

RESEARCH ARTICLE

Are torpid bats immune to anthropogenic noise?

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ABSTRACT

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

KEY WORDS: Anthropogenic noise, Habituation, *Myotis myotis*, Roosting bat, Torpor, Traffic noise

INTRODUCTION

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

Bats, the second largest and ecologically most diverse mammalian order, are both ecologically and economically important as agents of pest control, pollination and seed dispersal (Boyles et al., 2011; Kunz et al., 2011). As bats are highly mobile and can travel large distances between roosts and foraging grounds, they can be considerably affected by road systems. Road effects on bats include

vehicle mortality (Kiefer et al., 1995; Lesiński et al., 2011), light pollution (Rydell, 1992; Stone et al., 2009; Stone et al., 2012), and the barrier and edge effects (Kerth and Melber, 2009; Abbott et al., 2012; Berthiusen and Altringham, 2012). Moreover, two recent studies found that bats may avoid foraging areas with loud traffic noise that can reduce their foraging efficiency (Schaub et al., 2008; Siemers and Schaub, 2011). Nevertheless, a great number of bat species roost in places exposed to loud anthropogenic noise (Altringham, 2012).

Two aspects of bat biology may minimize the potential adverse effects of anthropogenic noise on roosting bats. First, many bat species conserve energy by engaging in torpor, which is characterized by a reduction of metabolic rate, body temperature (T_b) and other physiological processes (Speakman and Thomas, 2003; Geiser, 2004). Although the central nervous system of hibernators and daily heterotherms continues to function at low T_b , the peripheral and brainstem auditory systems become less responsive as T_b declines (Coats, 1965; Rossi and Britt, 1984). This is true for hibernating bats and it has been corroborated in one neurophysiological and one behavioural experiment independently (Harrison, 1965; Speakman et al., 1991). Specifically, results from *Myotis lucifugus* suggested that the frequency above which no responses were elicited decreased continually with decreasing body temperature (Harrison, 1965). Second, nearly all bats are most sensitive to frequencies $>10\text{ kHz}$ (Neuweiler, 1990; Pfalzer and Kusch, 2003; Hoffmann et al., 2008), which is well above the frequencies in anthropogenic sounds (such as traffic noise) that typically contain the most sound energy. Consequently, the purpose of our study was to assess whether torpid bats were sensitive to anthropogenic noise. We experimentally tested this hypothesis with the greater mouse-eared bat (*Myotis myotis* Borkhausen 1797), a species that is commonly found to roost in noisy areas such as under bridges (Dietz et al., 2009). Virtually all animal species show a decrease in behavioural responses to repeated stimulation (Rankin et al., 2009; Thompson, 2009), therefore we additionally assessed whether torpid bats became habituated to noise with repeated exposure.

RESULTS

In the 5 min experiment, torpid bats responded to noise by raising their skin temperature (T_{sk}). The lowest skin temperature at the onset of the playback ($T_{sk,start}$) at which one bat responded to the noise stimuli (bat colony noise and bird noise) by elevating T_{sk} within the 5 min noise exposure period was 17.6°C . For bats with a $T_{sk,start}$ above 17.6°C , an increase in T_{sk} was observed for all noise types. There was a clear effect of noise type on roosting bats (Fig. 1; supplementary material Fig. S1). All three parameters showed that colony and vegetation noise had the strongest effects on torpid bats, whereas bats showed the weakest responses to traffic noise treatments. Specifically, the greatest change in skin temperature after the playback ($\Delta T_{sk,max}$), the greatest sum of the change in skin temperature ($\Delta T_{sk,sum}$) and the highest proportion of responses were all associated with colony and vegetation noise treatments. The

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List of abbreviations

T_a	ambient temperature
T_b	body temperature
T_{sk}	skin temperature
$T_{sk,max}$	highest skin temperature after the onset of the playback
$T_{sk,start}$	skin temperature at the onset of the playback
ΔT_{sk}	$T_{sk} - T_{sk,start}$
$\Delta T_{sk,max}$	$T_{sk,max} - T_{sk,start}$
$\Delta T_{sk,sum}$	the sum of the ΔT_{sk} for 15 min

effects between colony and vegetation noise treatments, however, did not differ significantly (sequential Šidák, all $P > 0.05$). However, the variation of the median values of $T_{sk,start}$ between different noise types was small ($\pm 1.26^\circ\text{C}$; supplementary material Fig. S2) relative to the average increase in T_{sk} for colony and vegetation noise treatments (Fig. 1C).

In addition to the effects of different types of noise, the generalized linear mixed models (GLMMs) indicated that the time of day when bats were tested (e.g. 08:00 h versus 20:00 h) affected the responses. From morning towards evening when the nocturnal active phase of bats approached, the responses of torpid bats became progressively stronger as indicated by greater $\Delta T_{sk,max}$ (GLMM, $P < 0.05$) and $\Delta T_{sk,sum}$ (GLMM, $P < 0.01$). However, all three parameters consistently suggested that lower $T_{sk,start}$ did not result in a lower proportion of responses, smaller $\Delta T_{sk,max}$ or smaller $\Delta T_{sk,sum}$ (GLMMs, all $P > 0.05$).

Based on both the 5 min and 1 h experiments, we found that bats rapidly habituated to noise. The proportion of responses in the 5 min experiment decreased significantly when bats were exposed to the same noise type a second time (Fig. 2A, GLMM, $P < 0.05$). In particular, habituation to traffic noise was more pronounced than to bird, colony and vegetation noise. Moreover, a profound decline in T_{sk} , averaged for all tested bats, occurred 11 min after noise onset and further dropped towards the end of the noise exposure (Fig. 2B).

DISCUSSION

Based on changes in T_{sk} , we found that (i) traffic noise was less disturbing than colony or vegetation noise for torpid bats; (ii) the time of day when bats were exposed to noise affected their response; (iii) an individual's $T_{sk,start}$ was not related to their responsiveness; and (iv) torpid bats showed rapid noise habituation capabilities. In this experiment we relied on three parameters (i.e. the proportion of responses, the $\Delta T_{sk,max}$ and the $\Delta T_{sk,sum}$) to make inferences, although the parameters were not absolutely independent from each other (e.g. supplementary material Fig. S3). We chose these three parameters for two reasons. First, despite the fact that in many cases all three parameters gave similar results, in two cases they did not (results i and iv above). Second, both the proportion of responses and the $\Delta T_{sk,max}$ represent only the physiological status of bats at a single time point, while the $\Delta T_{sk,sum}$ may approximate the overall response more closely. Thus, by ignoring any single parameter, some information would have certainly been lost.

Noise type and the strength of response

Bats responded differently to different noise stimuli. They responded least to traffic noise and the most to the vegetation and colony noise playbacks. As the vegetation noise, which had the lowest playback amplitude, impacted the bats most strongly, we are confident that playback amplitude alone was not a good predictor. Rather, the frequency with the highest energy might be more important in determining the responses of the bats. In general, all bats have good hearing sensitivity in the frequency ranges of both their social and echolocation calls and these frequencies are usually above 10 kHz (Neuweiler, 1990; Pfalzer and Kusch, 2003; Hoffmann et al., 2008). Both traffic noise and bird noise stimuli had the most energy below 5 kHz (Fig. 3), which falls outside of the bats' range of greatest hearing sensitivity (Schwarz, 2007). Thus, the different responses of bats to noise stimuli may simply result from the unequal hearing sensitivity to the different frequency ranges represented in the different noise types.

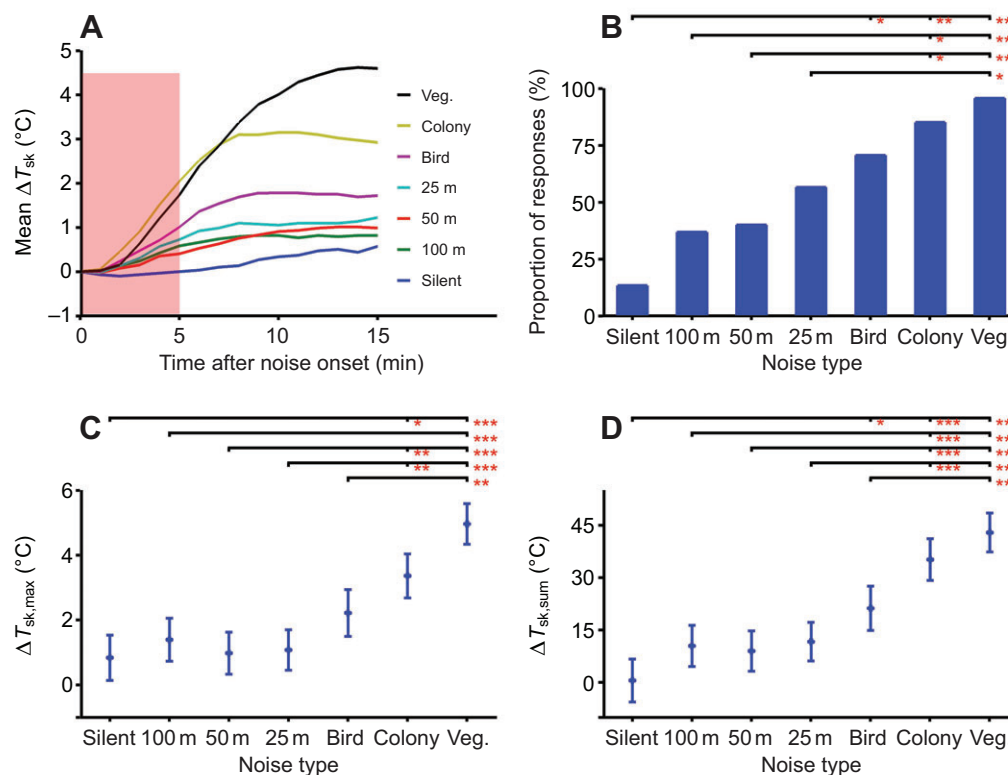


Fig. 1. Responses of torpid bats to different noise stimuli. (A) Average change in skin temperature (T_{sk}) of all bats during the 15 min after the onset of the playback. The red-shaded area shows the 5 min playback period. Each line shows the average response of bats to one noise type. (B) The proportion of responses, (C) the change in maximum T_{sk} ($\Delta T_{sk,max}$, mean \pm s.e.m.) and (D) the sum of ΔT_{sk} ($\Delta T_{sk,sum}$, mean \pm s.e.m.) for the different noise types. Asterisks above a noise condition indicate a significant difference from the condition to the left, indicated by the line (* $0.01 \leq P < 0.05$, ** $0.001 \leq P < 0.01$ and *** $P < 0.001$).

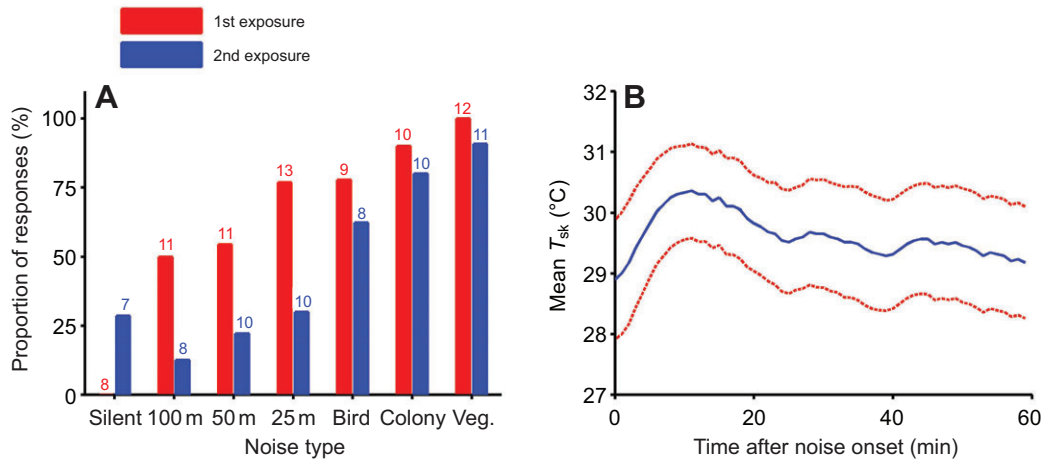


Fig. 2. Habituation capability of torpid bats to repeated and to prolonged noise exposure. (A) The proportion of responses of torpid bats to the first and second exposure to different noise stimuli in the 5 min experiment. Numbers above the bars indicate the sample size (i.e. the number of individuals) for each condition. (B) Mean skin temperature of all bats over time in the 1 h experiment. Dotted lines show ± 1 s.e.m.

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

Torpid bats and noise disturbance

There has been a long-standing interest in the response of hibernating bats to non-tactile human disturbances such as noise, light and environmental temperature (Ransome, 1971; Speakman et

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

Moreover, it was surprising that we found that $T_{sk, \text{start}}$ did not affect the response of torpid bats to noise disturbance. Several neurophysiological studies have shown that both the peripheral and brainstem auditory systems become less responsive when T_b declines (Coats, 1965; Rossi and Britt, 1984). It is known that torpid animals become more sensitive to stimuli at the end of the torpor bout, when they have higher T_b (Kristofferson and Soivio, 1964; Twente and Twente, 1968). At first sight, it appears that our finding contradicts these well-established views. However, this is not completely true. In this experiment, differences in the T_b of the bats

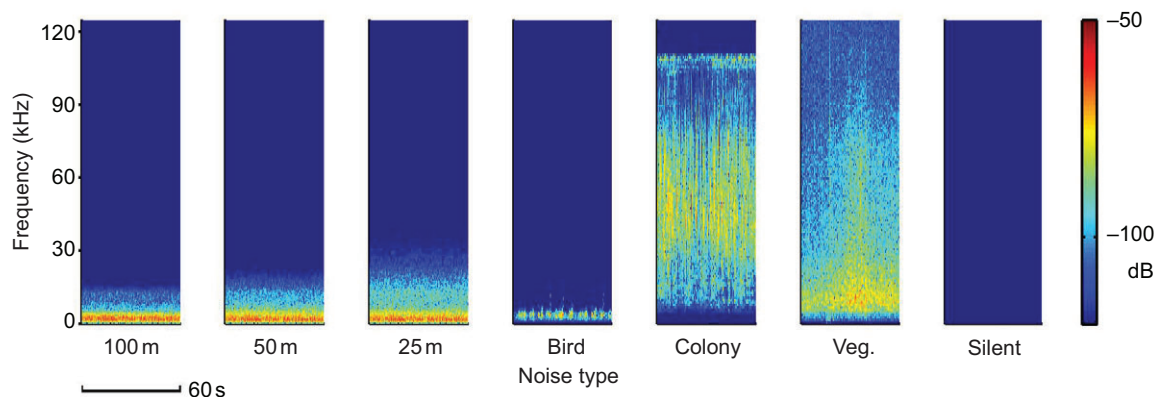


Fig. 3. Spectrograms of acoustic stimuli used in the experiment. The three types of traffic-like noise correspond to real traffic noise recorded at 100, 50 and 25 m distance from a highway.

at the onset of noise disturbance, as indicated by $T_{sk,start}$, do not necessarily mean that they were in different phases of torpor. In other words, bats with a lower $T_{sk,start}$ did not necessarily correspond to the earlier phase of torpor, and vice versa. This is fundamentally different from the natural situation in which T_b might be correlated with the phase of torpor. Nevertheless, we agree that bats with a lower $T_{sk,start}$ might have had reduced hearing sensitivity in this experiment, considering the neurophysiological results obtained from *M. lucifugus* at a similar T_b (Harrison, 1965). As indicated by our results, it seems that a slightly reduced hearing sensitivity may not necessarily correspond to a weaker physiological or behavioural response. However, it is logical to predict that torpid bats would become totally unresponsive when T_b is sufficiently low, such as in hibernation situations (Speakman et al., 1991).

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

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

MATERIALS AND METHODS

Study site and animals

This study was conducted at the Tabachka Bat Research Station of the Sensory Ecology Group (MPI Seewiesen), which is run in cooperation with the Directorate of the Rusenski Lom Nature Park in the district of Ruse, northern Bulgaria. The experiment was performed between late May and July 2012. Fifteen male greater mouse-eared bats (body mass at capture, 27.8 ± 2.1 g) were captured with a harp trap at the Orlova Chuka cave. One bat managed to remove its temperature logger during the night before the test and hence only 14 of them were tested in the laboratory. When not being tested, the bats were housed together in a cage ($49 \times 35 \times 37$ cm, length \times width \times height) at an ambient temperature between 18 and 24°C and

a relative humidity around 75%, and had access to water *ad libitum*. All bats were fed mealworms (larval instars of *Tenebrio molitor*) *ad libitum* at 22:00 h every evening. Capture, husbandry and behavioural studies were carried out under the licence of the responsible Bulgarian authorities (licence no. 465/29.06.2012). All bats were released in good health into the wild after the experiment.

Acoustic stimuli

We created a series of sound files for playback, containing different types of noise: bird noise, bat colony noise, vegetation noise, traffic-like noise and silence. All playback files (Fig. 3) were created in Adobe Audition 5.5 and had a sampling rate of 250 kHz. With the exception of the traffic noise and the silent sound files, which were digitally generated, the frequency and amplitude of the noise files varied with time. To standardize variation in the noise recordings, we created 14 different playback files for each noise type (i.e. bird noise, colony noise and vegetation noise) by randomly arranging sound segments cut from the original recordings. The silent sound (control) was generated by setting all amplitude values to zero. All files were high-pass filtered at 1 kHz (digital fast Fourier transform filter, 2048 points, Blackman window) to remove sounds that were probably not audible to the bats and to avoid damage to the speakers (Siemers and Schaub, 2011).

For the 25 and 50 m traffic noise, we digitally generated noise that would correspond to the loudest average 0.5 s sound made by a passing vehicle 25 and 50 m away on a highway (Siemers and Schaub, 2011). Although the traffic noise was audible at 100 m distance, our recordings were unsuitable for playback because the traffic noise was totally masked by the natural background noise and the thermal noise of the microphone itself. Therefore, to reproduce realistic playback files for traffic noise at 100 m, we first made recordings at a distance of 7.5 m from the highway edge (Schaub et al., 2008), which had a high signal-to-noise ratio to serve as a baseline. We then computed the average power spectral density of 50 passing cars and 50 passing trucks at speeds of ~ 80 km h⁻¹. Based on these values and the decay of amplitude over distance, we generated the 100 m traffic noise digitally. For the bird noise, recordings of the vocalizations of four bird species (*Hirundo rustica*, *Oriolus oriolus*, *Passer domesticus* and *Sturnus vulgaris*), which are typically found in our study area were extracted from a CD (Bergmann et al., 2008). Each bird noise file contained the vocalizations of all four species arranged randomly. For the bat colony noise, we made recordings between 15:00 h and 16:00 h in an afternoon under the large colony from which our bats were collected. This colony consists primarily of four species, *Myotis myotis*, *Myotis blythii oxygnathus*, *Rhinolophus mehelyi* and *Rhinolophus euryale*. For the vegetation noise, we made recordings ~ 10 cm from a tree (*Tilia platyphyllos*) on a windy afternoon, with average and maximum wind speeds of 2.77 and 4.07 m s⁻¹, respectively (Skywatch Atmos Anemometer, Skyview Systems Ltd, Suffolk, UK). Both the colony noise and the vegetation noise were recorded with a broadband microphone (UltraSoundGate CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) connected to an ultrasound recording interface (UltraSoundGate 116H, Avisoft Bioacoustics) at a sampling frequency of 250 kHz. The microphone had a flat frequency response between the frequency range 10–200 kHz, but frequencies below 10 kHz were under-represented to varying extents.

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

Experimental setup

We used T_{sk} as a means to determine whether environmental noise disturbs torpid bats. Changes in T_b are reflected by changes in T_{sk} (Audet and Thomas, 1996; Barclay et al., 1996). Given a T_a that is below the T_b of torpid animals and a lack of an external heat source, internal heat production is the

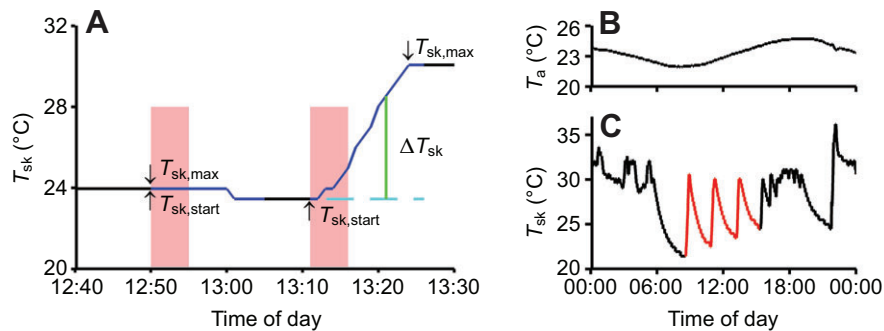


Fig. 4. Illustrations of parameter definition, room temperature and bat skin temperature. (A) Illustration of parameter definitions used for the analysis of skin temperature. The red-shaded area indicates the 5 min acoustic playback period. $T_{sk,start}$ is defined as the T_{sk} at the onset of the playback (arrows below the line). $T_{sk,max}$ is defined as the maximum T_{sk} after the onset of the playback (arrows above the line). When there is no increase in T_{sk} during the 5 min playback (left shaded area), $T_{sk,max}$ equals $T_{sk,start}$. When there is an increase in T_{sk} during the 5 min playback (right shaded area), $T_{sk,max}$ is the maximum T_{sk} after the onset of the playback. At each time point, ΔT_{sk} is the change in T_{sk} relative to $T_{sk,start}$, which is depicted by the height of the green line. The line sections in blue highlight the time periods used for calculating $\Delta T_{sk,sum}$, the sum of the change in skin temperature. (B) The ambient temperature of the experimental room, and (C) the corresponding T_{sk} of a bat during one exemplary experimental day. The red line section shows the time of day when trials were conducted.

only way to raise T_b (Lyman et al., 1982). In our experiment, T_{sk} of the bats (and hence T_b) was never below T_a , which was fairly stable over the course of a day (maximum $\Delta T_a = 2.8^\circ\text{C}$). As a result, an increase in T_{sk} in response to stimuli must be active and may be taken as a sign of disturbance.

One night before the experiment, a small patch of fur between the scapulae of the respective bat was removed and a miniature temperature logger [*ca.* $18 \times 12.5 \text{ mm}^2$, modified iButton (after Lovegrove, 2009), Maxim Integrated Products Inc., Sunnyvale, CA, USA] was glued to the exposed skin with mastic spirit gum (COIDRO-AG, Kems, Switzerland). Below the interscapular skin, bats have a large repository of brown adipose tissue (Neuweiler, 2000), which is involved in active heat production (Withers, 1992). Even if warming up the whole body takes some time, this spot becomes very warm compared with the surrounding skin, particularly at the beginning of the warming up process. Thus, measuring changes in T_{sk} at this spot allows high accuracy in determining the timing of the start of the warming up process. The temperature loggers recorded T_{sk} every 60 s to the nearest 0.5°C . The loggers were three-point calibrated in a cooling incubator (KB 53, Binder GmbH, Möhringen-Tuttlingen, Germany) at 0, 25 and 50°C . After the experiment, the logger was removed with mild spirit gum remover (COIDRO-AG, Kems, Switzerland).

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

Two types of tests were conducted that differed in the duration of the noise exposure during each trial. The 5 min experiment was used to assess the sensitivity of torpid bats to different types of noise and to assess their potential for habituation. The 1 h experiment was only used to assess the habituation ability. In the 5 min playback experiment, each individual was tested on its own on two consecutive days. On each day, the bat was subjected to a pseudo-randomized sequence of playbacks of the seven acoustic stimuli of 5 min duration each (Fig. 3). As playbacks were only triggered when the bat was completely still for at least 1 min, sometimes not

all seven playbacks could be presented within the time frame of the first experimental day. These playbacks were presented on the second day followed by a second presentation of the same noise sequence. Again, sometimes not all of the playbacks of the second sequence could be presented within the time frame of the second experimental day. Hence, only 12 of the 14 individuals were subjected to the same stimulus twice in 2 days. For the other two individuals, only two and three types of stimuli could be repeated on the second day because of their continuous active status. For the 1 min playback experiment, seven bats were subjected to 1 h noise treatment twice and four bats once. For these treatments, only the 25 m traffic noise and the colony noise were used. The 1 h playbacks were conducted after finishing the 5 min trials above, either in the morning between 07:30 h and 08:30 h or in the afternoon between 17:00 h and 18:00 h, which correspond with the typical rush hour traffic peaks.

Data analysis

To quantitatively represent the physiological status of bats in response to noise treatments, multiple parameters based on T_{sk} were defined and extracted (Fig. 4). The $T_{sk,start}$ was defined as the T_{sk} at the onset of each playback, which represented the initial physiological status of the bats before noise treatments. $T_{sk,max}$ was the highest T_{sk} achieved after each trial in the 5 min playback experiment. In our experiment, $T_{sk,max}$ always occurred before the start of the subsequent trial. The interval between two trials within a testing day varied from 9 to 258 min, with the median of 39 min. When no increase in T_{sk} occurred within the 5 min playback period (Fig. 4A, red-shaded area), $T_{sk,max}$ was equal to $T_{sk,start}$. A trial was categorized as ‘no response’ if $T_{sk,max} = T_{sk,start}$ or as ‘response’ when $T_{sk,max} > T_{sk,start}$. The proportion of responses for an individual bat was the number of ‘response’ trials for that subject divided by the number of total trials that the bat participated in. $T_{sk,max}$ only represents the physiological status of a bat at a single time point, instead of over a period of time, and thus may not be a close approximation of the overall response. Also, by our definition, $T_{sk,max}$ could not be used to distinguish between trials in which the bats did not change T_{sk} , or between trials in which the bats lowered the T_{sk} . To overcome these limitations, we also computed $\Delta T_{sk,sum}$ by summing ΔT_{sk} across a certain time period after the onset of the playback. The change in T_{sk} relative to $T_{sk,start}$ for each sampled time point was denoted as the ΔT_{sk} for that parameter (Fig. 4A). For example, $\Delta T_{sk,max} = T_{sk,max} - T_{sk,start}$. For the 5 min playback experiment, there was a trade-off between data extrapolation and under-representation in determining the time period for calculating $\Delta T_{sk,sum}$. The time, i.e. the minutes after the onset of the playback when $T_{sk,max}$ was reached, varied between 2 and 127 min in different trials, with the median of 7 min. We chose a time period of 15 min, which balanced data extrapolation versus under-representation, based on our dataset. In other words, setting the time period at 15 min minimized the number of trials that ended before the time limit, and thus required data extrapolation, and also minimized the number of trials for which the $T_{sk,max}$ occurred later than the

time limit. Specifically, in about 6% of the trials (all of which were 'no response' trials), a new trial started sooner than 15 min after the onset of the previous trial. For these trials, we extrapolated the missing values for the last few data points (between 1 and 6) by taking the last recorded T_{sk} . In about 7% of the total trials the $T_{sk,max}$ occurred more than 15 min after the onset of the playback. For these, we only considered data points within 15 min of the onset of the playback.

The T_b distinguishing torpor from normothermy was calculated following the equation proposed by Willis (Willis, 2007) ($T_{b,onset}-1$ s.e.). To be conservative, we used the minimum values for both body mass (M_b , minimum value 21.5 g) and T_a (minimum value 15°C) in our calculations. Moreover, T_b might be as much as 2.6 or 3.3°C higher than the corresponding T_{sk} (Audet and Thomas, 1996; Barclay et al., 1996). Taken together, the calculated T_{sk} differentiating torpor from normothermy in our experiment was 30 or 29.3°C. Based on a threshold of 29°C, 137 of 180 trials in this experiment were performed when bats were in torpor. The analyses for the 5 min experiments were thus limited to these 137 trials.

Statistical analysis was performed for the 5 min experiment in SPSS (version 21, IBM Corporation, NY, USA). Except for the Pearson correlation analysis, all other statistical analyses were conducted with GLMMs, and both linear and binary probit link functions were selected according to the data probability distribution. Initially, we built a GLMM for each of the three response variables (i.e. the proportion of responses, $\Delta T_{sk,max}$ and $\Delta T_{sk,sum}$) by setting the individual identity as a random effect and by including all the potential explanatory factors as a fixed effect (i.e. $T_{sk,start}$, noise type, the time of day for noise exposure, the order of noise presentation, the first or second time of noise repetition and the waiting time between the trials). Each model was then optimized by stepwise variable removal to achieve the smallest value of the corrected Akaike information criterion (AICc) (Burnham and Anderson, 2002; Burnham et al., 2011). For pair-wise comparisons, the sequential Šidák method was used (Holm, 1979).

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Competing interests

The authors declare no competing financial interests.

Author contributions

J.L., B.M.S. and B.M.C. conceived and designed the experiment; J.L. and I.M.B. performed the experiment; J.L. drafted the paper; J.L. and B.M.S. revised the paper.

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Supplementary material

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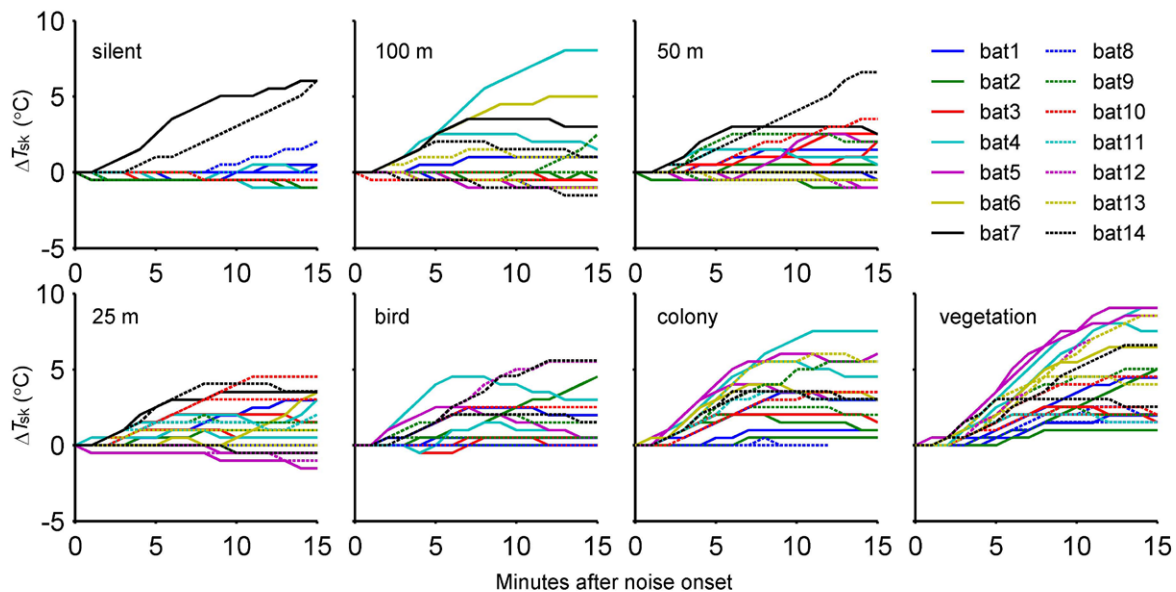


Fig. S1. The change in skin temperature of bats in response to the playback of different types of acoustic stimuli. Each line represents a single playback event (trial) and it shows the relative skin temperature of the tested bat to the skin temperature at the onset of the playback.

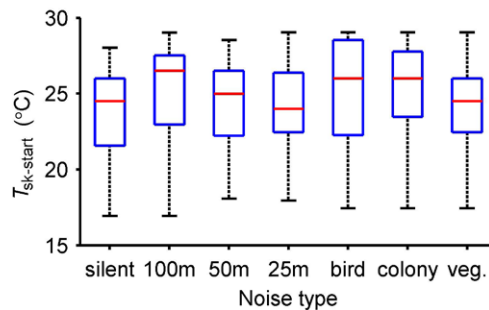


Fig. S2. Skin temperature at the onset of noise playback for each noise type. Medians, horizontal lines inside boxes; first and third quartiles, upper and lower margins of boxes, respectively; 5% and 95% quantile, small horizontal bars above and below boxes, respectively.

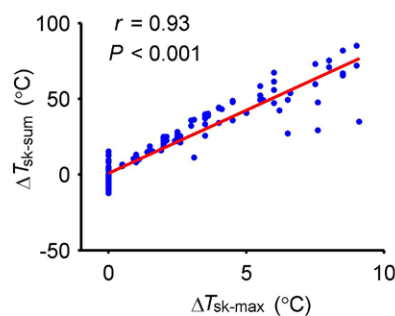


Fig. S3. Two response variables (ΔT_{sk-max} and ΔT_{sk-sum}) are positively correlated. The red line is plotted based on the results of the linear polynomial fit. ' r ' is the correlation coefficient and ' P ' is the related statistical significance derived from Pearson correlation.