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Effect of chronic traffic noise on behavior and physiology of plateau pikas (*Ochotona curzoniae*)

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During the last two decades, numerous studies have shown the effects of traffic noise on animal vocal communication. However, studies on the influences of traffic noise on wildlife behavior and physiology are scarce. In the present study, we experimentally manipulated the traffic noise exposure of plateau pika, a native small mammal widely distributed in the alpine meadow of Qinghai-Tibet Plateau, to explore the effects of traffic noise exposure on its behavior and physiology. We showed that noise exposure increased the pika's exploration and cortisol concentration (CORT) but decreased the resting metabolic rate (RMR). In addition, the relationships between RMR and exploration or CORT appeared under traffic noise treatment. This study suggests that traffic noise plays a large role in the behavior and physiology of plateau pikas and may have a long-term negative effect on the fitness of rodent populations. Generalizing these non-lethal effects to different taxa is crucial for the conservation and management of biodiversity in this increasingly noisy world.

KEYWORDS

behavior, cortisol, metabolism, road ecology, Qinghai-Tibet Plateau, rodent, small mammal, non-lethal effect

Introduction

From the last century, worldwide human population growth dramatically influenced the Earth through urbanization and industrialization (Sih et al., 2011). Transportation construction developed in an unprecedented scale, which helped us live a more convenient life. Transportation has become a fundamental element of human well-being. Although about 80% of Earth's terrestrial surface remains roadless, more than half is located in <1 km² buffer to roads (Ibisch et al., 2016). Simultaneously, traffic brings significant impacts, which are usually negative, on native species and ecosystems;

such impacts include natural landscape change (Fahrig and Rytwinski, 2009; Kati et al., 2020), biological invasion (Iacarella et al., 2020), habitat fragmentation (Ware et al., 2015), biodiversity loss (Secco et al., 2022). Following a huge anthropogenic road-building program, road ecology is now increasingly being studied globally (Ibisch et al., 2016). Road and traffic can affect terrestrial animal populations via fragmenting them into smaller sub-populations, which are more vulnerable to local extinction, increasing mortality directly or decreasing habitat amount and quality (Rytwinski and Fahrig, 2015). Around 194 million birds and 29 million mammals may be killed each year on European roads (Grilo et al., 2020); with the increase in traffic density, roadkills consisting of mammals and birds increased in Southern Spain (Canal et al., 2019); road reduced the natural area and space use of jaguars (Panthera onca) and wolf (Canis lupus), which prefer to cross low-speed and low-traffic-volume roads (Cerqueira et al., 2021; Dennehy et al., 2021); forest road can serve as barriers that impede movements and space use of red squirrel (Tamiasciurus hadsonicus grahamensis; Chen and Koprowski, 2016). Traffic also decreases animals' accessibility to food, mate, and resources, thus reducing the reproductive fitness and threatening population persistence (Fahrig and Rytwinski, 2009; Rytwinski and Fahrig, 2015; Secco et al., 2022). In 2050, a 60% increase of global road length or 25 million kilometers of new-building roadway is predicted compared with that in 2010 (Dulac, 2013). Therefore, road ecology should pay more attention to the effects of traffic on the biodiversity and conservation in the world.

In the last two decades, an increasing number of studies demonstrated that traffic noise has become one of the most important noise pollutions and has significant effects on wildlife (Halfwerk et al., 2011). The impact of traffic noise has been expended to variety of taxa, such as frogs (Troïanowski et al., 2017), bats (Berthinussen and Altringham, 2012), and songbird (Potvin and MacDougall-Shackleton, 2015). Traffic noise has negative effects on animals, i.e., disturbing their acoustic communication, reducing their reproductive success, embryo survival rate, and nestling growth, increasing stress hormone level, and influencing the population maintenance (Crino et al., 2013; Caorsi et al., 2017). Birds show behavioral flexibility under noise and adjust their song features to adapt to the variable-noise environment (Slabbekoorn, 2013). Bischoff's treefrog (Boana bischoffi) moves away from the noise source and decreases its advertisement call rate and dominant frequency (Caorsi et al., 2017), whereas the European treefrog (Hyla arborea) increases corticosterone levels and decreases immune function under traffic noise (Troïanowski et al., 2017). Shipping noise drives right whale (Eubalaena spp.) to shift their call frequencies, whereas male fin whales (Balaenoptera physalus) modify their song characteristics (Pirotta et al., 2019). However, most studies on noise effects on animals focus on the acoustic communicating species, with approximately two-thirds of such research mentioning songbirds and marine

mammals (Shannon et al., 2016); a limited number of studies centered on non-lethal effects of traffic noise on terrestrial mammals (Naidenko et al., 2021), especially on small mammals such as rodents. Rodents represent around 2,277 (42%) of the world's mammalian species with different life forms (Wilson and Reeder, 2005), and they are widely distributed in variable environments of the Earth. Studying the effects of traffic noise on rodents is crucial for their conservation and management.

The transportation construction has developed rapidly in China. By 2018, more than 5,280,700 km of highways, including 117,000 km of national expressways and 40,000 km of highspeed railways, have been built (Ministry of Transport of China, 2022). In 2035, the lengths of railway and expressway are predicted to reach 70,000 and 160,000 km, respectively (Xinhua News Agency, 2021). Sanjiangyuan National Park is one of the first batch of National Parks and also the largest National Park in China, occupying an area of 190,700 km². This park is a biodiversity hotspot with more than 270 wild animals; thus, balancing the ecological conservation and livelihood development is a crucial issue in Sanjiangyuan National Park (Cheng et al., 2020). Qinghai-Tibet highway and railway, which cross the Sanjiangyuan National Park, are major human and transportation routes and are thus also extremely busy traffic channels. The streams of traffic produce noise and light pollution along traffic channels. It has demonstrated that both richness and abundances of ground-dwelling birds was decreased near the Qinghai-Tibet highway and railway (Li et al., 2010), and the behaviors of Tibetan antelope were obviously affected by the highway (Ru et al., 2022). Plateau pikas are native small Lagomorphs that are widely distributed in the alpine meadow of Qinghai-Tibet Plateau (Smith and Xie, 2008; Wei et al., 2022). They are regarded as keystone species in the ecosystem of the plateau (Wei et al., 2019), and commonly found near the highway slope (Zhang et al., 2021). Previous studies have demonstrated that the Qinghai-Tibet highway and railway have changed the local rodent community structure (Yang et al., 2006) and restricted plateau pika's genetic exchange, leading to several genetic differentiations (Zhou et al., 2006). However, the knowledge of how traffic noise affects the plateau pikas is limited. This study aimed to test whether traffic noise affects the plateau pika's behavioral and physiological traits and their correlations. We predicted that (i) chronic exposure to traffic noise induces stress in plateau pika; (ii) traffic noise increases pika's exploration and metabolism; (iii) the correlations between behavior and physiology may change under traffic noise.

Materials and methods

Experiment animals

Plateau pikas were live-trapped using the using nooses anchored at burrow entrances with chopsticks (see details in the

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Qu et al., 2018) at the alpine meadow of Geermu, Qinghai in 10th-12th August 2019, brought back and raised in the animal laboratory at the Northwest Institute of Plateau Biology, Chinese Academy of Sciences. The trap site was about 2.0km away from the road to ensure that the plateau pikas were not exposed to traffic noise in the natural environment.

Plateau pikas were maintained in separate polypropylene cages ($47 \text{ cm} \times 35 \text{ cm} \times 20 \text{ cm}$) under natural light cycle condition, and the ambient temperature was kept $23 \pm 1^{\circ}$ C. Their gender was tested, and ID information were marked on the cages. The pikas were fed standard rabbit pellet (Beijing Ke Ao Food, Co., China) and pure water *ad libitum*. They were kept in the laboratory and adapted for 2 weeks. Then, 16 adult males and 12 adult females were used for the following experiment.

Traffic noise preparation and broadcasting

We recorded the traffic noise for playback experiments at Qinghai-Tibet highway (G109) at about 3,002 milestone. Recordings were collected 20 m away from the edge of the paved road at a height of 1 m, where plateau pikas are frequently found, on August 15th, 2019 from 09:00 am to 12:00 am, and lasted for 3 h. A total of 1,430 vehicles were recorded during this period. We selected such date and time because they represented the normal vehicle flux during the season, according to the records of the local department of transportation. A portable sound level meter (DELIXI 2201, 0.1 dB precision) was used to measure the mean amplitude (dB) of the traffic noise, and SONY PCM–D100 recorder was utilized to obtain sounds. From the 2h recording, a 30 min segment was selected and normalized to a mean amplitude level using Praat v 6.2 software.

Experimental procedure

The behavior and physiology of all pikas were measured at the start of the experiment in early September. Then, the pikas were randomly divided into two treatment groups ("treat" for short), i.e., "*Noise*" and "*Control*" groups, and housed in separate but adjoining rooms (18 m²) with the same conditions. In the "*Noise*" group room, traffic noise was broadcasted. In the "*Control*" group room, a silent broadcast was conducted during the whole experiment. The treatment lasted for 30 days. At the end of the experiment, the behavior and physiology of all pikas were measured once again using the same procedure.

Traffic noise was broadcasted using an amplified loudspeaker connected to a radio at the center of the "*Noise*" group room, with all the cages spread around the room to ensure that all the pikas heard the same noise. The noise was loop broadcasted during daytime from 07:00 am to 19:00 pm at 80 ± 2 dB sound pressure level to simulate the natural acoustic environment. Although a certain traffic noise was observed at night in G109, we did not

broadcast it in the experiment because pikas are inactive and live underground overnight (Zong and Xia, 1987).

Behavioral and physiological measurement

We conducted behavioral and physiological assays in early September and early October 2019, i.e., "*Pro*" and "*Post*" ("trial" for short) the traffic noise treatments, respectively. The behavioral and physiological parameters were tested in the following sequence:

Heart-rate test

A digital voice recorder (Sony PCM-D100) was placed on the left thorax of a pika to record the heart rate. Individuals were held gently by an experimenter, and the heart rate was measured for 30 s by another experimenter. The intervals between two adjacent heartbeats at 12 randomly selected points were detected from each audio file using Praat v 6.2 software. Previous studies demonstrated that this approach is feasible for the heart-rate measurement of plateau pikas (Qu et al., 2018).

Open-field test

After the heart-rate test, pikas were gently transferred to an open-field arena. The arena was a black acrylic box $(50 \text{ cm} \times 50 \text{ cm} \times 50 \text{ cm})$ with a white floor. We recorded the behavior exhibited by each pika for 2 min using a digital camera that was fixed above the arena using a tripod. Then, the pika was removed from the arena, and its ID and gender were recorded. After each trial, the arena was cleaned using 75% alcohol and air-dried for the subsequent trial. Using EthoVision XT 9.0 (Noldus Inc., Beijing, China) software, we measured the distances covered by the pika for 2 min as a proxy of exploration (Réale et al., 2007).

Resting metabolic rate (RMR)

We used an 8-channel FMS (Sable Systems International, Henderson, NV, United States) portable respiratory metabolism system to measure the RMR at 27° C using a biochemical incubator. Oxygen consumption per hour (mL O₂/[g h]) was expressed as the index of RMR. After the pikas had acclimatized in the chamber for 30 min, the RMR of plateau pikas was measured. Four rounds of metabolism were measured in 120 min, with each round lasting for 30 min. We obtained the average of the lowest consecutive records as the RMR (Zhu et al., 2021).

Fecal cortisol concentration (CORT)

After each test, fresh feces were immediately collected and transferred to a -20° C refrigerator. Fecal CORT was measured using rabbit cortisol enzyme-linked immunosorbent assay kit following the operation manual (Shanghai DUMA Biotechnology Co., Ltd., China). The assay sensitivity was 1.0 ng/ml with the degree of confidence above 99.8%. The intra-and inter-assay coefficients of variance were 10.3 and 6.8%, respectively.

TABLE 1 Unadjusted and adjusted repeatability (R) estimates with 95% CI.

Variables	Unadjusted R	Adjusted R	
Heart rate	0.333 (0.115, 0.702)	0.333 (0.099, 0.707)	
Exploration	0.324 (0.087, 0.735)	0.303 (0.068, 0.673)	
RMR	0.333 (0.112, 0.702)	0.333 (0.120, 0.698)	
CORT	0.322 (0.018, 0.674)	0.321 (0.025, 0.667)	



Statistical analysis

We calculated the repeatability of the heart rate, exploration, RMR, and CORT as an intra-class correlation coefficient from one-way ANOVA with treat as a fixed factor using the "*rptR*" package (Stoffel et al., 2017). Linear mixed-effects models (LMMs) were used to test the potential effect of traffic noise on these behavioral and physiological traits. We assessed the existence of correlations between the heart rate, exploration, RMR, and CORT of each individual from different treatment and trial groups by Spearman rank correlations using the "*Hmisc*" package. The LMM allows the estimation of the effects of explanatory variables and their interactions as fixed effects, with ID as random effects. LMMs were fitted using the "*lmer*" package, and "*lmerTest*" package was used to obtain the summary table and *p*-values for LMMs *via* Satterthwaite's degrees of freedom method (Kuznetsova et al., 2017).

R 4.2.1 software (R Development Core Team, 2022) was used for all statistical analyses.

Ethical statement

The animal study was reviewed and approved by The Ethics Committee of Northwest Institute of Plateau Biology, Chinese Academy of Sciences (NWIPB-20190727). The procedure for capturing, feeding, and operating on the plateau pikas followed the guidelines of the ethical approval standard of the committee. No effect of transport and experiment was observed on pika mortality.

Results

Repeatability and correlations

According to the variance proportions explained by betweenindividual variations, the behavioral and physiological traits of plateau pikas were repeatable (Table 1). The repeatability of heart rate and RMR showed similar patterns (R_{adj} = 0.333 [0.099, 0.707], R_{adj} = 0.333 [0.120, 0.698], respectively), followed by CORT (R_{adj} = 0.321 [0.025, 0.667]) and exploration (R_{adj} = 0.303 [0.068, 0.673]), which suggest that all behavioral and physiological traits measured were medium repeatable over time. At the start of the experiment, all traits were not significantly correlated with each other in the two groups. However, at the end of the experiment, exploration and CORT were significantly positively correlated, and RMR was significantly negatively correlated with exploration or CORT in the *Noise* group (p < 0.001; Figure 1A). However, all traits were not significantly correlated with each other in the *Control* group (Figure 1B).

Main and interaction effects

Before the experiments, the heart rates of plateau pikas were in the range of 140.70-413.82 beats per min, with an average of 342.22 ± 2.13 beats per min. At the end of the experiment, the average exploration of the *Noise* and *Control* groups changed to 383.60 ± 4.24 and 372.93 ± 2.67 beats per min, respectively. Treatment, trial, and their interaction and gender had no significant effects on heart rate (Figure 2A; Table 2).

Before the start of experiments, the exploration ranged from 0.50 to 6.09, with an average of 4.44 ± 0.08 . By contrast, at the end of the experiment, the average exploration of *Noise* and *Control* groups changed to 42.93 ± 0.76 and 4.13 ± 0.06 , respectively. The treatment and its interaction with the trial had significant effects on exploration (Figure 2B; Table 2).



Before the experiments, the RMR of plateau pikas ranged from 100.83 ml $O_2/[g:h]$ to 348.01 ml $O_2/[g:h]$, with the mean of 244.07 ± 2.26 ml $O_2/[g:h]$. At the end of the experiment, the mean RMR of the *Noise* and *Control* groups changed to 205.83 ± 4.40 and 228.74 ± 3.17 ml $O_2/[g:h]$, respectively. Although the trial had no significant effects on RMR, treatment, gender, and the interaction between treatment and trial had significant effects on RMR (Figure 2C; Table 2).

Similarly, before the start of the experiment, the CORT of plateau pikas ranged from 5.612 ng/mg to 11.799 ng/mg, with a mean of $8.32 \pm 0.06 \text{ ng/mg}$. At the end of the experiment, the mean CORT of the *Noise* and *Control* groups changed to 9.88 ± 0.06 and $7.76 \pm 0.09 \text{ ng/mg}$, respectively. The treatment and its interaction with trial had significant effects on CORT (Figure 2D; Table 2).

Discussion

The present study aimed to examine whether traffic noise has effects on the behavioral and physiological traits of plateau pikas. We observed significant increases in exploration and CORT but a significant decrease in the RMR of plateau pikas. Correlations between several traits were detected after exposure to traffic noise compared with the controls, supporting our prediction that traffic noise constitutes a chronic stressor to rodents. The results of one-month experimental studies focusing on the effects of artificial noise on rodent behavior and physiology (e.g., cortisol) were consistent with those of previous research (Cui et al., 2016, 2018).

Test sequence: Habituation to trial

When wild animals face repeated stimulus, their behavior may be modified, a process called habituation (Rankin et al., 2009). Heart rate and RMR tests involve the capture and handling of subjects, which may elicit significant stress responses (Careau et al., 2008). In this study, although neither behavioral (i.e., exploration) nor physiological traits (heart rate, RMR, and CORT) were singly affected by trial, exploration, RMR, and CORT were interactively affected by trial and traffic noise, suggesting that plateau pikas habituated to the experimental trial, which may

Variables	Fixed effects	Estimate	SE	<i>t</i> -value	<i>p</i> -value
Heart rate	Intercept	366.884	17.683	20.747	<0.001
	treat	10.670	21.658	0.493	0.624
	trial	-31.298	21.658	-1.445	0.155
	gender	10.574	15.473	0.683	0.497
	treat×trial	-9.488	30.629	-0.310	0.758
Exploration	Intercept	7.470	3.161	2.363	0.022
	treat	26.910	3.871	6.952	<0.001
	trial	-0.633	3.871	-0.163	0.871
	gender	-4.737	2.766	-1.713	0.093
	treat×trial	-26.930	5.474	-4.919	<0.001
RMR	Intercept	174.9	16.95	10.317	< 0.001
	treat	48.64	19.87	2.448	0.018
	trial	22.91	15.8	1.45	0.159
	gender	54.12	16.6	3.26	0.003
	treat×trial	-43.7	22.35	-1.965	0.049
CORT	Intercept	7.875	0.515	15.305	< 0.001
	treat	2.306	0.619	3.724	0.001
	trial	0.153	0.573	0.267	0.791
	gender	0.541	0.473	1.144	0.263
	treat×trial	-2.330	0.810	-2.875	0.008

TABLE 2 Results from the LMM analyses with traffic noise and time as fixed effects and ID as the random effect.

The last column reports the marginal R^2 with 95% *CI* of the model (row: fixed effects) and the variance explained by treatment and trial (reported as semi-partial R^2 , see Methods for details). The bold value means significant effect of variables.

weaken the impacts of traffic noise, as shown by the decreases or homogenizations in the exploration, RMR, and CORT of the *Control* group (Figure 2). In this scenario, we still detected the significant effects of traffic noise on exploration, RMR, and CORT, suggesting the crucial effects of traffic noise on pikas.

Numerous studies have reported the high repeatability estimate for animal behavior over time and/or context environment, which is termed personality (Réale et al., 2010). When animals face external challenges or disturbances, they may reflect behavioral variations, which can be explained through individual consistency and plasticity (Clermont et al., 2019; Couchoux et al., 2021). Using meta-analysis, Bell et al. (2009) observed a mean repeatability of 0.37; our previous study showed that the repeatability of exploration and CORT ranged from 0.34 to 0.63 in the natural population of plateau pikas (Qu et al., 2018). In the present study, the repeatability of exploration, heart rate, RMR, and CORT was around 0.32, indicating that plateau pikas showed lower behavioral and physiological consistency, but higher plasticity under traffic noise stress compared with the natural environment. Plasticity benefits animals to alter their behavior and physiology to better cope with variable environments. For example, urban birds show behavioral plasticity of the acoustic phenotype to increase their survival and reproduction rate during the urbanization process following artificial noise (Slabbekoorn, 2013). A number of insect species show behavioral plasticity in foraging, reproduction, habitat choice, and modified social

behavior to cope with artificial rapid environmental changes (van Baaren and Candolin, 2018). This study demonstrated that rodents can change their behavior and physiology to mitigate the potential adverse effects of traffic noise.

Behavioral, physiological variations and correlations

Heart rate under handling indicates the individual physiological index of coping style and reflects the response of the animal autonomic nervous system to predation risk or fearful stimuli rather than disturbance stress (Koolhaas et al., 2010). Our previous studies demonstrated that in the natural population, the heart rate and personality were correlated in plateau pikas, where the predation risk is high (Qu et al., 2018; Wei et al., 2020), as pikas are the primary prey for almost all predators on the Qinghai-Tibetan Plateau (Harris et al., 2014). In this study, the heart rate was consistent at the start and end of the experiment, indicating that plateau pikas maintained a stable response to human handling, regardless of whether they were exposed to traffic noise exposure; thus, artificial traffic noise may have different affecting mechanisms for wildlife compared with predation risk, and animals show variable coping styles to predation and artificial challenges (Francis and Barber, 2013).

As an external stressor, noise can hyperactivate the sympathetic autonomic nervous system and activate the hypothalamic-pituitary-adrenal axis, which increase the organism CORT and finally alter metabolism (Schmidt et al., 2013). Two weeks of noise enhanced the blood glucose and CORT levels and varied the glucose metabolism and gut microbiota composition in rats (Cui et al., 2016). Noise exposure also increased the corticosterone level of male treefrog but decreased their immune function (Troïanowski et al., 2017). Our results showed that at the end of the experiment, the CORT of plateau pikas in the Noise group was significantly higher than that at the start, whereas no significant variations in the CORT pre and post experiment were detected in the Control group. Thus, traffic noise caused evident stress to the pikas, and the significant interactions between treatment and trial on CORT suggested that pikas might have accustomed to the experimental environment over 1 month, which relieved the potential stress from an unfamiliar environment. Exploration reflects an animal's response to space, and it is associated with anxiety; anxious animals consistently show heightened exploration (Karl et al., 2006). In this study, the increased CORT and exploration suggest that persistent traffic noise is an interference pressure resource to pikas, and it affects their hormone sensitivity and anxiety, finally influencing their body conditions.

Metabolism is a proximate mechanism underlying behavioral and physiological variations (Careau et al., 2008). According to the Pace-of-life Syndrome (POLS) hypothesis, animal life history, behavior, and physiology are correlated, and their coupling is used to cope with external challenges (Réale et al., 2010; Boulton et al., 2015; Houslay et al., 2017). In this study, at the start of the experiment in both groups and at the end of experiment in the *Control* group, no significant correlations were detected among traits, whereas significant correlations between exploration, RMR, and CORT appeared in the *Noise* group at the end of the experiment, suggesting that traffic noise promoted the correlations among traits, that is, the occurrence of coping style. Although the presence of personality traits has been found in different external stresses, such as predation risk (Dingemanse et al., 2007, 2009) and artificial harvest (Biro and Post, 2008), we emphasized for the first time a significant relationship among behavior, RMR, and CORT induced by traffic noise.

Two distinct conceptual models explain the relationships between animal metabolism and behavior (Careau et al., 2008). The *performance model* refers to the situation in which a high metabolic rate requires more active behavioral traits. Thus, exploration, aggressiveness and boldness should positively correlate with energy metabolism. A positive correlation was detected between boldness and metabolism in wild-caught orb-weaving spider (Larinioides cornutus; Shearer and Pruitt, 2014) and fall field crickets (Gryllus pennsylvanicus; Careau et al., 2019). By contrast, the allocation model argues that with individuals having to allocate a fixed amount of energy to maintain their normal metabolism, limited energy is expended in the behaviors associated with activity, exploration, etc. The active mosquitofish (Gambusia holbrooki) has a low RMR, which suggests that high activity rates require individuals to allocate less energy toward maintenance (Biro et al., 2020). In this study, the decrease of RMR, as well as negative relationship between RMR and exploration in the Noise group at the end of experiment, provided evidence for the allocation model of energy management. Under ad libitum food conditions in the laboratory, traffic noise caused trade-offs between RMR and exploration. The relationships between behavior and metabolism are not always stable (Mathot et al., 2019). For instance, the risktaking behavior (i.e., boldness) and basal metabolic rate of the great tit (Parus major) changed from positive to negative in two consecutive years (Mathot et al., 2015). Under artificial light stimulus, the common toad (Bufo bufo) allocates less energy on activities but more energy on RMR (Touzot et al., 2019). Similarly, given that hormone production is a high-energyconsuming process, the negative relationships between CORT and RMR occurred under noise exposure conditions, which is consistent with the result of Guenther et al. (2014), who observed negative correlations between CORT and RMR in juvenile and mature cavies (Cavia aperea).

Conclusion

This study provided experimental evidence of the effects of chronic noise exposure on the behavior and physiology of wild rodents. Our results indicate that traffic noise exposure altered the exploration, metabolism, and CORT and their correlations in plateau pikas. The behavioral and physiological effects of traffic noise are currently overlooked in rodents, and their ecological consequences on the conservation and management are unclear. Our study showed that they should receive more attention, and traffic noise should be regarded as a potential influencing factor when assessing the effects of transportation on ecosystems. In the future, generalizing the non-lethal effects of traffic noise to different taxa, especially non-flagship or endangered species, is also crucial for the conservation and management of the whole ecosystem in this increasingly noisy world.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by The Ethics Committee of Northwest Institute of Plateau Biology, Chinese Academy of Sciences (NWIPB-20190727). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

JQ and YW conceived the study. HZ, YY, and LZ completed the majority of the experiments, and collected samples. JQ wrote the manuscript. YW reviewed the manuscript. All authors contributed to the articles and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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