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Acute high-intensity noise exposure exacerbates anxiety-like behavior via neuroinflammation and blood brain barrier disruption of hippocampus in male rats

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Abstract

The health risks associated with acute noise exposure are increasing, particularly the risk of mental health. This study aims to identify the association between acute high-intensity noise exposure and anxiety behavior in male rats, and to explore the associated neurobiological mechanisms. Male rats were subjected to different levels of acute high-intensity noise to determine the intensity that causes long-lasting anxiety-like behaviors. Anxiety-like behaviors were evaluated using the open field test (OFT) and the elevated plus maze test (EPMT) on the third day and 1month post-exposure, respectively. A range of techniques, including immunofluorescence staining, western blot, ELISA, and real-time quantitative PCR, were used to investigate neuronal apoptosis, glial cell activation, neuroinflammation, and blood-brain barrier (BBB) disruption in the hippocampus. Upon exposure to 135 dB of acute noise, male rats exhibited enduring anxiety-like behaviors. Subsequent investigations discovered that this noise intensity not only activated glial cells and triggered neuroinflammation within the hippocampus but also decreased the expression levels of ZO-1, claudin-5, and occludin, suggesting a disruption of the BBB. Additionally, this exposure was associated with the induction of neuronal apoptosis in the hippocampal region. In conclusion, acute exposure to 135 dB noise may cause persistent anxiety in male rats through a cyclical interaction between neuroinflammation and BBB disruption, potentially leading to neuronal apoptosis.

Keywords Acute noise, Anxiety, Hippocampus, Neuroinflammation, Blood brain barrier

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Introduction

Noise pollution is becoming increasingly severe with the development of traffic noise, ambient community noise, and the expansion of modern industry [1]. Although numerous studies have documented the health status of workers in chronic noise environments, there is a dearth of research on the acute noise exposure prevalent in military and industrial settings. This type of noise is distinguished by its high intensity (exceeding 100 dB), brief exposure duration, and the suddenness that makes it difficult to protect against. Acute high-intensity noise exposures pose significant health hazards and threaten human health [2, 3, 4].

Epidemiological studies have revealed a link between environmental noise and mental health issues [5], and a systematic review and meta-analysis by Yuliang Lan et al. emphasizes that anxiety is a common response to long-term exposure to low-level environmental noise [6] Animal model studies further indicate that environmental noise can impact inflammatory and oxidative stress pathways in the brain, leading to anxiety and depression-like behaviors [7, 8, 9] Recently, researches on high-intensity acute noise exposure have predominantly focused on damage to the auditory organs [10]. Additionally, studies have shown that three consecutive days of acute high-intensity noise exposure can alter gene expression in the hippocampus [11], a region intricately associated with the pathophysiology of mood and anxiety disorders [12]. Furthermore, research by Sonia et al. demonstrates that a single 2-hour exposure to moderate-intensity noise did not affect rats' auditory function but exacerbated their anxiety symptoms [13]. This suggests that even brief exposures to noise can influence long-term anxiety behavior. However, there is a paucity of research on the effects of short-term, acute high-intensity noise exposure on anxiety in organisms.

Noise exposure is intricately linked to adverse mental health outcomes through complex psychological and behavioral mechanisms. In this study, we employed an integrated approach from behavioral science, pathology, and molecular biology to investigate the immediate and long-term effects of graded acute high-intensity noise exposure on anxiety behavior. Concurrently, we delved into the potential pathological mechanisms that may mediate the impact of acute high-intensity noise exposure on anxiety, aiming to provide a more comprehensive scientific basis for understanding the mental health implications of noise pollution. Through these investigations, we aim to fill the existing research gap, particularly in the area of acute high-intensity noise exposure's impact on mental health, which is crucial for a holistic understanding of the full impact of noise pollution on human health.

Materials and methods

Animals

The study was approved by the Animal Care and Use Committee at Xi'an Jiaotong University (approval number: XJTU 2021 – 1501) and adhered to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978). 100 male Sprague Dawley rats, each weighing around 400 g, were housed in groups of up to six per cage with a 12-hour light/dark cycle. All procedures were conducted during the light phase, with rats having free access to food and water.

Noise exposure and experimental groups

One-third octave band noise with a center frequency of 1000 Hz was generated by Signal Processing Toolbox's filter design tool of MATLAB R2021a software, amplified by CTRLPA JG202HD-G/JG1010CS amplifier, and then emitted by a high-power loudspeaker. The loudspeaker was placed in an anechoic chamber with rat cages positioned 30 cm in front of it. Rats were given a 15-minute acclimatization period before being exposed to 6 min acute noise at varying intensities. Cages were cleaned with a 75% ethanol solution before and after each session to prevent olfactory interference.

The rats were randomly divided into five groups: the control group and the 105 dB, 115 dB, 125 dB, and 135 dB noise exposure groups, with 20 rats in each group. On day 3 after noise exposure, 10 rats from each group were randomly selected for behavioral testing. These rats were euthanized after the behavioral experiments, and tissues were collected for histological and protein-level analyses. On day 30 after noise exposure, the remaining 10 rats in each group underwent the same set of experiments. The experimental protocol is detailed in Fig. 1a.

Open-field test (OFT)

The OFT was conducted in a 100 cm × 100 cm × 40 cm black square enclosure. Rats were placed in the center and allowed to explore for 5 min (approximately 120 lx). The enclosure was sanitized with a 30% ethanol solution before each trial. The experimenter left the room after placing the rats in the arena. Trials were recorded and analyzed using Lab maze V3.0 software (Beijing Zhongshi Dichuang Technology Development Co), measuring distance traveled in the central zone (a 50 cm × 50 cm square), time spent in the center, and frequency of entries into the central area to assess anxiety levels.

Elevated plus maze test (EPMT)

The EPMT consists of open (50 cm × 10 cm) and enclosed arms (50 cm × 10 cm × 30 cm), both elevated 70 cm and intersecting at a central square (10 cm × 10 cm). Rats were placed in the central area, facing an open arm, and allowed to explore for 5 min. Visits to open and enclosed

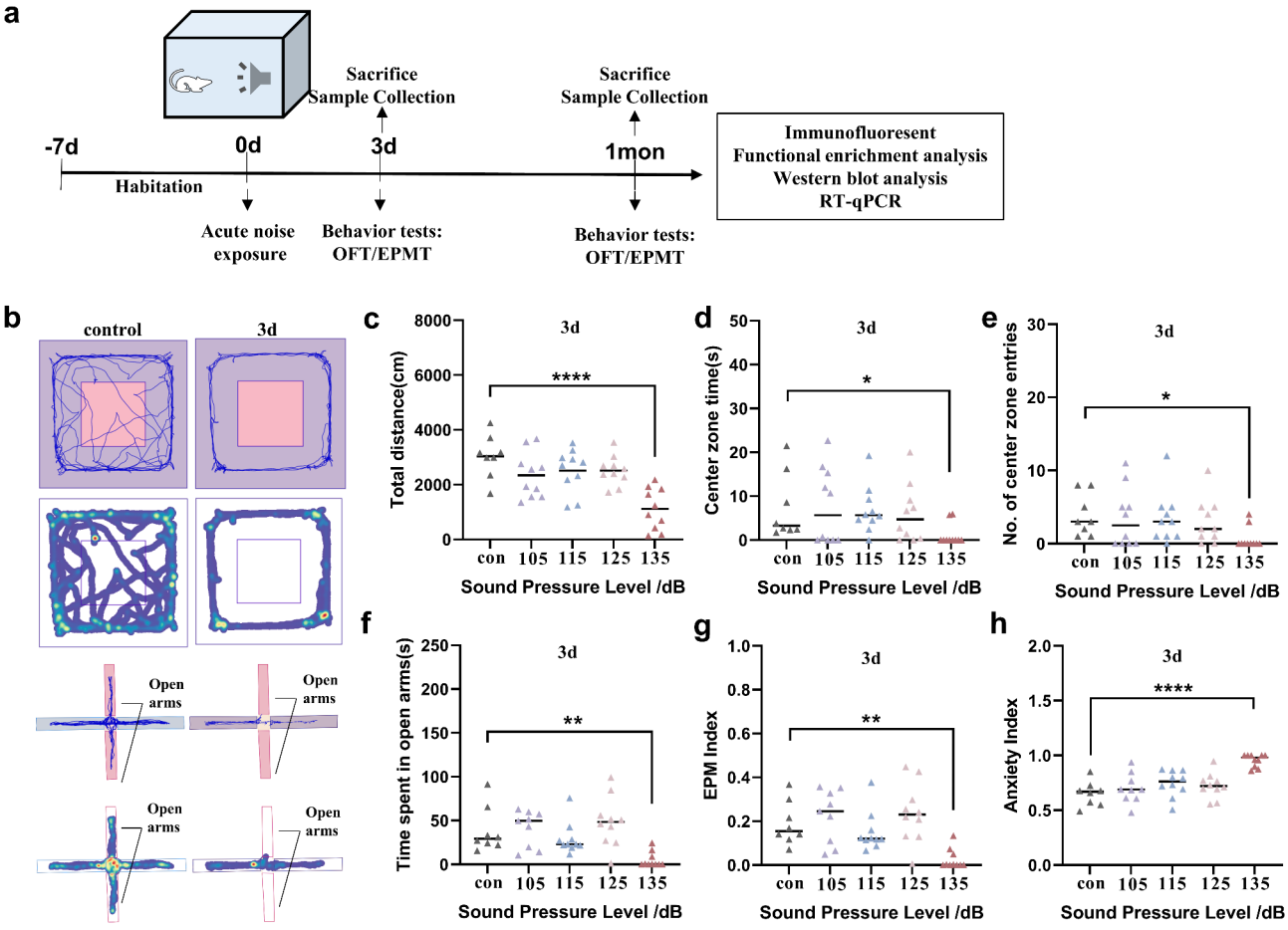

Fig. 1 Experimental design and changes in anxiety behavior of male rats on the third day after exposure to acute noise of different intensities. (**a**) Timeline for experiment. (**b**) Representative motion trajectories of male rats in the OFT (top) and EMPT (bottom). (**c**) Total distance, (**d**) center zone time, (**e**) NO. of center zone entries in OFT after different sound pressure level of acute noise exposure. (**f**) Time spent in open arms, (**g**) EPM index, (**h**) Anxiety index in EPMT after different sound pressure level of acute noise exposure. $N = 8–10$ rats per condition. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$

arms were recorded and analyzed using Labmaze V3.0 software (Beijing Zhongshi Dichuang Technology Development Co). The maze was sanitized with a 30% alcohol solution after each trial. The anxiety index was calculated using the following formula:

$$\text{Anxiety Index} = 1 - \frac{\left(\frac{\text{time spent in open arms}}{\text{total time}} + \frac{\text{number of open arms entries}}{\text{total entries}}\right)}{2}$$

The standard EPM index was calculated by the formular:

$$\text{EPM Index} = 100 \times \frac{\text{Time spent in open arms}}{(\text{time spent in open arms} + \text{time spent in closed arm})}$$

Brain tissue Preparation

Rats were anesthetized via intravenous injection of 1.5% isoflurane solution (RWD, China), followed by perfusion with 4% paraformaldehyde (PFA). Subsequently, the entire brain was harvested and processed. It was then fixed in 4% paraformaldehyde for 48 h and embedded in paraffin after processing in a tissue processor. Selects of the related brain regions were selected with the atlas of George Paxinos and Charles Watson (6th edition from the rat brain in stereotaxic coordinates) [14]. Tissue sections of 4 μm were cut on a microtome (Leica, China) and mounted on slides (Servicebio, China).

Immunofluorescence staining

Brain tissue sections were deparaffinized and then washed twice with PBS buffer. Subsequently, the sections were blocked with 10% normal goat serum (EMD Millipore) at room temperature for 1 h. After washing again with PBS buffer, the sections were incubated overnight at 4 °C with the following primary antibodies: neuronal nuclei-specific protein (NeuN, 1:200, Servicebio, GB11138), ionized calcium-binding adapter molecule 1 (Iba-1, 1:1000, Servicebio, GB113502), and glial fibrillary acidic protein (GFAP, 1:1000, Servicebio, GB15096). Following the primary antibody incubation, the sections

were thoroughly washed three times with PBS buffer and then incubated with the corresponding secondary antibodies for 50 min at room temperature: goat anti-rabbit CY3 (1:300, Servicebio, GB21303) or Alexa Fluor 488 goat anti-mouse IgG (1:400, Servicebio, GB21401). After the secondary antibody incubation, the sections were washed three times again with PBS buffer, placed on slides, and air-dried. The nuclei were counterstained with DAPI (Vector Laboratory, Inc. Burlingame, CA, USA). Finally, the sections were washed three times with PBS and mounted with an anti-fade fluorescence mounting medium. The mounted sections were imaged using a digital slide scanner (Pannoramic DESK p250, Hungary) for subsequent image analysis.

To quantify the immunopositive cells, we employed two distinct approaches using Image-Pro Plus 6.0 for analysis. For $NeuN^+$ cells, which were relatively sparse and required apoptosis number analysis, we conducted a cell counting analysis. Conversely, for Iba-1^+ microglia and $GFAP^+$ astrocytes, which exhibited high density, relatively uniform distribution, and bright immunostaining, we performed fluorescence intensity analysis. This method was chosen due to the high density and uniformity of these cells, which made fluorescence intensity a more suitable measure. For each rat, three sections were used, and three to six rats per experimental group were utilized for the histological analysis.

Western blot analysis

Proteins from the hippocampus were extracted utilizing RIPA buffer (Beyotime), enriched with a cocktail of protease and phosphatase inhibitors (Beyotime). Total protein concentrations were determined by the BCA protein assay kit (Thermo Fisher Scientific). An equal quantity of total protein (10 μg) was resolved on 4–12% polyacrylamide gels and electrophoretically transferred onto polyvinylidene fluoride (PVDF) membranes (MilliporeSigma) with the aid of TransBlot Turbo transfer system (Bio-Rad). Membranes were blocked with a 5% skim milk solution in 0.1% TBST for 1 h at room temperature, then incubated with primary antibodies at 4 °C overnight, followed by a 1-hour incubation with respective secondary antibodies at room temperature. Immunoreactive bands were detected using the ECL-plus chemiluminescent substrate (MilliporeSigma), and band intensities were quantified utilizing ImageJ analysis software. The primary antibodies utilized were zonula occludens-1 (ZO-1, 1:800, Affinity, AF5145), occludin (1:800, Affinity, DF7504), and claudin-5 (1:1000, Affinity, AF5216), B-cell lymphoma protein 2 (BCL-2, 1:2000, Abmart, 26593-1-AP) and Bcl-2-associated X (BAX, 1:800, Cell signaling technology, 2772 S), Caspase- 3 (1:800, Cell signaling technology, 9662 S) and cleaved Caspase- 3 (1:500, Cell signaling technology, 9664T).

Real-time polymerase chain reaction (qPCR)

qPCR was performed using QuantiTect SYBR green PCR kit (Qiagen, Crawley, UK), and the primer sequences were listed in Table S1. Total mRNA was isolated using TRIzol reagent (Ambion, United States) and reverse transcribed with the PrimeScript RT reagent kit (Takara, Japan) following the supplier's protocol. qPCR was conducted employing the SYBR Green detection method (Roche, Germany). Triplicate reactions for each sample were normalized against β-actin expression. Relative mRNA expression levels were determined employing the $2^{-\Delta\Delta Ct}$ comparative quantification method.

Measurement of biochemical indicators in blood

S100β concentrations were determined in the blood serum using enzyme-linked immunosorbent assay (ELISA) kits from eBioscience (R&D Systems, Minneapolis, MN).

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 25. The normality of the data was assessed using the one-sample Kolmogorov-Smirnov test. For normally distributed data, comparisons between two groups were performed using the two-tailed independent samples t-test (Student's t-test), while comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA). If a significant difference was detected, Tukey's multiple comparisons test was employed to determine the significance between each pair of groups. For data that did not follow a normal distribution, the Mann–Whitney U test was used for comparisons between two groups, and the Kruskal-Wallis one-way ANOVA was applied to evaluate differences among three or more groups. When the Kruskal-Wallis test indicated significant differences, Dunn's multiple comparisons test was selected to perform all pairwise comparisons. The significance threshold was set at $p < 0.05$. Data visualization was performed using GraphPad Prism 9.0.0. For small sample sizes ($n < 15$), individual values were presented using scatter plots to display the distribution of data points [15].

Results

Anxiety-Related behavior in male rats Post-Acute noise exposure at 3 days

We assessed anxiety behavior in male rats three days after acute noise exposure using the OFT and EPMT ($n = 8$ for minimum sample size; $n = 10$ for maximum sample size), and representative trajectory and heatmap images are shown in Fig. 1b. OFT data indicated that rats subjected to 135 dB noise exhibited a significant decrease in total distance (One-way ANOVA, $F(4, 43) = 8.680$, $P < 0.0001$; Fig. 1c) moved and time in the center (Kruskal

Wallis one-way ANOVA on ranks and post hoc Dunn's test, $P<0.05$; Fig. 1d) compared to controls. Moreover, fewer rats from the 135 dB group entered the center area (Kruskal Wallis one-way ANOVA on ranks and post hoc Dunn's test, $P<0.05$; Fig. 1e).

In the EPMT, rats exposed to 135 dB noise showed increased time in closed arms (one-way ANOVA, F (4, 42) = 7.212, $P<0.001$; Fig. S1), reduced time and entries in open arms (Kruskal Wallis one-way ANOVA on ranks and post hoc Dunn's test, $P<0.01$; Fig. 1f), lower EPM index (Kruskal Wallis one-way ANOVA on ranks and post hoc Dunn's test, $P<0.01$; Fig. 1g), and higher anxiety index (Kruskal Wallis one-way ANOVA on ranks and post hoc Dunn's test, $P<0.0001$; Fig. 1h). No significant differences were observed between control and groups exposed to 105–125 dB noise.

These results suggest that 135 dB noise exposure induces anxiety-like behavior in male rats three days post-exposure.

Anxiety-Related behavior in male rats Post-Acute noise exposure at 1 month

Anxiety behavior was also evaluated one month after noise exposure ($n=8$ for minimum sample size; $n=9$ for maximum sample size), and representative trajectory and heatmap images are shown in Fig. 2a. OFT results showed a significant reduction in distance (Kruskal Wallis one-way ANOVA on ranks and post hoc Dunn's test, $P<0.05$; Fig. 2b), time in the center (Kruskal Wallis one-way ANOVA on ranks and post hoc Dunn's test, $P<0.01$; Fig. 2c), and entries into the center (Kruskal Wallis one-way ANOVA on ranks and post hoc Dunn's test, $P<0.01$; Fig. 2d) for rats exposed to 135 dB noise.

EPMT results for the 135 dB group revealed decreased time in open arms (One-way ANOVA, F (4, 38) = 4.674, $P<0.01$; Fig. 2e), lower EPM index (one-way ANOVA, F (4, 38) = 4.881, $P<0.01$; Fig. 2f), and higher anxiety index (One-way ANOVA, F (4, 38) = 3.226, $P<0.05$; Fig. 2g). No significant changes were found between control and 105–125 dB exposed groups.

These findings indicate persistent anxiety-like behavior in male rats one month after 135 dB noise exposure.

Hippocampal neuron damage in male rats Post-135 dB acute noise exposure at 1 month

Hippocampal neuron damage was assessed using immunofluorescence staining ($n=6$). NeuN staining, a marker for mature neurons [16], was used to evaluate neuron density (Fig. 3a). No significant changes were observed three days post-exposure, but a significant decrease in NeuN-positive cells was seen one month later in the hippocampal regions (CA1: one-way ANOVA, F (2, 15) = 24.56, $P<0.0001$; CA3: one-way ANOVA, F (2, 15) = 110.3, $P<0.0001$; DG: one-way ANOVA, F (2, 15) = 45.24, $P<0.0001$; Figs. 3b-d). In addition, Fig. 3e presents the correlation analysis results between the reduction in the number of NEUN-positive cells and the difference in anxiety index one month after noise exposure (Pearson Correlation Analysis, $R=0.8864$, $P=0.0186$).

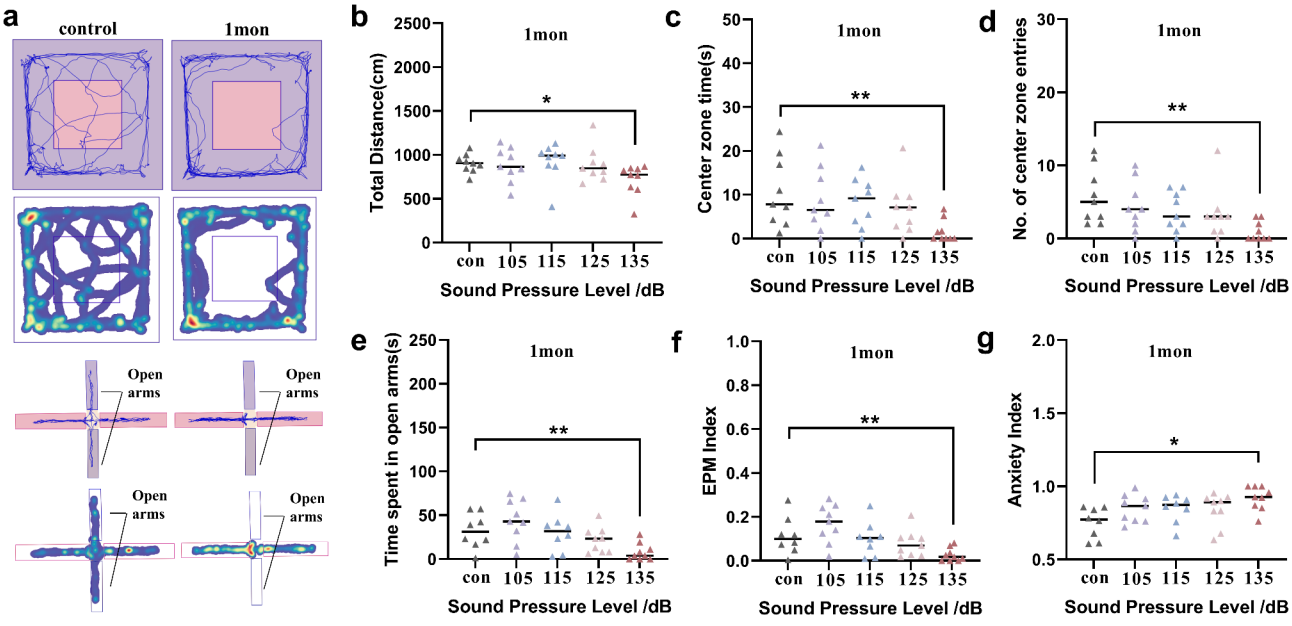

Fig. 2 The changes in anxiety behavior and body weight of male rats one month after exposure to acute noise of different intensities. (**a**) Representative motion trajectories of male rats in the OFT (top) and EMPT (bottom). (**b**) Total distance, (**c**) center zone time (**d**) NO. of center zone entries, in OFT after different sound pressure level of acute noise exposure. (**e**) Time spent in open arms, (**f**) EPM index, (**g**) Anxiety index in EPMT after different sound pressure level of acute noise exposure. $N=8$–10 rats per condition. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

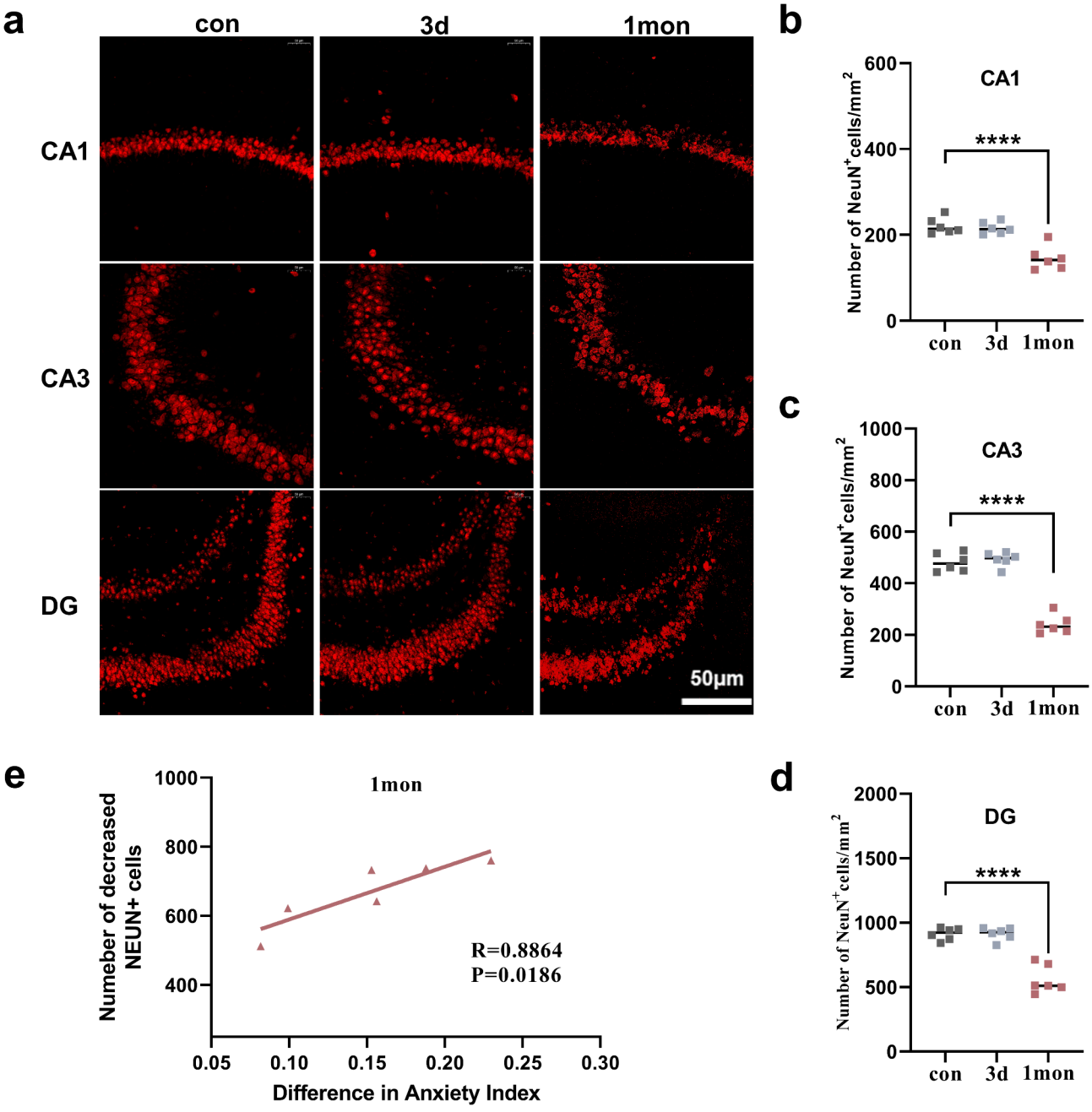

Fig. 3 The neural injury and the number of NeuN$^+$ cells in the hippocampus in male rats at 135dB on the third day and 1month later. (**a**) Representative pictures of NeuN immunofluorescence staining (red) (Scale bar: 50 μm). The number of NeuN$^+$ cells in the CA1 (**b**), CA3 (**c**), DG (**d**) of hippocampus (Scale bar: 50 μm). (**e**) Correlation analysis between the reduction in the number of NEUN-positive cells and the difference in anxiety index (Pearson Correlation Analysis). $N = 6$ rats per condition. ****$P < 0.0001$

These results suggest that exposure to 135 dB noise exacerbates hippocampal neuron damage over time and is persistently associated with anxiety-like behaviors.

Glia activation in the Hippocampus of male rats Post-135 dB acute noise exposure

Iba-1 and GFAP, markers for microglial and astrocytic activation, were assessed via immunofluorescence staining ($n = 3$). GFAP staining showed increased density in CA1, CA3, and DG regions three days post-135 dB noise exposure, with more pronounced effects one month later (One-way ANOVA, CA1: F (2, 6) = 36, $P < 0.001$; CA3: F (2, 6) = 23.18, $P < 0.01$; DG: F (2, 6) = 35.96, $P < 0.001$; Figs. 4b-d). Iba-1 staining also indicated increased microglial activation three days post-exposure, with a more severe increase one month later

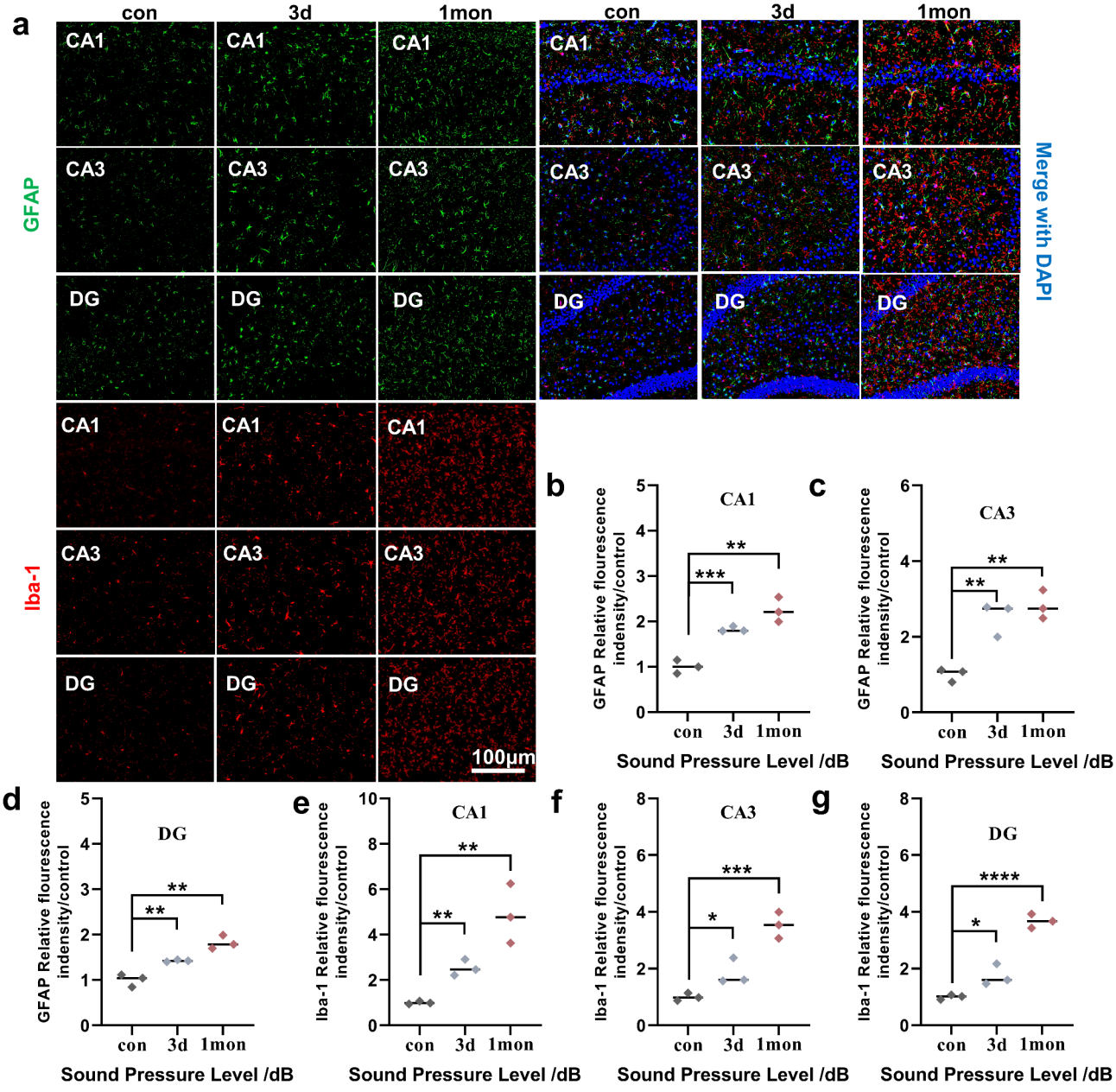

Fig. 4 Effects of 135dB acute noise exposure on hippocampal glia activation of male rats. (**a**) Representative microphotograph of immunofluorescence staining showed the expression of Iba-1 and GFAP in the CA1, CA3, and DG of hippocampus. Fluorescence quantification of normalized GFAP in CA1 (**b**), CA3 (**c**), and DG (**d**). Fluorescence quantification of normalized Iba-1 in CA1 (**e**), CA3 (**f**), and DG (**g**). $N = 3$ rats per condition. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

(One-way ANOVA, CA1: F (2, 6) = 18.59, $P < 0.01$; CA3: F (2, 6) = 33.63, $P < 0.001$; DG: F (2, 6) = 82.86, $P < 0.0001$; Figs. 4e-g).

These results indicate that 135 dB noise exposure triggers glial cell activation in the hippocampus of male rats, with effects persisting up to one month.

Neuroinflammation and neuronal apoptosis induced by 135 dB acute noise exposure in male rats

Cytokines play a pivotal role in orchestrating inflammatory responses, with pro-inflammatory cytokines initiating or exacerbating inflammation, and anti-inflammatory cytokines performing the opposite function [17]. To ascertain whether glial cell activation leads to the release of inflammation-related factors, we measured the gene expression levels of IL-1β, TNF-α, and IL-10 in the rat hippocampus using RT-qPCR ($n = 4$). Quantitative

analysis revealed that, compared to the control group, the expression of pro-inflammatory factors IL-1β (one-way ANOVA, F (2, 9) = 44.2, $P < 0.0001$; Fig. 5a) and TNF-α increased (one-way ANOVA, F (2, 9) = 51.44, $P < 0.0001$; Fig. 5b), while anti-inflammatory IL-10 decreased (Kruskal Wallis one-way ANOVA on ranks and post hoc Dunn's test, $P < 0.01$; Fig. 5c), both three days and one month after noise exposure.

There is substantial literature supporting the close association of Bcl-2 with anti-apoptotic functions and Bax with pro-apoptotic functions, while caspase-3 is recognized as a key executor of apoptosis [18]. In this study, western blot analysis was used to investigate noise exposure's impact on neuronal apoptosis ($n = 4$, Fig. 5d). No significant apoptosis was observed three days post-exposure, but a significant increase was seen one month later, indicated by increased Bax (One-way ANOVA, F (2, 9) = 12.97, $P < 0.01$; Fig. 5e), Bcl-2 (One-way ANOVA, F (2, 9) = 39.94, $P < 0.0001$; Fig. 5h), and cleaved-caspase-3 expression (One-way ANOVA, F (2, 9) = 7.481, $P < 0.05$; Fig. 5g). The expression of caspase-3 showed no significant changes (One-way ANOVA, F (2, 9) = 0.5821, $P = 0.5784$; Fig. 5f).

These findings suggest that 135 dB noise exposure induces neuroinflammation and neuronal apoptosis in the hippocampus of male rats.

Blood-Brain barrier (BBB) permeability increased by 135 dB acute noise exposure in male rats

ZO-1, claudin-5, and occludin are essential components that sustain the functional properties of the BBB [19]. Western blot analysis ($n = 4$, Fig. 6b) indicated that acute

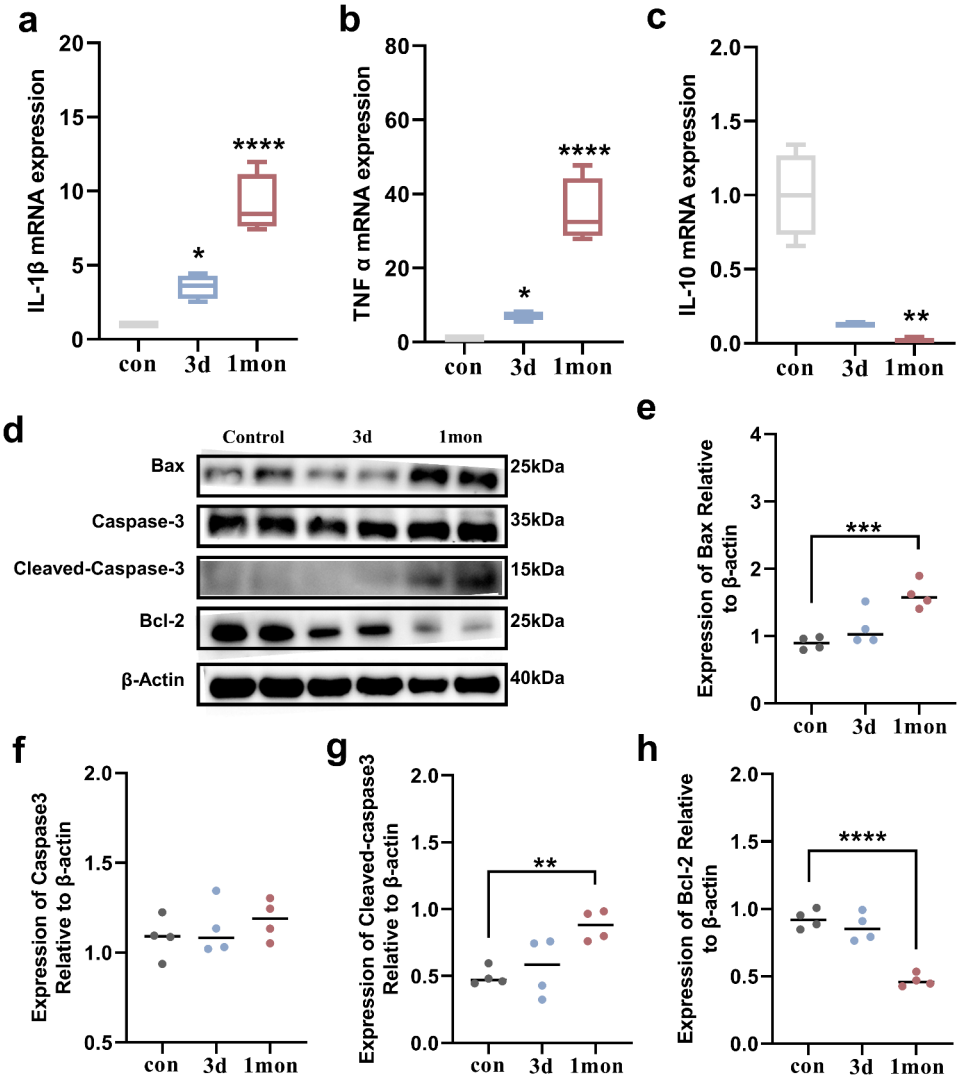

Fig. 5 Effects of 135dB acute noise exposure on hippocampal neuroinflammation and neuronal apoptosis in male rats. qRT-PCR analysis of IL-1β (**a**), TNF-α (**b**), and IL-10 (**c**). (**d**) The protein expression levels of apoptosis. Quantification of Bax (**e**), Caspase3 (**f**), Cleaved-Caspase3 (**g**), and Bcl-2 (**h**) protein expression in the hippocampus of male rats. $N = 4$ rats per condition. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

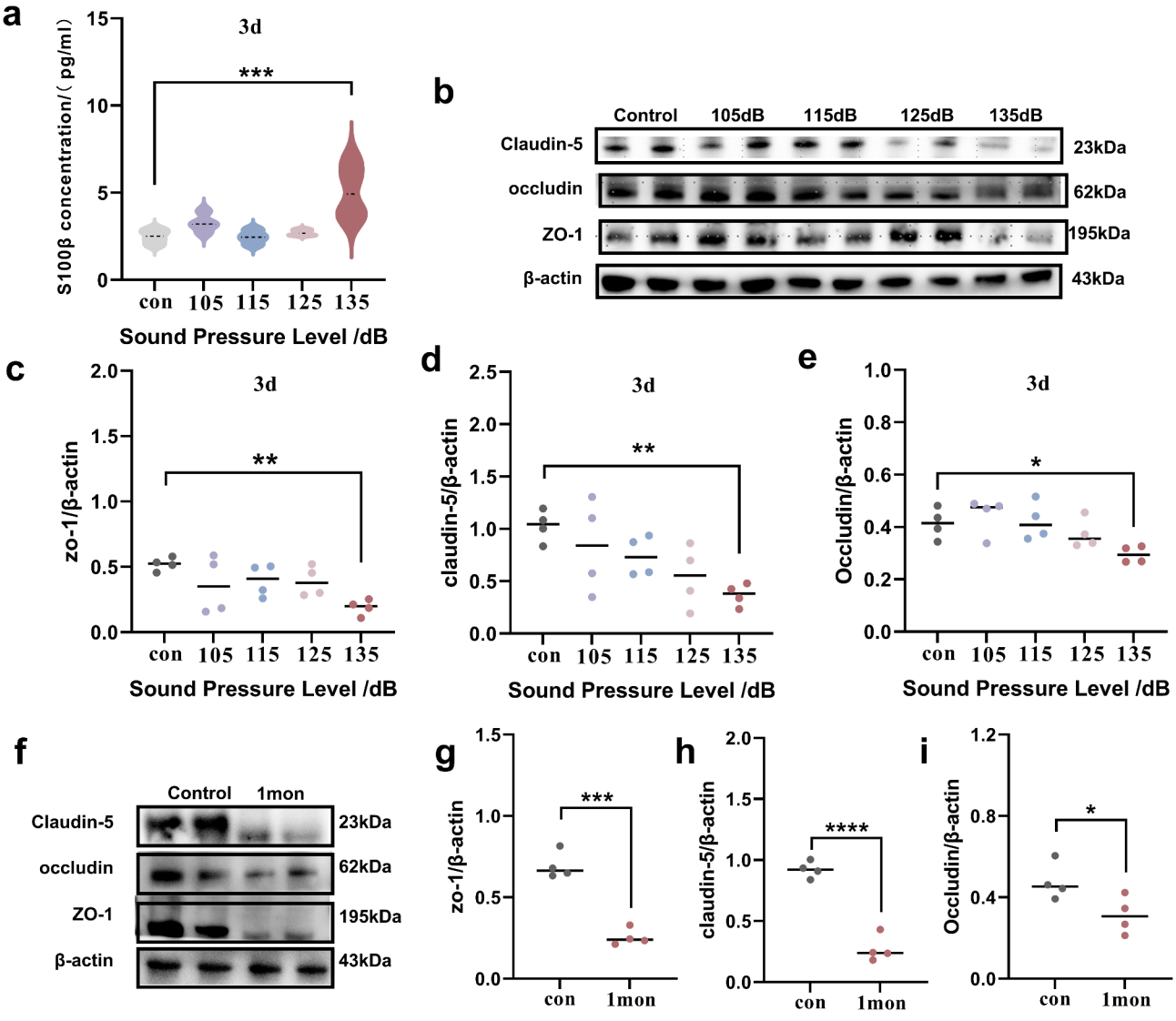

Fig. 6 Effects of 135dB acute noise exposure on blood brain barrier of male rats. (**a**) The content of S100β in hippocampus on the third day following exposure to acute noise at different intensities. (**b**) Expression levels of blood-brain barrier-related proteins in the hippocampus of male rats on the third day following exposure to acute noise at different intensities. Quantification of ZO-1 (**c**), Claudin-5 (**d**), and Occludin (**e**) protein expression. (**f**) Expression levels of blood-brain barrier-related proteins in the hippocampus of male rats one month after exposure to acute noise at 135dB. Quantification of ZO-1 (**g**), Claudin-5 (**h**), and Occludin (**i**) protein expression. $N = 4$ rats per condition. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

noise exposure at 135 dB significantly reduced their expression three days post-exposure, with no significant changes at 105–125 dB noise levels (zo-1: one-way ANOVA, F (4, 15) = 3.325, $P < 0.05$; claudin-5: one-way ANOVA, F (4, 15) = 3.651, $P < 0.05$; occludin: Kruskal Wallis one-way ANOVA on ranks and post hoc Dunn's test, $P < 0.05$; Figs. 6c-e). Serum S100β concentration, measured by ELISA, was significantly elevated ($n = 4$, one-way ANOVA, F (4, 15) = 7.822, $P < 0.01$; Fig. 6a). 1month post-exposure ($n = 4$, Fig. 6f), the expression of these tight junction proteins remained significantly decreased (Student's t-test, zo-1:$P = 0.0001$; claudin-5: $P < 0.0001$; occludin: $P = 0.0457$; Figs. 6g-i).

These findings suggest that acute noise exposure at 135 dB may induce BBB dysfunction within the hippocampus of male rats.

Discussion

This study explored the association between acute high-intensity noise exposure and anxiety behavior in male rats. The results indicated that acute noise-induced anxiety is mediated by neuroinflammation and BBB impairment, culminating in hippocampal neuronal apoptosis. The main findings are summarized as follows: (1) Exposure to 135 dB of acute noise induced anxiety, which persisted even after one month. (2) Noise exposure activated microglia and astrocytes, triggering a neuroinflammatory

response. (3) Noise exposure increased the permeability of the BBB. (4) Hippocampal neuronal apoptosis was observed one month post-exposure, with a significant correlation to anxiety levels.

We employed the OFT and the EPMT, both recognized as standard experimental paradigms, to evaluate rodent anxiety-like behaviors. Anxiety levels were determined by the frequency and duration of entries into zones perceived as aversive, a methodology validated in our prior research [20]. These tests were also employed to evaluate the impact of noise exposure on anxiety levels [21]. Our findings revealed that rats exhibited significant anxiety-like behaviors three days after exposure to 135 dB noise (Fig. 1, Fig. S1), with these behaviors persisted up to one month post-exposure (Fig. 2).

Neuroinflammation has been confirmed to induce anxiety-like behaviors [22, 23]. Glial cells, as the innate immune cells of the central nervous system, play a central role in regulating neuroinflammatory responses and are activated under specific neuropathological conditions [24]. Activation of glial cells can lead to the release of pro-inflammatory cytokines, such as TNF-α and IL-1β [24, 25]. This supports our findings of acute noise exposure inducing microglial and astrocytic activation in the hippocampal region (Fig. 4), along with increased expression of pro-inflammatory factors TNF-α and IL-1β and decreased expression of the anti-inflammatory factor IL-10 (Figs. 5a-c). These results suggest that acute noise exposure may induce anxiety behavior through the neuroinflammatory response mediated by the activation of glial cells.

In addition, the quantity and morphological characteristics of neurons in the hippocampal region are closely associated with anxiety symptoms [26, 27, 28]. In this study, we used NeuN, a neuron-specific biomarker [16] to assess changes in the number of hippocampal neurons following high-intensity acute noise exposure. Our observations revealed no significant neuronal damage three days post-exposure; however, a significant reduction in hippocampal neuron count was observed one month after exposure, indicating that noise exposure induced neuronal loss and damage (Fig. 3). Concurrently, there was a significant increase in the density of microglia and astrocytes one month later (Fig. 4). This prolonged glial cell activation is consistent with the observed phenomenon of neuronal apoptosis one month post-exposure (Fig. 3), suggesting that acute noise exposure may trigger a widespread chronic neuroinflammatory response. The research by Zhang Kai et al. [29] has demonstrated that the neurotoxicity mediated by microglia and astrocytes significantly exacerbates neuronal death. In addition, our previous studies have confirmed that anxiety-like behavior induced by acute noise exposure is closely related to neuronal apoptosis in the hippocampus [30]. Therefore, it can be hypothesized that acute noise exposure may promote neuronal apoptosis and the development of persistent anxiety-like behavior through neuroinflammation caused by the activation of neuroglial cells.

The BBB, a selectively permeable interface composed of endothelial cells, strictly governs the transfer of cells, molecules, and ions between the bloodstream and the brain [31]. BBB integrity is essential for maintaining the brain's microenvironment, and its disruption has been linked to anxiety-related behaviors [32, 33]. In our study, we observed that acute high-intensity noise exposure led to an increase in serum S100β levels (Fig. 6e), a protein that may leak from brain tissue into the bloodstream when the BBB is compromised, resulting in elevated serum concentrations. This finding suggests that noise exposure induces neuronal damage and increased BBB permeability. A study has confirmed that high-intensity noise exposure can directly increase BBB permeability in pigs [34]. To further investigate the impact of noise exposure on BBB permeability, we conducted relevant research. Our results found that acute exposure to 135 dB noise significantly reduced the expression of tight junction proteins ZO-1, claudin-5, and occludin in the hippocampal region of rats (Figs. 6a-d), and this effect persisted for at least a month (Figs. 6f-i). These findings indicate that acute noise exposure directly damages the BBB, enhancing the permeability of the BBB in the hippocampal region of male rats. BBB damage has been shown to exacerbate neurotoxicity induced by environmental exposures, potentially leading to anxiety [35, 36]. Additionally, studies have shown that neuroinflammation can aggravate BBB damage [5]. Considering that noise exposure can activate microglia and astrocytes in the hippocampal region, leading to the release of pro-inflammatory cytokines, we believe this may be the primary cause of changes in BBB permeability. Notably, high-intensity acute noise exposure may directly alter BBB permeability, allowing more inflammatory mediators to flow in and exacerbate the inflammatory response. This could initiate a vicious cycle where neuroinflammation and BBB disruption reinforce each other, potentially leading to neuronal apoptosis and increasing the risk of psychological health conditions, including anxiety and depression.

In summary, the results demonstrate that acute noise exposure at 135 dB can activate neuroglial cells, exacerbate neuroinflammatory responses, and potentially increase the permeability of the blood-brain barrier directly. These sustained pathological changes may lead to long-term hippocampal dysfunction and neuronal apoptosis, thereby triggering persistent anxiety-like behavior. Our findings provide novel biological insights into the link between acute high-intensity noise exposure and long-term mental health issues. However, these results have only been observed in rats, and their

application in human experiments remains unknown. Additionally, the stress response may also play a significant role in this process. In the future, we plan to establish more experiments to further investigate the effects of different stress paradigms on inducing anxiety-like behavior and hippocampal dysfunction. Meanwhile, damage to the auditory pathway may also have an indirect impact on anxiety-like behavior. Therefore, studying the transmission of noise from the external auditory canal to the central nervous system is equally crucial.

Conclusion

In summary, our study's findings indicate that acute noise exposure at 135 dB induces long-term anxiety behavior in rats through the activation of microglia and astrocytes, which mediates neuroinflammation and exacerbates blood-brain barrier (BBB) damage, ultimately leading to hippocampal neuronal apoptosis. Furthermore, the disruption of the BBB and the activation of glial cells may act in concert to enhance neurotoxicity.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Author contributions

JZ, YS, and XW designed the study. YS and HZ took care of the SD rats, collected, analyzed and interpreted the data. LH, YK, and ZF participated in behavior tests and data collection. HZ and FZ participated in the establishment of the animal model. YS wrote the manuscript. JZ, HZ and XW provided technical assistance in experimental work and corrected the manuscript. All authors read and approved the final manuscript to be submitted.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the Animal Care and Use Committee at Xi'an Jiaotong University (approval number: XJTU 2021 – 1501) and adhered to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978).

Competing interests

The authors declare no competing interests.

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References

1. Arjunan A, Rajan R. Noise and brain. Physiol Behav. 2020;227:113136.
2. Yehudai N, et al. Acute acoustic trauma among soldiers during an intense combat. J Am Acad Audiol. 2017;28(5):436–43.
3. Parameshwarappa V, Norena AJ. The effects of acute and chronic noise trauma on stimulus-evoked activity across primary auditory cortex layers. J Neurophysiol. 2024;131(2):225–40.
4. Manning C, Mermagen T, Scharine A. The effect of sensorineural hearing loss and tinnitus on speech recognition over air and bone conduction military communications headsets. Hear Res. 2017;349:67–75.
5. Hahad O et al. Noise and mental health: evidence, mechanisms, and consequences. J Expo Sci Environ Epidemiol, 2024.
6. Lan Y, et al. Transportation noise exposure and anxiety: A systematic review and meta-analysis. Environ Res. 2020;191:110118.
7. Jafari Z, Kolb BE, Mohajerani MH. Chronic traffic noise stress accelerates brain impairment and cognitive decline in mice. Exp Neurol. 2018;308:1–12.
8. Cheng Y, et al. Noise induced Depression-Like behavior, neuroinflammation and synaptic plasticity impairments: the protective effects of Luteolin. Neurochem Res. 2022;47(11):3318–30.
9. Said MA, El-Gohary OA. Effect of noise stress on cardiovascular system in adult male albino rat: implication of stress hormones, endothelial dysfunction and oxidative stress. Gen Physiol Biophys. 2016;35(3):371–7.
10. Babisch W. The noise/stress concept, risk assessment and research needs. Noise Health. 2002;4(16):1–11.
11. Lee CH, et al. Effect of acute noise trauma on the gene expression profile of the hippocampus. BMC Neurosci. 2020;21(1):45.
12. Phelps EA. Human emotion and memory: interactions of the amygdala and hippocampal complex. Curr Opin Neurobiol. 2004;14(2):198–202.
13. Molina SJ, et al. Exposure of developing male rats to one or multiple noise sessions and different housing conditions: hippocampal thioredoxin changes and behavioral alterations. Front Behav Neurosci. 2019;13:182.
14. <6版大鼠脑图谱.pdf>.
15. Weissgerber TL, et al. Beyond bar and line graphs: time for a new data presentation paradigm. PLoS Biol. 2015;13(4):e1002128.
16. Wolf HK, et al. NeuN: a useful neuronal marker for diagnostic histopathology. J Histochem Cytochem. 1996;44(10):1167–71.
17. Shi Z, et al. Chronic exposure to environmental pollutant ammonia causes damage to the olfactory system and behavioral abnormalities in mice. Environ Sci Technol. 2023;57(41):15412–21.
18. Yan Z, et al. Combined exposure of heat stress and Ozone enhanced cognitive impairment via neuroinflammation and blood brain barrier disruption in male rats. Sci Total Environ. 2023;857(Pt 3):159599.
19. Sweeney MD, Sagare AP, Zlokovic BV. Blood-brain barrier breakdown in alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018;14(3):133–50.
20. Kang Y, et al. Exercise ameliorates anxious behavior and promotes neuroprotection through osteocalcin in VCD-induced menopausal mice. CNS Neurosci Ther. 2023;29(12):3980–94.
21. Gogokhia N, et al. Gender differences in anxiety response to high intensity white noise in rats. Neurosci Lett. 2021;742:135543.
22. Guo B, et al. Neuroinflammation mechanisms of neuromodulation therapies for anxiety and depression. Transl Psychiatry. 2023;13(1):5.
23. Zou L, et al. High-cholesterol diet promotes depression- and anxiety-like behaviors in mice by impact gut microbe and neuroinflammation. J Affect Disord. 2023;327:425–38.
24. Glass CK, et al. Mechanisms underlying inflammation in neurodegeneration. Cell. 2010;140(6):918–34.
25. Zhang H, et al. Co-exposure of chronic stress and alumina nanoparticles aggravates hippocampal microglia pyroptosis by activating cathepsin B/NLRP3 signaling pathway. J Hazard Mater. 2022;436:129093.
26. Alinaghipour A, Salami M, Nabavizadeh F. Nanocurcumin substantially alleviates noise stress-induced anxiety-like behavior: the roles of tight junctions and NMDA receptors in the hippocampus. Behav Brain Res. 2022;432:113975.
27. Barzegar M, et al. Prenatal exposure to noise stress: anxiety, impaired Spatial memory, and deteriorated hippocampal plasticity in postnatal life. Hippocampus. 2015;25(2):187–96.
28. Rashno M, et al. Correction to: Chrysin attenuates traumatic brain injury-induced recognition memory decline, and anxiety/depression-like behaviors in rats: insights into underlying mechanisms. Psychopharmacology. 2023;240(6):1387.

29. Zhang K, et al. Targeting microglial GLP1R in epilepsy: A novel approach to modulate neuroinflammation and neuronal apoptosis. Eur J Pharmacol. 2024;981:176903.
30. Song Y, et al. High-intensity acute noise exposure causes anxiety in female rats by inducing hippocampal neuron apoptosis. Ecotoxicol Environ Saf. 2025;291:117833.
31. Blanchette M, Daneman R. Formation and maintenance of the BBB. Mech Dev. 2015;138(Pt 1):8–16.
32. Gal Z, et al. [Anxiety and depression - the role of blood-brain barrier integrity]. Neuropsychopharmacol Hung. 2019;21(1):19–25.
33. Dion-Albert L, et al. Vascular and blood-brain barrier-related changes underlie stress responses and resilience in female mice and depression in human tissue. Nat Commun. 2022;13(1):164.
34. Wang X, et al. Effect of low-frequency but high-intensity noise exposure on swine brain blood barrier permeability and its mechanism of injury. Neurosci Lett. 2018;662:122–8.
35. Wang A, et al. High fried food consumption impacts anxiety and depression due to lipid metabolism disturbance and neuroinflammation. Proc Natl Acad Sci U S A. 2023;120(18):e2221097120.
36. Hubbard WB, et al. Resilience of females to acute blood-brain barrier damage and anxiety behavior following mild blast traumatic brain injury. Acta Neuropathol Commun. 2022;10(1):93.

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