ANXIETY IN MURINE CAUSED BY COMBINED AND SINGLE TRAFFIC NOISES: A CONTRASTIVE STUDY

Guoqing Di

College of Environment & Resource Sciences, Zhejiang University, China
email: dgq@zju.edu.cn

Yaqian Xu

College of Environment & Resource Sciences, Zhejiang University, China

This research aims to compare the influences of combined and single traffic noises on receptors' anxiety. Institute of cancer research mice were exposed to combined traffic noise (CTN) from highway and high-speed railway for 52 days, whose day-night equivalent continuous A-weighted sound pressure level (L_{dn}) was 70dB(A). The impacts of CTN on anxiety were explored by behavior tests and monoamine neurotransmitter assays, which were compared with the results from two previous studies on the impacts of single high-speed railway noise (HSRN) and aircraft noise (AN). No significant differences were shown in the behavioral indicators and the monoamine levels between experimental and control groups after CTN exposure, indicating no anxiety was caused by the 70 dB(A) CNT in mice. When L_{dn} was approximately 70 dB(A), CTN had less obvious impacts on anxiety than HSRN and AN, which is mainly related to that both the acoustical parameters of noise events [maximum noise level (L_{Amax}), noise events duration, slope of rise, difference of L_{Amax} from 1-min background equivalent continuous A-weighted sound pressure level] and modified day-night equivalent continuous R-weighted sound pressure level (considering animal auditory sensitivity to different sound frequencies and circadian rhythms) of CTN are smaller than those of HSRN and AN.

Keywords: noise effect, anxiety, traffic noises, highway, high-speed railway

1. Introduction

Recently, with the rapid development of highway and high-speed railway in China, the density of traffic networks has increased continuously. To save land resources, transport lines, such as highways and high-speed railways, are always constructed in parallel, which results in the increasingly prominent pollution of combined traffic noise. There are significant differences in both time and frequency domain properties between single and combined traffic noise from highway and high-speed railway. Highways with large traffic flow produce continuous noise; while high-speed railways produce intermittent noise with a shorter duration, a higher peak sound level and more low-frequency components (Di and Zheng, 2013). Noises with different acoustic characteristics could induce different influences on the receptors (Di *et al*., 2014). In order to confirm whether noise emission standards for combined and single traffic noises need to be established separately, it is necessary to conduct a comparative study on the effects due to combined and single traffic noises.

To investigate anxiety of receptors caused by combined traffic noise (CTN) from highway and high-speed railway, both behavioral responses (the OFT and the LDBT) and levels of plasma monoamine neurotransmitters (NE, DA, 5-HT) were evaluated in this study. Meanwhile, in the years 2011 and 2013, it was conducted that similar animal experiments on single aircraft noise (AN) and single high-speed railway noise (HSRN) whose day-night equivalent continuous A-weighted sound pressure level (L_{dn}) was (70 ± 1.5) dB(A). Relevant research results have been reported (Di *et* *al*., 2011a; Di and He, 2013). At present study, those results will be quoted directly to compare the impacts on anxiety between combined and single traffic noise.

2. Materials and methods

2.1 Animals

Healthy male Institute of Cancer Research (ICR) mice (n = 60, 4 weeks of age, weighting 15~20 g) obtained from Experimental Animal Center of Zhejiang Province (Hangzhou, China) were used for the experiments. The mice were randomly assigned into two groups: the control group (CG, n = 30) and the experimental group (EG, n = 30). They were housed five per cage and kept under controlled ambient temperature (22 ± 2℃), humidity (50%~60%) and a 12 h/12 h light/dark cycle (light on from 08:00 to 20:00). They had free access to water and food. All procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals established by the National Institutes of Health. Every possible effort was made to minimize animal suffering and to reduce the number of animals used.

2.2 Combined traffic noise sampling and exposure

A four-channel dynamic signal analyzer (Photon II, Royston, England) was used to record highway and high-speed railway noises at different time of the day, respectively. The two single traffic noises collected were reasonably arranged and superimposed to get CTN according to the 24 h traffic flux of highway (2092 passenger car units per hour) and high-speed railway (24 vehicles per hour in the daytime and 12 vehicles per hour at night).

The CTN was played through a dodecahedron non-directional sound source (Nor270, Norsonic, Lierskogen, Norway) in a sound insulation lab. The L_{dn} of the EG was (70 ± 1.5) dB(A) (the intensity of noise exposure varied slightly at different cages). The equivalent continuous A-weighted sound pressure level (L_{Aeq}) of the background noise was no more than 35 dB(A), which was the intensity presented to the CG. After 7-day adaptation in the laboratory, the EG was exposed to the CTN 24 h per day for 52 days, while the CG was not exposed.

2.3 Behavioral tests and monoamine neurotransmitter assays

In order to avoid circadian rhythm induced variations, behavioral tests and blood collection were conducted at the same time of the day (between 17:00 and 19:00) every time. The OFT was carried out on days 4, 24, and 44 during the period of noise exposure, and the LDBT on days 5, 25, and 45. For the purpose of reducing the possible impacts of behavioral tests on monoamine levers, blood sampling from the same mice examined in the behavioral tests was carried out 7 days later of the LDBT (on days 12, 32, and 52). Ten mice from each group were randomly chosen for each experiment.

The OFT was performed in a well illuminated wooden square arena (72 cm × 72 cm) with 30 cm high walls. The floor and walls were painted black and the floor was divided into 64 grids (9 cm × 9 cm) by white lines. The thirty-six girds located in the center of arena were regarded as “center area”. The test was conducted in a sound insulation lab. The individual mouse was placed in the center of arena and its behaviors were recorded by a camera for 5 min. Through playing back videos, the center time, number of line crossing, rearing and defecation, was calculated by three individuals independently. The mean values of the results from the three individuals were considered as the final results.

The light-dark box was one third for the dark and two thirds for the lit compartment with an exterior size of 18 cm × 10.5 cm × 10.5 cm (l × w × h). The lit and dark compartments were separated by a partition. An opening in the center of the partition at floor level allows the animal to shuttle freely from one part to another. The lit compartment was painted white and illuminated by a 20-W light source at the height of 30 cm above the floor, while the dark compartment was painted black and covered by a light-proof lid. The animal was placed in the center of the lit compartment with its back to the opening at the beginning of the test, and its behaviors were also recorded by a camera for 5 min. The time spent in the lit compartment and the number of transitions between the two compartments was analyzed.

1.0 mL blood was collected in a 1.5 mL centrifuge tube by eyeball removal. 40 μL disodium ethylene diamine tetraacetic acid solution of 50 g/L was added to the sample and oscillated. After 15 min of quiescence, the mixture was centrifuged at 3000 r/min for 20 min at 4℃. Then, 150 μL supernatants were extracted, to which 150 μL perchloric acid of 5% was added. The mixture was thoroughly shaken and stood for 20 min to fully precipitate the plasma proteins, followed by centrifugation at 10000 r/min for 15 min at 4℃. 150 μL supernatants were extracted for the determination of plasma NE, DA, 5-HT levels by high performance liquid chromatography-fluorimetric detection (Di *et al*., 2011b; Di and He, 2013).

2.4 Statistical analysis

All data are expressed as mean ± SEM. Comparison between the two groups was performed by unpaired Student's t-tests using SPSS 20.0. Differences were considered significant when $P<0.05$.

3. Results

3.1 The behavioral tests

After CTN of 70 dB(A) exposure for different time, the results of the OFT and LDBT are shown in Table 1 and Table 2. There were no significant differences in the center time, the number of line crossing, rearing, and defecation from the OFT between the EG and CG under different noise exposure durations, as well as the time spent in the lit compartment and the number of transitions from the LDBT ($P>0.05$).

Table 1: The results of OFT in experimental group (EG, n = 10) and control group (CG, n = 10)

Noise exposure duration	Center time/s		Number of line crossing		Number of rearing		Number of defecation	
	CG	EG	CG	EG	CG	EG	CG	EG
4d	112.9±5.9	122.5±4.2	392.0±16.6	403.1±24.9	5.8±2.2	2.6±0.7	3.2±0.6	2.8±0.6
24d	89.9 ±10.1	89.3±6.2	313.0±11.8	355.2±17.6	9.2±2.8	5.5±0.9	3.1±0.6	1.8±0.4
44d	78.0±3.9	78.4±6.8	361.2±11.0	356.6±17.9	2.5±0.9	4.2±1.7	4.6±0.9	3.3±0.7

Table 2: The results of the LDBT in experimental group (EG, n = 10) and control group (CG, n = 10)

Noise exposure duration	Time spent in the lit compartment/s		Number of transitions	
	CG	EG	CG	EG
5d	135.0±10.0	141.9±11.0	35.0±1.0	39.9±4.2
25d	120.5±10.5	139.6±8.6	29.4±1.5	31.5±3.4
45d	133.5±8.0	118.4±9.6	30.9±2.2	25.6±1.6

3.2 Concentrations of plasma monoamines

Figure 1 shows the average levels of plasma monoamines (NE, DA, 5-HT) of the EG and CG. There were no significant differences in plasma NE (Figure 1a), DA (Figure 1b), and 5-HT (Figure 1c) levels between the two groups over the period of CTN exposure ($P>0.05$).

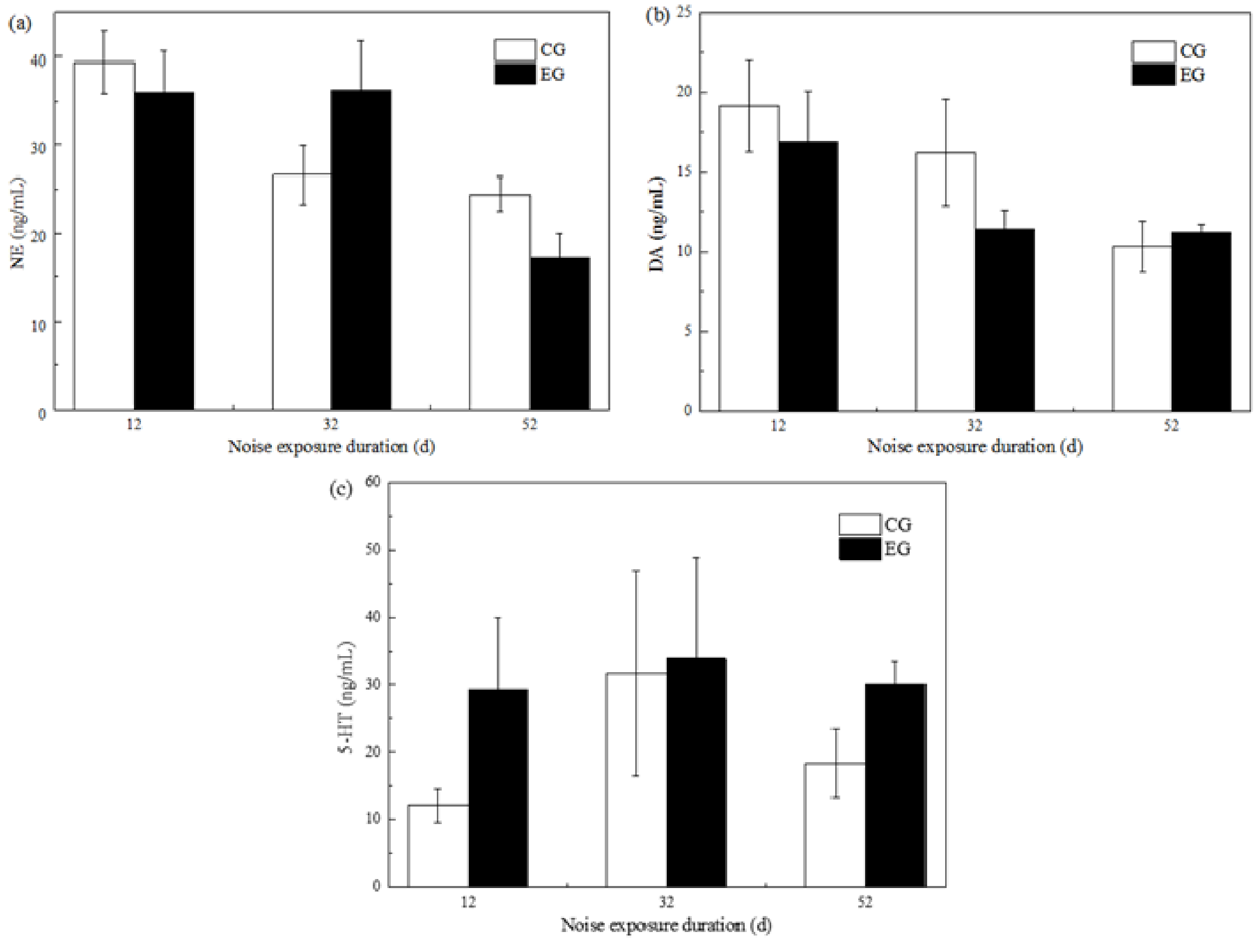

Figure 1: Concentrations of plasma monoamines after noise exposure for different time, (a) norepinephrine (NE), (b) dopamine (DA), (c) serotonin (5-HT)

3.3 Comparing combined and single traffic noise impacts on anxiety

The results from animal experiments on CTN, HSRN (Di and He, 2013) and AN (Di *et al.*, 2011a), whose L_{dn} = (70 ± 1.5) dB(A), were compared in Table 3. Generally, in the OFT, the animal anxiety level is positively correlated with the center area duration and the number of defecation, whereas it is negatively correlated with the center time, the number of line crossing, grooming and rearing; in the LDBT, the animal anxiety level is negatively correlated with the time spent in the lit compartment and the number of transitions; in the experiment of plasma monoamines, the animal anxiety level is positively correlated with NE, DA, and 5-HT concentrations.

As shown in Table 3, over the duration of CTN exposure, all the behavioral indicators from the OFT and LDBT, as well as the levels of the three kinds of plasma monoamines showed no significant differences between the EG and CG, which implied that no anxiety was induced by 70 dB(A) CNT exposure in mice. After HSRN exposure for a certain time, behavioral indicators (center time, the number of line crossing, grooming and rearing, the time spent in the lit compartment and the number of transitions) of the EG were significantly less those of the CG, while the plasma DA level was obverse. The results indicated that HSRN of 70 dB(A) would cause anxiety in mice. After AN exposure for a certain time, the number of line crossing was reduced significantly in the EG, whereas the center area duration and the plasma NE level were significantly increased in the EG. Those results suggested that AN, whose Ldn approximated 70 dB(A), would cause anxiety in rats. In summary, CTN had less obvious impacts on anxiety than HSRN and AN when the L_{dn} was 70 dB(A) approximately.

Table 3: Comparison of results from the three animal experiments on combined traffic noise (CTN) from highway and high-speed railway, single high-speed railway noise (HSRN) and single aircraft noise (AN)

Exposure sound sources	Exposure duration	Experimental animals	Main results
CTN	24h/d 52d	ICR mice	There were no significantly differences in center time, the number of line crossing, rearing and defecation (4d, 24d, 44d)[a], the time spent in the lit compartment and the number of transitions (5d, 25d, 45d), the levels of plasma NE, DA and 5-HT (12d, 32d, 52d) between the EG and CG ($p>0.05$).
HSRN	24h/d 53d	ICR mice	Center time (18d, 53d), the number of line crossing (53d), the number of grooming (8d, 18d), the number of rearing (28d), the time spent in the lit compartment (53d) and the number of transitions (53d) was reduced significantly in the EG ($p<0.05$). The plasma DA level (40d) was significantly increased in the EG ($p<0.05$), whereas the plasma NE and 5-HT levels (3d, 10d, 20d, 30d, 40d) were not significantly different between the EG and CG ($p>0.05$).
AN	24h/d 36d	SD rats	Noise exposure caused a significant decrease in the number of line crossing (8d) ($p<0.05$) and a significant increase in center area duration (8d) and the plasma NE level (29d) ($p<0.05$).

[a] 4d, 24d, 44d indicates noise exposure duration, similarly hereinafter.

4. Discussion

This study showed that, under the noise exposure of (70 ± 1.5) dB(A), apparently, CTN was less influential to anxiety compared with SHRN (Di and He, 2013) and AN (Di *et al*., 2011a). The result above could be caused by the differences in noise acoustic characteristics and experimental subjects, which will be concretely discussed below.

4.1 The impact of noise events acoustic properties on anxiety levels of the receptors

Concerning the relationship between acoustic characteristics of traffic noise events and noise effects, studies mainly focus on annoyance and sleep disturbance. Scarce number of studies about the impact of noise events acoustic properties on receptors' anxiety has been found. Strong links between noise annoyance, sleep disturbances and anxiety was discovered; in consequence, acoustic characteristics of noise events which are related to the degree of annoyance and sleep disturbance, would also affect anxiety levels.

A noise event was defined as an increase in sound pressure level that exceeded statistic sound level L_{90} by 3 dB(A); slope of rise for a noise event was represented by the steepest slope of the noise event curve; the 1-min background L_{Aeq} was defined as L_{Aeq} of 1-min time which spanned directly preceding the start of a noise event. The acoustic properties of noise events of CTN in this study, and HSRN (Di and He, 2013) or AN (Di *et al*., 2011a) in previous studies were analyzed (Table 4).

As displayed in Table 4, except the number of noise events and 1-min background L_{Aeq}, all the acoustic parameters of noise events analyzed [noise events duration, slope of rise, maximum noise level (L_{Amax}), difference of L_{Amax} from 1-min background L_{Aeq}] of CTN are much smaller than those of HSRN and AN. Studies have shown that traffic noise annoyance significantly increases with the slope of rise. Sleep disturbance due to the traffic noise significantly increases with the noise events duration, the slope of rise, L_{Amax} and the difference of L_{Amax} from 1-min background L_{Aeq}, among which L_{Amax} plays a decisive role. Thus, the fact that L_{Amax}, the noise events duration, the slope of rise and the difference of L_{Amax} from 1-min background L_{Aeq} of CTN are smaller than those of HSRN and AN, is one of the important reasons for CTN being less influential on anxiety than HSRN and AN when L_{dn} = (70 ± 1.5) dB(A). With regard to the correlation between the number of noise events and annoyance, as well as between the number of noise events and sleep disturbance, Sato *et al*. and Janssen *et al*. indicated that the number of traffic noise events was irrelevant to annoyance and sleep quality, respectively. However, some other studies suggested that the number of traffic noise events was positively correlated with noise annoyance and sleep disturbance. In the present study, anxiety was less affected by CTN than by HSRN and AN, whose number of noise events is far less than that of CTN. That is, the number of traffic noise events is not related to anxiety.

The above analysis implies that when evaluating noise effects and developing noise limits, researchers should not only concern the parameters representing macroscopic exposure intensity of noise (e.g., L_{dn}), but also the microstructure of noise (e.g., the acoustic characteristics of the single noise event).

Table 4: Acoustic parameters of noise events of combined traffic noise (CTN) from highway and high-speed railway, single high-speed railway noise (HSRN) and single aircraft noise (AN)

Parameters	Exposure sound sources	Range	Median	25th percentile	75th percentile
The number of noise events	CTN	10800/d	/	/	/
	HSRN	524/d	/	/	/
	AN	205/d	/	/	/
Noise events duration (s)	CTN	0.1~56.6	0.2	0.1	9.2
	HSRN	7.8~12.4	11.0	7.8	12.3
	AN	25.1~77.9	52.8	43.4	63.4
Slope of rise [dB(A)/s]	CTN	9.6~279.6	20.5	19.0	64.7
	HSRN	100.4~648.7	315.4	100.4	648.7
	AN	84.4~1335.4	272.1	132.3	430.8
L_{Amax} [dB(A)]	CTN	61.2~82.6	61.3	61.2	65.6
	HSRN	84.5~90.9	87.7	84.5	90.8
	AN	81.3~90.2	82.8	86.6	89.2
1-min background L_{Aeq} [dB(A)]	CTN	62.0~69.2	64.1	63.6	68.1
	HSRN	38.5~42.8	42.8	38.5	42.8
	AN	29.2	/	/	/
Difference of L_{Amax} from 1-min background L_{Aeq} [dB(A)]	CTN	-7.6~16.2	-2.8	-2.9	1.2
	HSRN	41.7~52.4	47.0	41.7	48.0
	AN	52.1~61.0	53.6	57.4	60.0

4.2 The impact of subject auditory sensitivity and circadian rhythms on anxiety levels

Differences in the hearing sensitivity and circadian rhythms of different receptors can also bring variances in anxiety degree under the same noise exposure intensity (L_{dn}). In the animal experiments on CTN and HSRN (Di and He, 2013) effects, ICR mice were used as subjects, while SD rats were used in the animal experiments on AN effect (Di *et al*., 2011a). According to the audiograms of humans, rats and mice, humans, rats and mice vary in hearing sensitivity to different sound frequencies. At frequencies of 20 Hz to 8000 Hz, hearing sensitivity decreases in the order of humans, rats and mice. At the same time, frequency spectrums vary with different traffic noises. Thus, the noise exposure intensity the receptors actually received depends both on the hearing ability of the receptors at each frequency and on the spectrum of the noise. Studies have introduced a sound pressure level weighting according to the animal auditory sensitivity, called R-weighting. Based on the R-weighting, the spectrums of CTN, HSRN and AN, as well as the audiograms of mice and rats, the actual noise exposure intensity which was presented in this study as the day-night equivalent continuous R-weighted sound pressure level ($L_{R,\,dn}$), in the three animal experiments on CTN, HSRN (Di and He, 2013) and AN (Di *et al*., 2011a),was calculated (Table 5) as Eq. (1)~Eq. (4).

$$F_{ij} = L_{ij} - T_i \tag{1}$$

$$L_{\mathrm{R}j} = 10\log\sum_{i=1}^{n}\left(10^{0.1F_{ij}}\right) \tag{2}$$

$$L_{\mathrm{Req},T} = 10\log\left(\frac{1}{m}\sum_{j=1}^{m}10^{0.1L_{\mathrm{R}j}}\right) \tag{3}$$

$$L_{\mathrm{R,dn}} = 10\log\left[\frac{16}{24}10^{0.1L_{\mathrm{Req,d}}} + \frac{8}{24}10^{0.1(L_{\mathrm{Req,n}}+10)}\right] \tag{4}$$

Where F_{ij} is the R-weighted sound pressure level of i-th 1/3 octave band within the j-th time interval of Δt; L_{ij} is the sound pressure level of i-th 1/3 octave band within the j-th time interval of Δt; T_{i} is the hearing threshold of mice or rats at the center frequency of i-th 1/3 octave band; $L_{\mathrm{R}j}$ is the R-weighted sound pressure level within the j-th time interval of Δt; n is the number of 1/3 octave bands; $L_{\mathrm{Req},T}$ is the equivalent continuous R-weighted sound pressure level within the time interval of T; m is the number of Δt within the time interval of T, $m = T/\Delta t$; $L_{\mathrm{R,dn}}$ is the day-night equivalent continuous R-weighted sound pressure level; $L_{\mathrm{Req,d}}$ is the equivalent continuous R-weighted sound pressure level of 16 h in the daytime between 06:00~22:00; $L_{\mathrm{Req,n}}$ is the equivalent continuous R-weighted sound pressure level of 8 h at night between 22:00~08:00 the next day.

Above calculation of $L_{\mathrm{R,dn}}$ is based on the circadian rhythm of humans. In other words, $L_{\mathrm{R,dn}}$ is computed by adding 10dB to $L_{\mathrm{Req,n}}$ considering that noise has a greater influence at night (22:00~06:00 the next day) for humans when they have a rest than in the daytime. However, the circadian rhythm of mice and rats is different from that of human. In general, mice and rats take a rest at 08:00~20:00 and stay active at 20:00~08:00 the next day. So $L_{\mathrm{R,dn}}$ needs to be modified to meet the circadian rhythm of mice and rats. The modified $L_{\mathrm{R,dn}}$ is represented as $L'_{\mathrm{R,dn}}$, which is calculated by adding 10dB to the equivalent continuous R-weighted sound pressure level of 08:00~20:00 ($L'_{\mathrm{Req,d}}$) (Table 5) as Eq. (5).

$$L'_{\mathrm{R,dn}} = 10\log\left[\frac{12}{24}10^{0.1L'_{\mathrm{Req,n}}} + \frac{12}{24}10^{0.1(L'_{\mathrm{Req,d}}+10)}\right] \tag{5}$$

Where $L'_{\mathrm{R,dn}}$ is the modified day-night equivalent continuous R-weighted sound pressure level; $L'_{\mathrm{Req,n}}$ is the equivalent continuous R-weighted sound pressure level of 12 h between 20:00~08:00 the next day; $L'_{\mathrm{Req,d}}$ is the equivalent continuous R-weighted sound pressure level of 12 h between 08:00~20:00.

Table 5 shows that, when the L_{dn} of CTN, HSRN and AN was approximately equal, the $L_{\mathrm{R,dn}}$ of CTN which was obtained by R-weighting according to animal auditory sensitivity, is 2.9dB (R) smaller than that of HSRN and 2.7dB (R) smaller than that of AN. On the basis of $L'_{\mathrm{R,dn}}$ which was achieved according to the circadian rhythm of mice and rats, the gap in noise exposure intensity between CTN and the two single traffic noises is further increased. The $L'_{\mathrm{R,dn}}$ of CTN is 7.6dB (R) and 4.6dB (R) smaller than that of HSRN and AN, respectively. Thus, influenced by noise frequency spectrums, animal auditory sensitivity and circadian rhythms, the noise exposure intensity experimental animals actually received ($L'_{\mathrm{R,dn}}$) of CTN is smaller than that of HSRN and AN, which may be another reason why anxiety from CTN is less than that from HSRN and AN when the L_{dn} of noise is approximately equal. It gives us an important enlightenment that there are great differences in effects of noise between different experimental animals even when they are exposed to the same noise. To make the results among each study comparable, the strain of experimental animals should keep consistent as far as possible. Likewise, in order to better analogize the noise effects of animal to those of human, it is recommended to use animals whose auditory sensitivity is similar to humans, such as the gerbil, when using the rodent as models to conduct noise effects experiments.

Table 5: Exposure levels of combined traffic noise (CTN) from highway and high-speed railway, single high-speed railway noise (HSRN) and single aircraft noise (AN) considering animal hearing sensitivity and circadian rhythms

Exposure sound sources	$L_{R,dn}$	$L'_{R,dn}$
CTN	42.7	45.8
HSRN	45.6	53.4
AN	45.4	50.4

5. Conclusions

The study has found no significant impacts of CTN on behaviors and plasma NE, DA, 5-HT levels, which indicated that no anxiety in ICR mice was caused by 70 dB(A) CTN. The impacts of combined and single traffic noises on anxiety were contrasted. The results showed that CTN had less obvious impacts on anxiety compared with SHRN and AN, which is mainly owing to two reasons. Firstly, acoustic parameters of noise events (L_{Amax}, noise events duration, slope of rise, difference of L_{Amax} from 1-min background L_{Aeq}) of CTN are much smaller than those of HSRN and AN. Secondly, $L'_{R,dn}$ (i.e. exposure intensity that animals actually received by taking noise frequency spectrums, animal auditory sensitivity and circadian rhythms into consideration) of CTN is smaller than that of HSRN and AN. Those suggest that when evaluating noise effects and establishing noise limits, not only should the parameters representing noise macroscopic exposure intensity (e.g., L_{dn}) be concerned, but also the noise microstructure (e.g., the acoustic characteristics of the single noise event) should be taken into account. In future similar animal studies, animals whose auditory sensitivity is similar to humans, such as gerbil, should be employed consistently for the comparison between different studies and applying the results into humans.

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