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## Influences of combined traffic noise on anxiety in mice

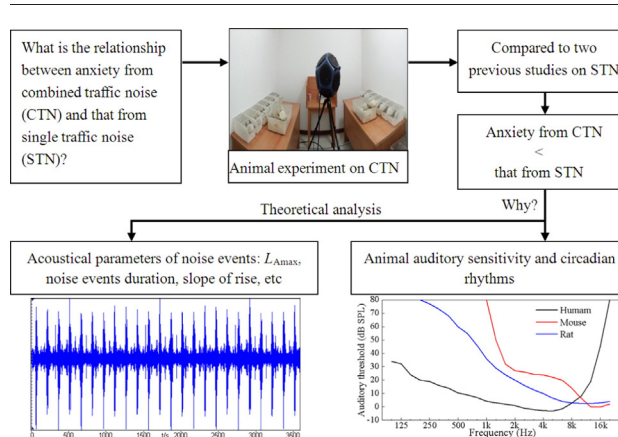
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### HIGHLIGHTS

- Combined traffic noise (CTN) impacts on anxiety were studied by an animal experiment.
- Results in this study were compared to prior studies on single traffic noise effects.
- No obvious impacts of 70 dB(A) CTN on anxiety in mice was found in this study.
- CTN had less impact on anxiety than single high-speed railway and aircraft noises.
- Noise events parameters and auditory sensitivity may affect anxiety levels.

### GRAPHICAL ABSTRACT



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### ABSTRACT

With the rapid development of traffic facilities in China, traffic noise pollution is increasingly prominent. This research aims to explore the influences of combined traffic noise 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 70 dB(A). The impacts of CTN on anxiety were explored by behavior tests and monoamine neurotransmitter assays. The results were in depth discussed in comparison to two previous studies on the impacts of single high-speed railway noise (HSRN) and aircraft noise (AN), but data from the three studies were not merged and statistically compared. No significant differences were shown in the behavioral indicators and the monoamine levels between the experimental and control groups after CTN exposure, indicating no obvious impacts of 70 dB(A) CTN on anxiety in mice were found in this study. 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.

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**Abbreviations:** AN, aircraft noise; CTN, combined traffic noise; DA, dopamine; HSRN, high-speed railway noise; ICR, Institute of Cancer Research;  $L_{Aeq}$ , equivalent continuous A-weighted sound pressure level;  $L_{Amax}$ , maximum noise level;  $L_{dn}$ , day-night equivalent continuous A-weighted sound pressure level; LDBT, light-dark box test; NE, norepinephrine;  $L_{R,dn}$ , day-night equivalent continuous R-weighted sound pressure level;  $L'_{R,dn}$ , modified day-night equivalent continuous R-weighted sound pressure level; OFT, open-field test; SD, Sprague-Dawley; 5-HT, serotonin.

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## 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 (CTN). There are significant differences in both time and frequency domain properties between single and combined traffic noises 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; Elmenhorst et al., 2012). 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.

Most studies regarding the health effects of traffic noises focus on annoyance (e.g., Gidlof-Gunnarsson et al., 2012; Bartels et al., 2015; Xie et al., 2016), sleep disturbance (e.g., Marks et al., 2008; Lercher et al., 2010) and cardio-cerebrovascular disease (e.g., Aydin and Kaltenbach, 2007; Belojevic et al., 2008). Only a few studies have investigated the impacts of traffic noises on receptors' anxiety. It has been found that positive and negative results coexist in those studies. Hardoy et al. (2005) discovered that the airport noise exposure of high levels was associated with an increased risk of syndromal anxiety states. In addition, Bocquier et al. (2014) reported that the exposure to nighttime road traffic noise might increase the individual use of anxiolytics-hypnotics. However, a study conducted by Halonen et al. (2014) received a negative result, namely, road traffic noise exposure was irrelevant to the usage of anxiolytics. All the research mentioned above adopted the method of field survey to explore the impacts of traffic noises on anxiety. Nevertheless, field surveys may be influenced by bias and confounding, and their observational nature makes it difficult to draw firm causal relationships. That may be an important reason of the inconsistent findings from the above studies. On the contrary, the research carried out through animal models in laboratories with controlled conditions may efficiently avoid the impacts of bias and confounding.

As an environmental stimulus, noise can cause stress responses on the receptors which are composed of alterations in behavior, autonomic function and the secretion of multiple neurotransmitters (Carrasco and de Kar, 2003). Stress responses are considered to be one of the major causes of mental disorders such as anxiety (McEwen, 2000). Due to the fact that the behaviors and related neurotransmitter concentrations of the animals are subject to changes in the situation of anxiety, the anxiety level can be assessed by particular behavior models (Ramos and Mormede, 1997; Gould et al., 2009) and measurements of related neurotransmitter levels (Lechin et al., 1998; Habr et al., 2014).

Behavioral models to evaluate stress and anxiety are well established in murine, among which the open-field and the light-dark box tests are two kinds of classic models (Archer, 1981; Belzung and Griebel, 2001; Crawley, 2007). Specifically, the open-field test (OFT) is potentially sensitive to both activity and exploration, and to emotionality, which tends to suppress the expression of the former (Deacon et al., 2002). The commonly used indices to assess emotionality, like anxiety, are center time, locomotion and defecation (Ramos and Mormede, 1997; Prut and Belzung, 2003; Gould et al., 2009). The light-dark box test (LDBT) rests on the conflict between the aversion to light of murine and the tendency to explore a novel environment, hence, the measures of exploration in the lit compartment (time and entrances) are often regarded as experimental indices of anxiety (Crawley, 1989; Bourin and Hascoet, 2003).

Many researchers characterize the degree of anxiety by monoamine neurotransmitters levels, among which norepinephrine (NE),

dopamine (DA) and serotonin (5-HT) concentrations are common indicators (Pitchot et al., 1992; Inoue et al., 1994; Lechin et al., 1998; Silva and Brandao, 2000). Noise stress has been documented to activate the autonomic nervous system and neuroendocrine system, and induce variations of plasma NE, DA, and 5-HT concentrations (Babisch, 2003; Di et al., 2011b; Di and He, 2013).

To investigate anxiety of receptors caused by combined traffic noise 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 2011 and 2013, two similar animal experiments were conducted (Di et al., 2011a; Di and He, 2013). In these two previous experiments, Sprague-Dawley (SD) rats and Institute of Cancer Research (ICR) mice were respectively exposed to single aircraft noise (AN) and single high-speed railway noise (HSRN), whose intensities measured by day-night equivalent continuous A-weighted sound pressure level ( $L_{dn}$ ) were both ( $70 \pm 1.5$ ) dB(A). According to the present experiment and the two previous experiments, the differences of impacts on anxiety between combined and single traffic noises were discussed, and the reasons for the differences were analyzed in terms of acoustical parameters of traffic noise events and subject auditory sensitivity.

## 2. Materials and methods

### 2.1. Animals

Healthy male ICR mice ( $n = 60$ , 4 weeks of age, weighing 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 ( $n = 30$ ) and the experimental group ( $n = 30$ ). They were housed five per cage and kept under controlled ambient temperature ( $22 \pm 2$  °C), 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 (1996). 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 experimental group was ( $70 \pm 1.5$ ) dB(A) (the intensity of noise exposure varied slightly in different cages, and 70 dB was the average exposure intensity in all cages, while  $\pm 1.5$  dB was the range of exposure intensity difference). The equivalent continuous A-weighted sound pressure level ( $L_{Aeq}$ ) of the background noise was no  $> 35$  dB(A), which was the intensity presented to the control group. After 7-day adaptation in the laboratory, the experimental group was exposed to the CTN 24 h per day for 52 days, while the control group was not exposed.

### 2.3. Behavioral tests and monoamine neurotransmitter assays

Behavioral tests, as well as monoamine neurotransmitter assays, were carried out at three different points in time during the period of noise exposure. Ten mice from each group were tested at each point in time. Detailed experiment schedule is shown in Table 1. The OFT

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