}$  after the onset of the playback (arrows above the line).  
10 When there is no increase of  $T_{sk}$  during the five-minute playback (left shaded area),  
11  $T_{sk-max}$  equals  $T_{sk-start}$ . When there is an increase of  $T_{sk}$  during the five minutes  
12 playback (right shaded area)  $T_{sk-max}$  is the maximum  $T_{sk}$  after the onset of the  
13 playback. At each time point  $\Delta T_{sk}$  is the change of  $T_{sk}$  relative to  $T_{sk-start}$ , which is  
14 depicted by the height of the green line. The line sections in blue highlight the time  
15 periods used for calculating  $\Delta T_{sk-sum}$ . (B) The ambient temperature of the  
16 experimental room, and (C) the corresponding  $T_{sk}$  of a bat during one exemplary  
17 experimental day. The red line section shows the time of day when trials were  
18 conducted.

19

20 Figure 3. Responses of torpid bats to different noise stimuli. (A) Average change in  
21 skin temperature of all bats during the 15 minutes after the onset of the playback. The  
22 red-shaded area shows the five-minute playback period. Each line shows the average  
23 response of bats to one noise type. The proportion of responses (B),  $\Delta T_{sk-max}$  (C), and  
24  $\Delta T_{sk-sum}$  (D) for different noise types. Asterisks above a noise condition indicate the  
25 statistical difference from the line-connected condition without an asterisk on the left.  
26 (\*  $0.01 \leq P < 0.05$ , \*\*  $0.001 \leq P < 0.01$  and \*\*\*  $P < 0.001$ ).

27

28 Figure 4. (A) The proportion of responses of torpid bats to the first and second  
29 exposure of different noise stimuli in the five-minutes experiment. The number  
30 associated with each bar indicates the sample size (i.e. the number of individuals) for  
31 each condition. (B) Mean skin temperature of all bats over time in the one-hour  
32 experiment. Dotted lines show 1 standard error of the mean (s.e.m).

33

1

**SUPPLEMENTARY MATERIAL**

2 Figure S1. The change in skin temperature of bats in response to the playback of  
3 different types of acoustic stimuli. Each line represents a single playback event (trial)  
4 and it shows the relative skin temperature of the tested bat to the skin temperature at  
5 the onset of the playback.

6 Figure S2. Skin temperature at the onset of noise playback for each noise type.  
7 Medians, horizontal lines inside boxes; first and third quartiles, upper and lower  
8 margins of boxes, respectively; 5% and 95% quantile, small horizontal bars above and  
9 below boxes, respectively.

10 Figure S3. Two response variables ( $\Delta T_{sk-max}$  and  $\Delta T_{sk-sum}$ ) are positively correlated.  
11 The red line is plotted based on the results of the linear polynomial fit. ' $r$ ' is the  
12 correlation coefficient and ' $P$ ' is the related statistical significance derived from  
13 Pearson Correlation.

14

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