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Effects of aircraft noise cessation on blood pressure, cardio- and cerebrovascular endothelial function, oxidative stress, and inflammation in an experimental animal model

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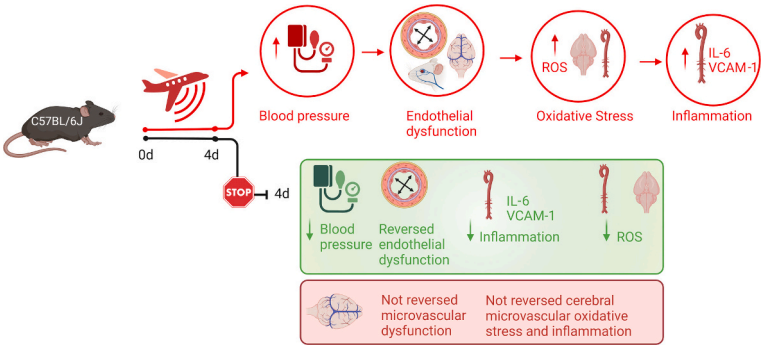
HIGHLIGHTS

- Short-term aircraft noise causes cardiovascular and cerebral damage in mice.
- Noise cessation by 4 days almost completely reverses noise inflicted cardiovascular damage.
- Damage in cerebral microvessels persists even after 4 days of noise cessation.
- Our data reveal differential longevity of adverse noise effects in vessels of the brain and the periphery.

GRAPHICAL ABSTRACT



Abbreviations: ACh, Acetylcholine; CD68, Cluster of Differentiation 68; dB(A), Decibel (A-weighted to reflect the perception by the human ear); DHE, Dihydroethidium; ET-1, Endothelin-1; GBD, Global Burden of Disease; IBA1, Ionized calcium-binding adapter molecule 1; IL, Interleukin; NIBP, Non-invasive blood pressure; NO, Nitric oxide; NOX, NADPH oxidase; PBS, Phosphate-buffered saline; PDBu, Phorbol 12, 13-dibutyrate; ROS, Reactive oxygen species; RT-qPCR, Quantitative Reverse-Transcription Polymerase Chain Reaction; VCAM-1, Vascular cell adhesion molecule; WHO, World Health Organization.

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ABSTRACT

Large epidemiological studies have shown that traffic noise promotes the development of cardiometabolic diseases. It remains to be established how long these adverse effects of noise may persist in response to a noise-off period. We investigated the effects of acute aircraft noise exposure (mean sound level of 72 dB(A) applied for 4d) on oxidative stress and inflammation mediating vascular dysfunction and increased blood pressure in male C57BL/6 J mice. 1, 2 or 4d of noise cessation after a 4d continuous noise exposure period completely normalized noise-induced endothelial dysfunction of the aorta (measured by acetylcholine-dependent relaxation) already after a 1d noise pause. Vascular oxidative stress and the increased blood pressure were partially corrected, while markers of inflammation (VCAM-1, IL-6 and leukocyte oxidative burst) showed a normalization within 4d of noise cessation. In contrast, endothelial dysfunction, oxidative stress, and inflammation of the cerebral microvessels of noise-exposed mice did not improve at all. These data demonstrate that the recovery from noise-induced damage is more complex than expected demonstrating a complete restoration of large conductance vessel function but persistent endothelial dysfunction of the microcirculation. These findings also imply that longer noise pauses are required to completely reverse noise-induced vascular dysfunction including the resistance vessels.

1. Introduction

Environmental noise is a transport-related health risk (Munzel et al., 2014; Munzel et al., 2018a; Munzel et al., 2021; WHO/EU). A large proportion of the population is exposed to noise levels exceeding the World Health Organization (WHO) guidelines. Although growing evidence links traffic noise to cardiovascular (CV) morbidity and mortality, transportation noise is neither mentioned as a health risk factor in the Global Burden of Disease (GBD) Study (only occupational noise is mentioned (GBD Collaborators, 2017)) nor in the report "Health at a Glance: Europe 2022" (OECD/EU, 2022). Strong evidence was provided by the WHO environmental noise guidelines for the European region, concluding that transportation noise stimulates the development of cardiometabolic disease (Kempen et al., 2018). According to the WHO guidelines, the pooled relative risk for ischemic heart disease (IHD) was 1.08 (95 % CI 1.01–1.15) per 10 dB(A) increase in noise exposure, starting at 50 dB(A) (Kempen et al., 2018). Transportation noise (railway and road traffic) was also associated with an increase in arterial stiffness, a subclinical marker of atherosclerosis and development of future cardiovascular disease (Foraster et al., 2017). Arterial hypertension, stroke, heart failure, arrhythmia and cognitive impairment, especially in children (Stansfeld et al., 2005) and impairment of mental health (Sygna et al., 2014) may also be associated with noise exposure (Munzel et al., 2014; Munzel et al., 2018a). According to the WHO, at least 1.6 million healthy life years are lost annually from traffic-related noise in Western Europe (Fritschi et al., 2011). A recent study from Switzerland concluded that years of life lost (YLL) due to traffic are dominated by air pollution, whereas adverse effects on morbidity and quality of life are dominated by noise (Vienneau et al., 2015). Road traffic is the dominant noise source, with 125 million people being affected, followed by rail traffic (nearly 8 million) and aircraft (almost 3 million). The WHO indicates the need for additional evidence for health impacts of noise by longitudinal studies (Kempen et al., 2018). Therefore, at least a more recent GBD Study publication acknowledges that environmental noise is among the risk factors not yet included and that "future rounds of GBD might evaluate whether these risk factors meet inclusion criteria" (GBD Collaborators, 2020).

Traffic noise-induced annoyance seems more pronounced in response to air versus road versus railway noise (Munzel et al., 2014) and annoyance could be an important modifier of noise health effects (Babisch et al., 2013). However, due to its prevalence, road traffic noise has a dominant impact on impaired cognition (Stansfeld et al., 2005), risk of stroke (Sorensen et al., 2011), arterial hypertension (van Kempen and Babisch, 2012) and diabetes (Sorensen et al., 2013). Whereas railway noise may cause severe health effects by the high sound pressure levels that may be reached (up to 100 dB(A)), it seems that the sound pattern of aircraft noise is more annoying. Aircraft noise, especially during nighttime, is associated with annoyance and hypertension as shown by the "Hypertension and exposure to noise near airports" (HYENA) study (Babisch et al., 2009; Jarup et al., 2008). In the present study we investigate the effects of aircraft noise in the brain and cardiovascular system since the Rhine-Main area suffers from a high aircraft noise burden due to the proximity to Frankfurt airport. In addition, we previously characterized the aircraft noise-mediated effects on the cardiovascular system in a mouse model (Kroller-Schon et al., 2018; Munzel et al., 2017) and human field studies (Herzog et al., 2019; Schmidt et al., 2013).

The mechanisms leading to vascular dysfunction in response to transportation noise are meanwhile better understood. According to the noise reaction model introduced by Babisch, an "indirect pathway", plays a crucial role in causing cardiovascular disease (Babisch, 2003). It represents the cognitive perception of noise, subsequent sympathoadrenal activation (Recio et al., 2016), increasing stress hormones, inflammation and pro-thrombotic pathways and endothelial dysfunction (Munzel et al., 2014; Munzel et al., 2018a) that contribute to cardiovascular disease, e.g. acute myocardial infarction (MI), heart failure, hypertension, arrhythmia and stroke (Babisch, 2014; Munzel et al., 2018a). Oxidative stress and tissue inflammation by infiltrated immune cells appears to be a hallmark of the adverse health effects of noise (Daiber et al., 2020; Frenis et al., 2021c), where noise promotes the adhesion and infiltration of blood leukocytes, mostly monocytes and macrophages, by shifting the vasculature and circulatory system to a pro-inflammatory phenotype (Eckrich et al., 2021a). Importantly, the adverse effects of noise can be completely prevented by genetic deletion of the phagocytic NADPH oxidase (Nox2) (Kroller-Schon et al., 2018) or by genetic ablation of LysM-positive myelomonocytic cells (Frenis et al., 2021a). Recently we demonstrated that during long-term noise exposure, these side effects remain constant and that no adaptation process may occur or that tolerance may develop concerning noise-induced hypertension, inflammation, oxidative stress, and endothelial dysfunction (Frenis et al., 2021b).

The time course concerning the reversibility of noise-induced adverse health effects in response to the noise-off period is of great clinical importance, which has yet to be addressed, including the impact on noise-induced hypertension, endothelial dysfunction, oxidative stress, and inflammation. From a preventive perspective, it is important to know how long the adverse health effects of noise persist, e.g., to recommend sufficiently long noise-free intervals for exposed populations by lowering nighttime noise exposure limits, prohibition of nighttime flights and railway traffic or other mitigation measures.

2. Materials and methods

2.1. Animals

All animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the U.S. National Institutes of Health with approval granted by the Ethics Committee of the University Medical Center Mainz and the Landesuntersuchungsamt Rheinland-Pfalz (Koblenz, Germany; permit number: 23177–07/G 18–1-084 E3 and G 20–1-103). All mice were male C57BL/6 J between 6 and 12 weeks old, purchased from Janvier (Le Genest-Saint-Isle, France). Only male mice were used because the menstrual cycle (via estrogen signaling) significantly influences endothelial function, our primary read-out. A total of 115 mice were used. The control group and 4d noise group consisted of 35 and 29 mice, respectively, as they were present on each experimental day. However, the 1, 2, and 4 day noise cessation groups were comprised of only 17 mice per group since they were not present on all experimental days. The mice were housed in a standard 12 h light/dark cycle in an institutional animal facility with ad libitum access to standard rodent chow and water. Following 4d noise exposure and subsequent discontinuation of exposure for 1, 2, and 4d, animals were sacrificed by cardiac puncture under ketamine/xylazine anesthesia. The blood, aorta, and brain were harvested. Whereas blood pressure was measured in all mice (as a general parameter to assess the successful noise exposure), the other parameters were not measured in all animals (e.g., due to limited availability of aortic tissue or logistic reasons such as the external measurements in retinal and cerebral microvessels) and for some measurements tissues and plasma were pooled.

2.2. Noise exposure

The noise chamber maintains the same housing conditions as the institutional animal facility to minimize transfer stress. Noise events (aircraft landing and take-off events) were 43 s long, irregularly distributed over a 2-h sequence, repeated constantly over 4d. The noise events were separated by irregularly distributed periods of relative silence (noise background level in the animal facility of 48 dB(A)) to prevent early adaptation and better to emulate the actuality of noise pollution in the environment. Noise was applied through downward-facing speakers positioned approximately 30 cm above open mouse cages as described (Munzel et al., 2018b). The sound system is a Grundig MS 540 with a total output of 65 W. The noise events had a maximum sound pressure level of 85 dB(A) and a mean of 72 dB(A) for 4d around-the-clock (Kroller-Schon et al., 2018; Munzel et al., 2017), sound pressure levels which are below the threshold for cochlear damage (Frenis et al., 2021b). Sound pressure levels were calibrated with a Class II Sound level meter (Casella CEL-246) within one of the cages at initial setup and were maintained constant throughout all exposure protocols. The duration of noise exposure varied by the experimental design, but the volume and sequence of noise intervals were maintained constant throughout all exposure protocols. The noise was either applied for 4d or mice remained unexposed, however with a background noise level in the animal facility of 48 dB(A). After 4d of exposure, mice were randomly assigned to four groups: 0d OFF (sacrificed directly after end of noise exposure), 1d OFF (sacrificed 1d after end of noise exposure), 2d OFF (sacrificed 2d after end of noise exposure), and 4d OFF (sacrificed 4d after the end of noise exposure). Also, during the "OFF" period or noise exposure cessation phase, the background noise level in the animal facility was 48 dB(A). The time window of noise cessation over up to 4d was chosen since also our noise exposure regimen over 4d is an acute model. Accordingly, we wanted to test here whether the fast onset of noise-mediated damage over 4d is also subject to fast recovery when noise is stopped for up to 4d. The schedule is shown in Fig. 1.

2.3. Non-invasive blood pressure (NIBP) measurement

Non-invasive blood pressure (NIBP) measurements were performed using a CODA High Throughput 2 Noninvasive Blood Pressure System (Kent Scientific, Torrington, USA) as published (Bayo Jimenez et al., 2021; Frenis et al., 2021a; Steven et al., 2020). Animals were allowed to enter the restraining tube freely, placed in restraining tubes on a pre-heated platform at 32 °C, and allowed to rest for 15 min. The occlusion cuff was fitted at the base of the tail and the volume-pressure recording

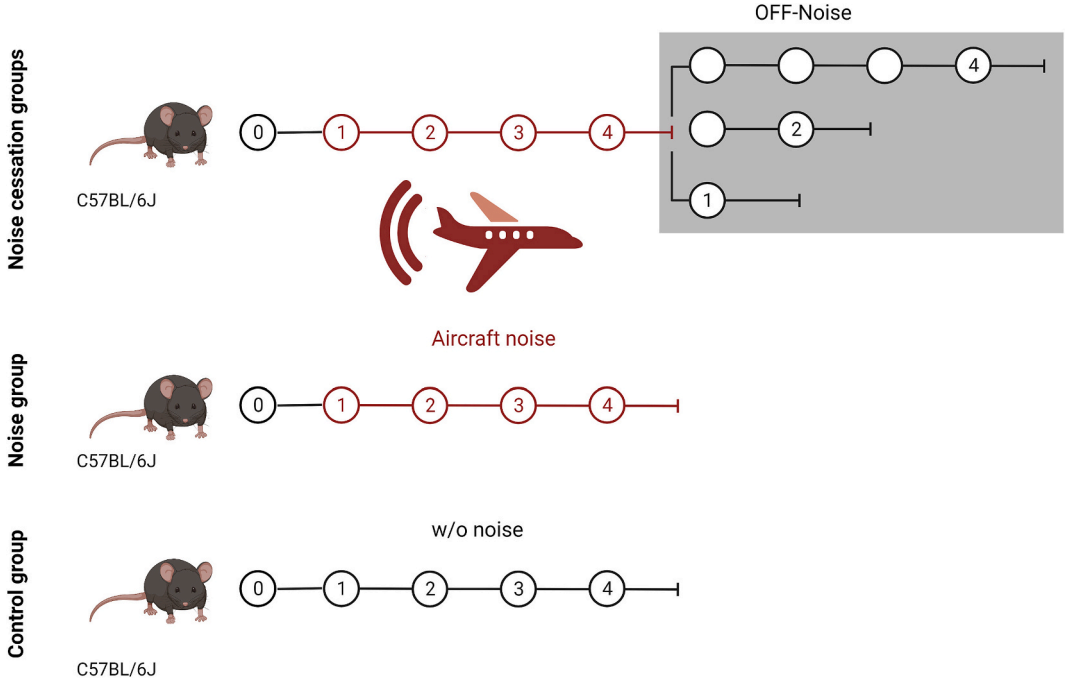

Fig. 1. Scheme for noise exposure. C57BL/6J mice were randomly assigned to five groups: the control (Ctr) group, 4d noise exposure (Noise), and the noise cessation groups, 1d OFF, 2d OFF, and 4d OFF. Created with BioRender.com.

cuff was placed distal to the occlusion cuff. The cuffs were adjusted in placement on the tail to be secure but not tight. Fifteen NIBP measurements were taken per animal at each time point, the first five of which were discarded as acclimation cycles. Measures took place in a quiet setting at a set time each day to account for the diurnal blood pressure variation. The mean values of ten NIBP readings were used for each animal.

2.4. Isometric tension studies in the aorta

Using isometric tension studies, we evaluated endothelial function in segments of the aorta. Upon subjection to different vasodilators and vasoconstrictors in organ bath chambers, this assay records different relaxation patterns of aortic ring segments (4 mm). The rings were then rewashed and pre-constricted with prostaglandin F2α ($PGF_{2\alpha}$) to yield approximately 80 % of the maximal tone induced by KCl bolus. Concentration-relaxation curves in response to increasing concentrations of acetylcholine (ACh) and nitroglycerin were performed as described (Kroller-Schon et al., 2018; Munzel et al., 2017). Relaxation responses are reported as a percentage of maximal $PGF_{2\alpha}$ constriction.

2.5. Microvascular function assessment

Cerebral arteriole isolation was recently described by us in detail (Eckrich et al., 2021a; Eckrich et al., 2021b). In brief, after careful isolation of the brain, a vascular tree of the middle cerebral artery (MCA) was isolated using fine-point-tweezers and microscissors. Subsequently, the MCA including its branches was transferred to a pressure myograph. Then, the main branch of the MCA was cannulated with a micropipette, and the pipette tip was guided into a branching arteriole by gently pulling the cannulated end of the MCA further on the pipette and by pushing the free end of the MCA against the pipette tip. After cannulation of the arteriole, the free end of the MCA was tied to the micropipette. Next, the non-cannulated end of the arteriole was tied to an opposite micropipette, and the pipette tips were moved apart to gently stretch the arteriole. After placing the pressure myograph on an inverted microscope, the arteriole was pressurized to 40 mmHg via the indwelling micropipette and visualized by a digital camera. A constant temperature of 37 °C and flow of carbogen gas (95 % oxygen, 5 % CO2 *v/v*) was maintained in the organ chamber throughout the experiment. Viability of the arteriole was assessed by adding KCl (100 mM) to the circulating bath solution. After an equilibration period of 30 min, the thromboxane mimetic, U46619 (10^{-11} to 10^{-6} M; Cayman Chemical, Ann Arbor, MI), was cumulatively applied to the circulating bath solution. After completion of the concentration-response curve, U46619 was washed out and the arteriole was preconstricted to 50–70 % of the initial diameter by titration of U46619. After preconstriction, responses to the endothelium-dependent vasodilator, acetylcholine, and to the nitric oxide (NO) donor, sodium nitroprusside, (both 10^{-9} to 10^{-4} M; Sigma-Aldrich, Munich, Germany) were measured to cumulative application of the respective substances into the organ chamber.

2.6. Detection of oxidative stress in whole blood by oxidative burst

Leukocyte-dependent oxidative burst is a readout for leukocyte-dependent hydrogen peroxide formation (mainly by phagocyte-type NADPH oxidase, Nox2) (Gollner et al., 2020). Hydrogen peroxide is converted by myeloperoxidase to highly reactive oxygen-metal complexes that lead to oxidation of L-012 (Wako; 120-04891) to a chemiluminescent intermediate (Zielonka et al., 2013). Heparinized blood harvested via cardiac puncture was diluted 1:50 with citrate and stimulated with 50 μg/ml zymosan A (Sigma; Z4250) or 10 μM phorbol ester dibutyrate (Sigma; P1269) in phosphate-buffered saline (PBS) containing 1 mM Ca^{2+}/Mg^{2+} by 100 μM L-012. Enhanced chemiluminescence (ECL) was measured using a Mithras2 chemiluminescence plate reader (Berthold Technologies, Bad Wildbad, Germany) as published (Kroller-Schon et al., 2018).

2.7. Detection of oxidative stress and inflammation in cortical and aortic tissue

Dihydroethidium (DHE)-dependent fluorescence microtopography was used to determine the vascular and cortical ROS formation in cryosections of the aorta and frontal cortex, as described (Oelze et al., 2006; Wenzel et al., 2008). To perform the staining, slides were thawed and warmed to 38 °C, incubated with 1 μM DHE solution for 30 min, covered with a coverslip. The reaction was stopped by placing the slides on ice for 10 min. ROS-derived red fluorescence was detected using a Zeiss Axiovert 40 CFL microscope, Zeiss lenses, and Axiocam MRm camera. ImageJ was used to quantify the images.

2.8. Detection of oxidative stress via DHE-HPLC

Oxidative stress from superoxide was also measured by a modified HPLC-based method to quantify 2-hydroxyethidium levels as previously described (Kalinovic et al., 2019; Kroller-Schon et al., 2018) and modified from another report (Zhao et al., 2005). Briefly, tissue of frontal cortex was incubated with 50 μM DHE for 30 min at 37 °C in PBS buffer. Tissues were homogenized in 50 % acetonitrile/50 % PBS using a glass homogenizer (brain frontal cortex) or pulverized in a mortar under liquid nitrogen and resuspended in homogenization buffer (aorta), centrifuged and 50 μl of the supernatant were subjected to HPLC analysis. The system consists of a control unit, two pumps, mixer, detectors, column oven, degasser and an auto sampler (AS-2057 plus) from Jasco (Groß-Umstadt, Germany) and a C18-Nucleosil 100–3 (125 × 4) column from Macherey & Nagel (Düren, Germany). A high pressure gradient was employed with acetonitrile and 50 mM citrate buffer pH 2.2 as mobile phases with the following percentages of the organic solvent: 0 min, 36 %; 7 min, 40 %; 8-12 min, 95 %; 13 min, 36 %. The flow was 1 ml/min and DHE was detected by its absorption at 355 nm whereas 2-hydroxyethidium was detected by fluorescence (Ex. 480 nm/Em. 580 nm).

2.9. Quantitative reverse transcription real-time PCR (qRT-PCR)

50 ng of total RNA from aortic tissue was used for quantitative reverse transcription real-time PCR (qRT-PCR) analysis using QuantiTect Probe RT-PCR kit (Qiagen) as described previously (Kroller-Schon et al., 2018; Munzel et al., 2017; Steven et al., 2020). TaqMan® primer-probe-mix purchased from Applied Biosystems (Foster City, CA) was used to analyze the mRNA expression patterns of vascular cell adhesion molecule 1 (VCAM-1, Mm00449197_m1). Each PCR reaction included 2 μl of RNA, 18 μl of iTaq Universal SYBR Green Real-Time PCR Supermix (Bio-Rad, Munich, Germany), resulting in a final volume of 20 μl per reaction. Samples were run in triplicates. A typical qRT-PCR thermal cycle consisted of a 30 min reverse transcription step at 50 °C and an initial denaturing step at 95 °C for 15 min, followed by 40 cycles of: denaturing at 95 °C for 15 s, and annealing/extension at 60 °C for 1 min. The housekeeper gene TATA box binding protein (Mm_00446973_m1) was used as an internal control. The comparative ΔΔCt method was used for relative mRNA quantification. Gene expression was normalized to the endogenous control TBP mRNA (One-step PCR), while the amount of target gene mRNA expression in each sample was expressed relative to that of control (ΔΔCt). Gene expression of target gene in each sample was expressed as the percentage of unexposed wild type.

2.10. Dot blot analysis of protein modification and expression

Following protein isolation from plasma and determination of concentration by Bradford assay, 20 μg of protein was transferred into each well to deposit on the nitrocellulose membrane (Sigma Aldrich, WHA10402506) via a Minifold I vacuum Dot-Blot system

(Schleicher&Schuell, 10484138CP) (Kroller-Schon et al., 2018; Munzel et al., 2017), washed twice with 200 μl of PBS then dried for 60 min at 60 °C to adhere the proteins. The membranes were then cut and incubated in Ponceau S solution (Sigma, P7170) for protein visualization. The stain was removed and the membrane blocked for one hour at room temperature with 5 % milk in PBS-T. The membranes were incubated with an antibody against interleukin 6 (Abcam, ab6672, 1:1000) overnight at 4 °C. Membranes were then washed 3× in TBS-T and incubated with anti-rabbit peroxidase-coupled secondary antibody (Vektor PI-1000, 1:10,000) for two hours at room temperature, at which point positive bands were detected using ECL development (Thermo Fisher, 32,106) and Chemilux Imager (CsX-1400 M, Intas). Densitometric quantification was performed using Gel-Pro Analyzer 6.0 software.

2.11. Staining of cerebral vessels

Cerebral arterioles together with surrounding brain tissue were isolated immediately after mice had been sacrificed, embedded in Tissue Tek OCT compound (Sakura Finetek Europe, Alphen aan den Rijn, Netherlands), frozen in liquid nitrogen and stored at −80 °C until use. For dihydroethidium (DHE) staining, cryosections of 10 μM thickness were placed on Superfrost Plus slides (Thermo Fisher Scientific, Menzel-Gläser, Braunschweig, Germany) and 1000 μL of 1 μM DHE solution were dropped on each slide. Next, slides were placed in a light-protected and humidified chamber and incubated at 37 °C for 30 min. Subsequently, the fluorescence was recorded by using an Eclipse TS 100 microscope (Nikon, Yurakucho, Tokyo, Japan) equipped with a DS–Fi1-U2 digital microscope camera (Nikon) and the imaging software NIS Elements (Nikon, Version 1.10 64 bit). Later, fluorescence intensity was measured in the vascular wall by using ImageJ (NIH, http://rsb.info.nih.gov/ij/) as described previously (Birk et al., 2021).

For immunohistochemical evaluation, frozen brain tissue sections of 10 μm thickness containing arteriole cross-sections were cut and fixed in 4 % paraformaldehyde (pH 7.4) solution for 20 min. Next, slides were rinsed with PBS and incubated at room temperature with a blocking solution containing 0.1 % Triton-X-100 and 0.1 % bovine serum albumin for 30 min. Next, primary antibodies directed against the prooxidant NADPH oxidase 2 (NOX2, 1:200, rabbit polyclonal, #B12365, LSBio, Seattle, WA, USA), interleukin 6 (IL-6, 1:100, rabbit monoclonal, #ab229381, Abcam, Waltham, MA, USA), the ionized calcium-binding adapter molecule 1 (IBA1, 1:400, rabbit monoclonal, #ab178846, Abcam), endothelin-1 (ET-1, 1:600, rabbit polyclonal, #ab117757, Abcam), vascular cell adhesion protein 1 (VCAM-1, 1:200, mouse monoclonal, #sc-13,160, Santa Cruz Biotechnology, Inc.) and cluster of differentiation 68 (CD68, 1:1000, mouse monoclonal, #ab955, Abcam), were diluted in blocking solution, transferred on the slides and incubated for 2 h at room temperature. Thereafter, each slide was washed with PBS three times for 5 min and incubated for 1 h at room temperature with a secondary antibody. As a secondary antibody either a Rhodamine Red-X-coupled antibody (1:200, goat anti-rabbit polyclonal, #111–295-003, Dianova GmbH, Hamburg, Germany) or an Alexa Fluor™ 488-coupled antibody (1:200, goat anti-mouse polyclonal, #A-11001, Thermo Fisher Scientific, Waltham, MA, USA) was applied for 1 h at room temperature. After incubation, residual antibody was removed by washing the tissue three times with PBS and each slide was mounted with 4′,6-diamidino-2-phenylindole (DAPI)-containing medium (VECTASHIELD® Mounting Medium with DAPI, H-1200, BIOZOL Diagnostica Vertrieb GmbH, Eching, Germany) and cover-slipped. Then, images of arteriole cross-sections were made by fluorescence microscopy Eclipse TS 100 microscope (Nikon) equipped with a DS–Fi1-U2 digital microscope camera (Nikon) and the imaging software NIS Elements (Nikon, Version 1.10 64 bit). Fluorescence intensity was measured in the vascular wall by using ImageJ (NIH, http://rsb.info.nih.gov/ij/).

2.12. Statistical analysis

Results are expressed as the means ± standard error of the mean (SEM). Two-way ANOVA (with Bonferroni's correction for comparison of multiple means) was used to compare concentration-relaxation curves (Prism for Windows, version 8.1, GraphPad Software Inc.) One-way ANOVA (with Bonferroni's correction for comparison multiple means) or, where appropriate, equivalent non-parametric test (Dunn/Kruskal-Wallis multiple comparisons) was used for comparisons of blood pressure, other oxidative stress parameters, aortic and brain ROS formation, protein and mRNA expression and whole blood oxidative stress (Prism for Windows, version 8.1, GraphPad Software Inc.) p-values <0,05 were considered as statistically significant and are provided in the figures or by symbol legends of tables.

3. Results

3.1. An aircraft noise pause of 4d normalizes blood pressure and endothelial dysfunction in the aorta

Aircraft noise exposure for 4d increased systolic, diastolic, and mean arterial blood pressure, which showed a minor trend of amelioration after 1-2d of noise cessation but was still significantly increased compared to the unexposed control mice (Fig. 2A). After 4d of noise cessation, diastolic and mean arterial pressure was not significantly increased anymore versus unexposed mice. In addition, systolic blood pressure was significantly decreased compared to the 4d noise exposure group. Noise exposure for 4d caused significant endothelial dysfunction (acetylcholine (ACh)-dependent relaxation) (Fig. 2B), which was completely reversed already on 1d after noise cessation. Whereas there were significant differences for some ACh-concentrations between the 4d noise exposed group and the groups of 1d, 2d and 4d noise cessation in the concentration relaxation curves (left panel in Fig. 2B), there was only a significant improvement of the maximal relaxation between 4d noise exposed mice and 1d of noise cessation group (right panel in Fig. 2B). Of note, in the concentration relaxation curves for 1d, 2d and 4d of noise cessation, no data point was significantly increased as compared to the respective values of unexposed mice. Endothelial-independent relaxation by nitroglycerin was neither affected by noise nor the noise-off period (data not shown).

3.2. Consequences of aircraft noise off on ROS production in aortic and cerebral tissues and messenger RNA expression and inflammation markers

Aortic and cortical superoxide formation in whole tissues were increased by 4d and decreased during the 4d noise-off period (Fig. 3A, B). Higher levels of superoxide formation were detected in cortical tissue of noise-exposed mice when compared to unexposed control mice as measured by quantitative HPLC analysis of the superoxide-specific dihydroethidium (DHE) oxidation product, 2-hydroxyethidium (Fig. 3C).

Aircraft noise caused an up-regulation of inflammatory parameters in aortic tissue and plasma. The gene expression of VCAM-1 in aortic tissue (Fig. 4A) and the expression of circulating IL-6 were significantly increased in response to noise (Fig. 4B). The noise-off period normalized the aortic VCAM-1 gene and IL-6 expression (Fig. 4A,B). Likewise, the oxidative burst in response to phorbol ester (via protein kinase C-mediated activation of NOX-2) and zymosan A (initiating Toll-like receptor 2-dependent NOX-2 activation and ROS formation) was normalized in the noise-off period (Fig. 4C).

3.3. Lack of effect of an aircraft noise pause on cerebral microvascular dysfunction, oxidative stress, and inflammation

In contrast to the normalization of endothelial dysfunction of arterial conductance vessels, noise-induced microvascular dysfunction of

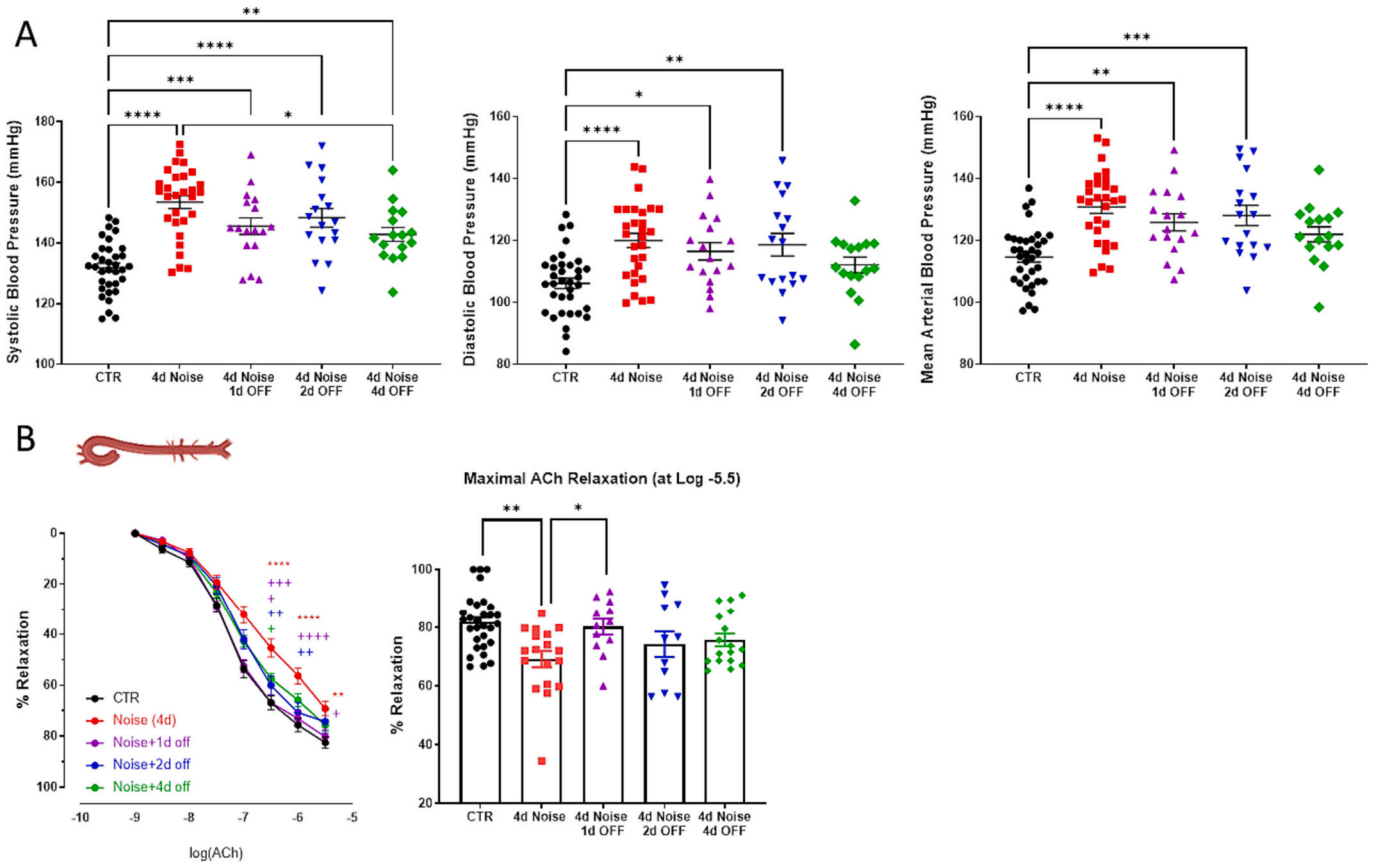

Fig. 2. Effects of 4d aircraft noise exposure and of 1, 2 and 4d noise cessation on blood pressure and aortic endothelial function. (A) Systolic, diastolic and mean arterial blood pressure measured on the final day of noise exposure and on each day after noise cessation. Data points are measurements from individual mice (twice daily); (B) Endothelium-dependent (ACh) relaxation of thoracic aortic rings was measured by isometric tension method, and quantification of maximum relaxation of all groups. Data points are measurements from 16 to 35 (A) and 11–20 (B) individual mouse samples. One-way ANOVA with Tukey's multiple comparison test (A and bar graph in B) and 2-way ANOVA with Bonferroni's multiple comparison test (concentration-relaxation curves in B). (A and B, right panel) $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ versus indicated group. (B, left panel) **p < 0.01, ****p < 0.0001 versus unexposed control; $^{+}$p < 0.05, $^{++}$p < 0.01, $^{++++}$p < 0.0001 for noise exposed versus respective noise cessation group (according to the color code). There were no significant differences in any dataset between the 1d, 2d and 4d noise cessation groups and no significant differences in the relaxation curves between the unexposed control group and any of the noise cessation groups.

cerebral arterioles persisted even after 4d of noise cessation (Fig. 5A). Microvascular vasodilation in response to the endothelium-independent nitric oxide donor, sodium nitroprusside (SNP), and vasoconstriction induced by the thromboxane receptor agonist, U46619, was neither affected by noise nor by cessation. Neither noise-induced ROS formation nor inflammation in cerebral microvessels was decreased after noise cessation reflected by persistent elevated DHE staining (Fig. 5B), protein expression of the phagocytic NADPH oxidase (NOX-2) (Fig. 6A) and protein levels of the inflammatory markers IL-6 (Fig. 6B) and VCAM-1 (Fig. 6E). The inflammatory markers even seem to be aggravated upon noise cessation. Microvascular protein expression of the vasoconstrictor endothelin-1 (ET-1) (Fig. 6D) and the monocyte/macrophage marker CD68 (Fig. 6F) only showed a trend of elevation by noise or noise cessation. Also, no increased expression of the marker for activated microglia cells, IBA1, was observed directly in cerebral microvessels of noise-exposed mice (Fig. 6C).

4. Discussion

With the present studies, we aimed to investigate the reversibility of aircraft noise-induced cardiovascular and cerebral side effects during a noise-off period in our well-characterized murine model. We have previously reported that just a single night of aircraft noise exposure induces endothelial dysfunction and increased oxidative stress in healthy human subjects (Schmidt et al., 2013), even more pronounced in patients with established coronary artery disease (Munzel et al., 2018a) and also within 1 day or even 1 night in our animal model (Kroller-Schon et al., 2018; Munzel et al., 2017). Importantly, we have also demonstrated that noise-induced endothelial dysfunction, inflammation, and oxidative stress are not subject to adaptation or tolerance development in response to long-term noise exposure for up to 4 weeks (Frenis et al., 2021b). The question remains whether the cardiovascular and cerebrovascular side effects induced by short-term noise will rapidly disappear in response to a noise-off period or whether they may persist.

With the present studies, we have established the following results. Firstly, a 4 days aircraft noise exposure causes hypertension, endothelial dysfunction of large conductance and small cerebral microvessels, increases oxidative stress in the brain arterioles and the aorta and increases the expression of inflammatory biomarkers. Secondly, the noise-off period reduced blood pressure and improved endothelial dysfunction of the aorta within 24 h, while interestingly, endothelial dysfunction of cerebral microvessels persisted. Thirdly, the 4 days noise-off period normalized ROS levels in the aorta and cerebral cortex but not in cerebral microvessels compatible with the concurrence of endothelial dysfunction. We have chosen four days of noise exposure and up to 4 days of noise cessation (OFF period) regimens to investigate the fast onset and potential recovery of noise-mediated damage. The fast onset of noise-dependent cardiovascular and cerebral damage was previously established (Kroller-Schon et al., 2018; Munzel et al., 2017), which is also in accordance with the fast onset of effects by one night of noise

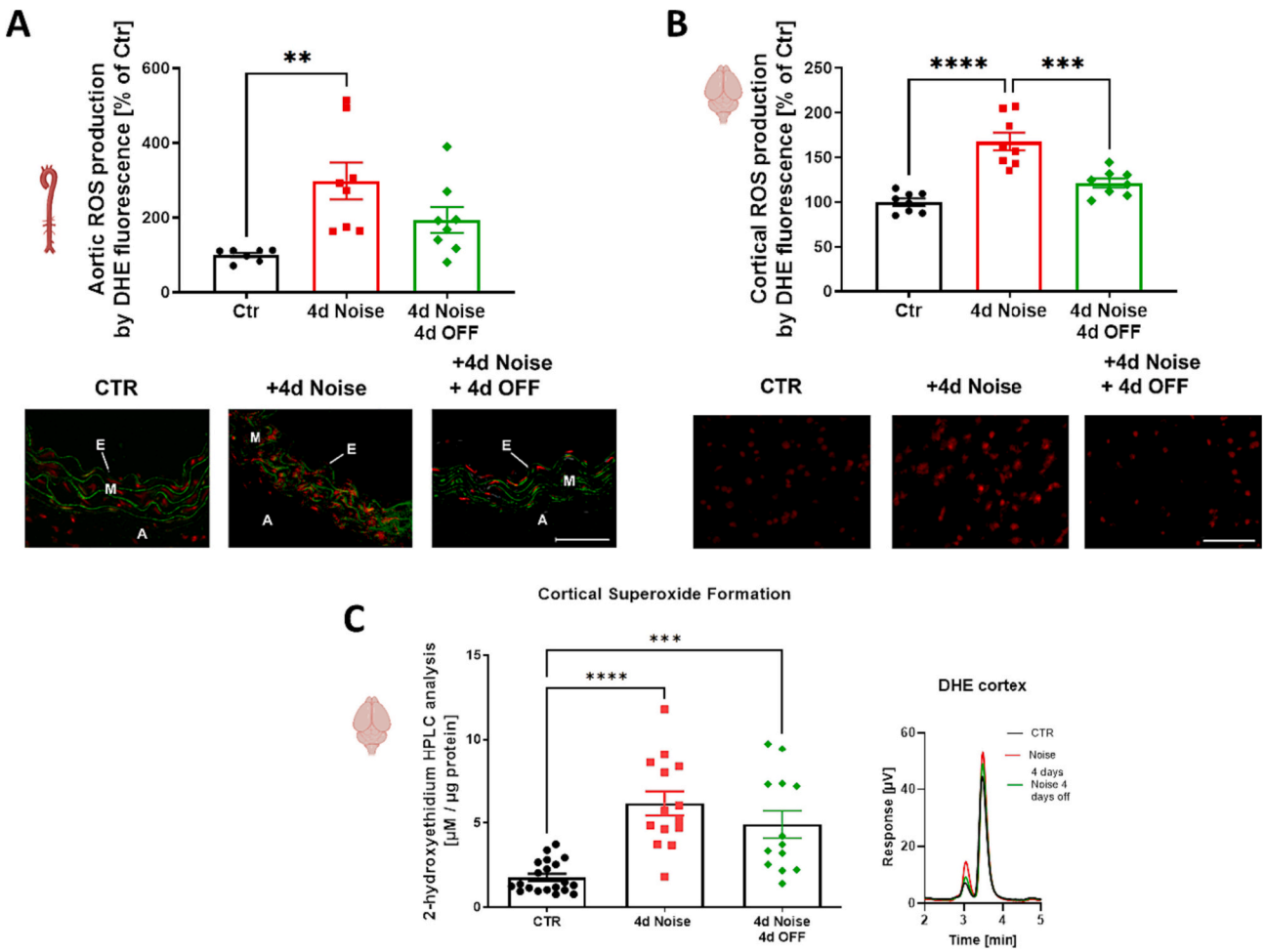

Fig. 3. Effects of 4d aircraft noise exposure and of 4d noise cessation on ROS formation. (A,B) Dihydroethidium stainings of aortic and cortical cryosections and their representative photomicrographs show ROS formation as red fluorescence and green autofluorescence from aortic laminae. (C) Quantification and representative chromatograms of superoxide levels in cortical tissue as measured by HPLC analysis of 2-hydroxyethidium formation. Data points represent 8 (DHE staining) or 13–20 (DHE HPLC) individual mouse samples. A, adventitia; E, endothelium; M, media. Data points represent individual mice; 1-way ANOVA with Tukey's multiple comparison test; *p < 0.05, **p < 0.01, ***p < 0.01, ****p < 0.0001.

exposure in our human field studies (Herzog et al., 2019; Schmidt et al., 2013). Also, concerning the human setting, we consider the 4 days noise exposure regimen relevant since we are continuously subject to short-term noise fluctuations, e.g. daytime versus nighttime exposure differences, hotel stay in a noisy downtown versus a quiet home at a rural site. The rare human and animal studies on reversibility of noise health effects investigated hours to days of recovery as discussed below. We consider short-term recovery from noise an important topic for studies since this may simulate the real-world situation where we are exposed to higher noise levels during the workdays (4-5d) and lower noise levels during the weekend (2-3d).

Thus, our previously reported findings that noise exposure for 4d induced vascular oxidative stress and endothelial dysfunction in the aorta as well as in cerebral, retinal, and mesenteric arterioles (Eckrich et al., 2021a; Frenis et al., 2021a) along with the persistent impairment of cerebral microvessel function upon ablation of lysozyme M-positive monocytic cells (Frenis et al., 2021a) indicates that the vascular repair mechanisms behind the blood-brain-barrier may differ significantly from that of the systemic circulation.

The lack of normalization of endothelial dysfunction, inflammatory biomarkers (IL-6 and VCAM-1), and oxidative markers (ROS levels and NOX2 expression) in the microvasculature of the brain as compared to arterial conductance vessels following noise-off periods suggests that the brain may be at particular risk from noise-induced damage being associated with long-term consequences including deterioration of cognitive function, neurodegenerative (Lyman et al., 2014) or cerebrovascular (Yang et al., 2018) disease and dementia, with focus on Alzheimer's disease.

One explanation for the differences in the reversibility of noise-induced changes between arterial conductance and cerebral resistance vessels may be a more significant impact of the microglia of the brain on vascular function, which is getting activated in response to noise exposure, also known to trigger prolonged ROS production (Frenis et al., 2021a; Lehmann et al., 2019). A recent study in a mouse stress model of social defeat, characterized by prolonged microglia-driven oxidative stress in the CNS (Lehmann et al., 2019), reported that endothelial function of retinal arterioles was largely compromised after 3d of stress discontinuation and did not completely recover even after 8 months of stress cessation (Wang et al., 2021). This observation may be explained by the prolonged activation of ROS-producing immune cells leading to endothelial cell activation. Since we also detected increased expression of the adhesion molecule VCAM-1 in cerebral arterioles following noise exposure and cessation but did not find microvessel-associated differences in IBA1 expression between the three mouse groups, the observed chronic endothelial changes appear to be induced by circulating leukocytes rather than by resident microglia. This fits to our previous observations that the cerebral microvascular dysfunction of noise-exposed mice was improved by genetic ablation of LysM-positive

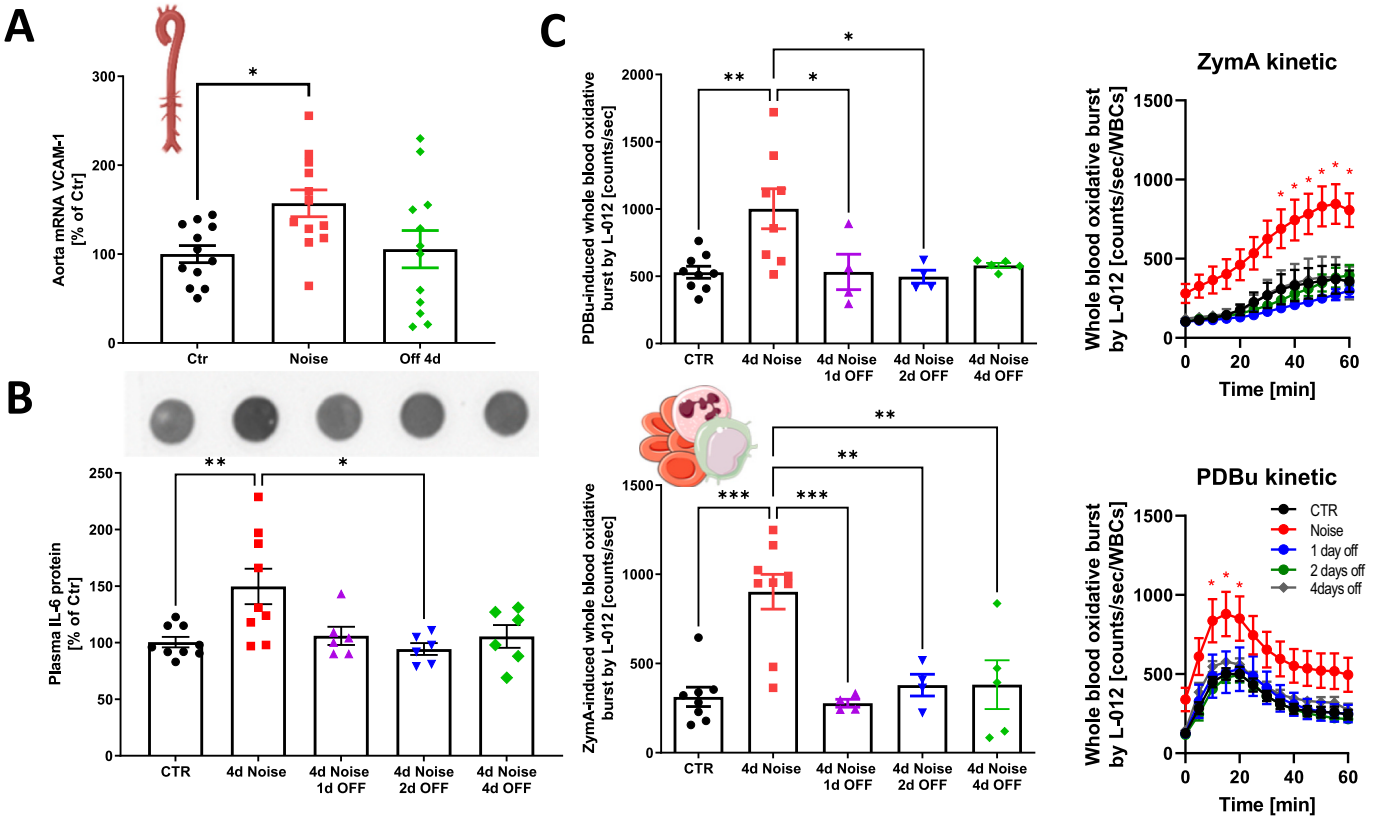

Fig. 4. Markers of inflammation show an attenuation after noise cessation for 1, 2 or 4d. (A) mRNA expression of VCAM1 in aortic tissue was measured via quantitative RT-PCR. (B) IL-6 protein expression was determined by dot blot analysis in plasma. Representative blotting images are shown above the densitometric quantification. (C) Oxidative burst (NOX-2 and myeloperoxidase activity) in whole blood after zymosan A and PDBu stimulation was determined by chemiluminescence (*L*−012) over 60 min. Data points from (A) represent 12 individual mouse samples, (B,C) represents pooled samples from 2 to 3 mice ($n = 6$–9 for IL-6; $n = 4$–9 measurements for oxidative burst); 1-way ANOVA with Tukey's multiple comparison test for all bar graphs; 2-way ANOVA with Bonferroni's multiple comparison test for the kinetic traces;* $p < 0.05$, ** $p < 0.01$. There were no significant differences between the 1d, 2d and 4d noise cessation groups and no significant differences between the unexposed control group and any of the noise cessation groups.

myelomonocytic cells even though microglia were not ablated in this model (Frenis et al., 2021a).

In addition, we cannot completely rule out that other parts of the glia or neurons surrounding the vessels may contribute to the prolonged endothelial dysfunction after noise cessation, which would also be supported by eNOS-independent regulation of vascular tone of cerebral microvessels (Birk et al., 2021; Capettini et al., 2010; Faraci et al., 2004; Pohl et al., 2000). This idea is supported by our previous data indicating microglia activation, neuronal NOS uncoupling and cerebral oxidative stress in multiple regions of the brain of noise-exposed mice, which could cause adverse effects on nearby vessels, e.g., via redox or inflammatory signaling (Frenis et al., 2021a; Kroller-Schon et al., 2018).

We and others have shown that night-time rather than daytime transportation noise is mainly responsible for the cardiovascular/cerebral damage (Munzel et al., 2020; Munzel et al., 2021), implying that fragmentation of sleep and/or too short sleep periods and the resulting stress are pivotal for the adverse health effects of noise (Kroller-Schon et al., 2018). The link between disrupted sleep rhythms and cardiovascular disease is well-established (Cappuccio et al., 2010; Crnko et al., 2019; Van Laake et al., 2018) and that shift workers are at higher risk and have worsened outcomes following a cardiovascular event (Furlan et al., 2000; Zhao et al., 2022). Noise and other environmental stressors and pollutants significantly impact the redox dysregulation of the circadian clock, e.g., by oxidation of critical thiol groups in clock components (Daiber et al., 2022; Li et al., 2020). Importantly, one night of aircraft noise exposure increased the serum levels of 3-nitrotyrosine-positive proteins and malondialdehyde levels, biomarkers of oxidative stress, in patients with established coronary artery disease significantly (Kroller-Schon et al., 2018; Schmidt et al., 2015). In addition, train noise exposure for one night caused pro-oxidative and pro-atherothrombotic changes in the plasma proteomes of healthy volunteers (Herzog et al., 2019). Next-generation sequencing (RNAseq) analysis also identified significant differences in regulating genes responsible for the circadian rhythm. Thus, it is tempting to speculate that a disruption of the circadian rhythm may be a critical necessary stimulus concerning the neuroinflammation of microglia (Griffin et al., 2019). E.g., *Rev-erbα*$^{-/-}$ mice with a genetic absence of a critical clock component display aberrant and spontaneous microglial activation, resulting in NF-κB-mediated inflammation (Griffin et al., 2019). Vasoconstriction within the cerebral vasculature displays rhythmicity and has an apparent role in the recovery following brain injury (Lidington et al., 2022). We thereby speculate that the sustained disruption of the circadian rhythm of mice brains, even after 4d of noise cessation, could be secondary to a prolonged "recalibration" of the core clock following the disruption.

Also of great importance is the reversibility of noise-inflicted damage after noise cessation. A clinical study on pilots speculated that the higher prevalence of hypertension in pilots was due to the basal blood pressure increase triggered by noise exposure that does not return to normal even after noise-free intervals, which may also be due to the lower sympathetic adaptability of these subjects (Tomei et al., 1996). Also, previous data suggest that noise-mediated shifts of the hearing threshold may be reversible (Gerhardt and Walton, 1986) by non-persistent damage of cochlear blood vessels and the blood cells therein (Nakai and Masutani, 1988). In contrast, noise-induced cochlear hypoxia is irreversible after

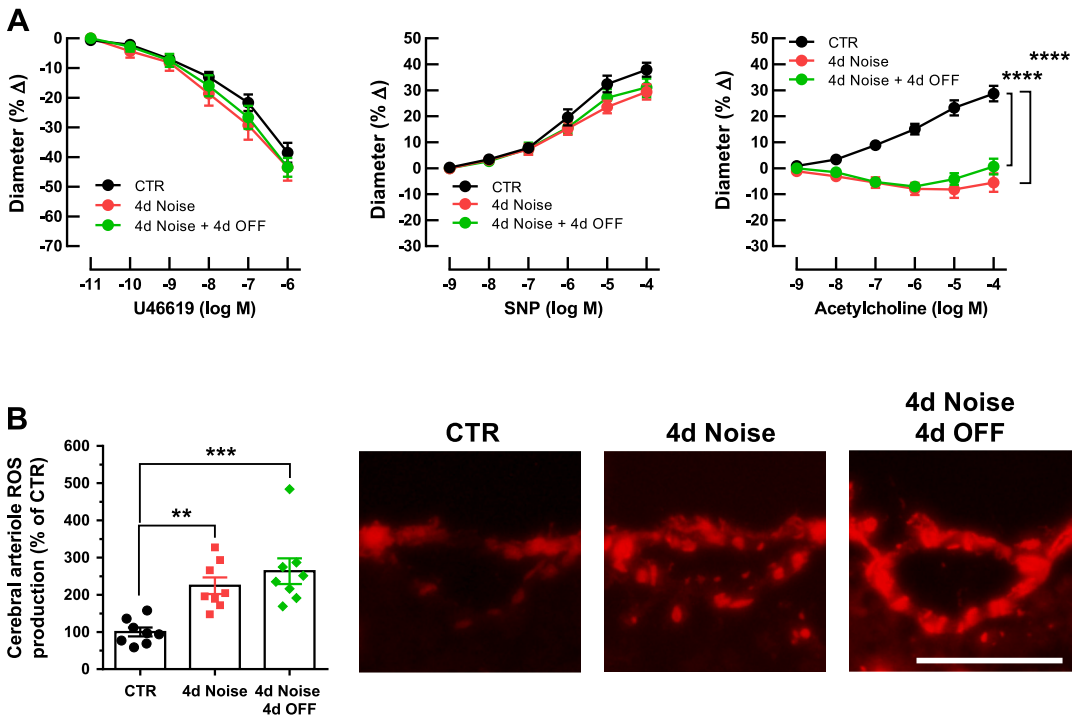

Fig. 5. Cerebral microvascular function and ROS production after 4d aircraft noise exposure and after 4d noise cessation. (A) Vasoconstrictor responses of cerebral arterioles to U46619 and the endothelium-independent vasodilator SNP were neither affected by noise exposure nor by cessation of noise. However, responses to the endothelium-dependent vasodilator, acetylcholine, were virtually abolished after 4d of noise exposure and did not markedly improve 4d after discontinuation of noise exposure. (B) After 4d of noise exposure, a marked increase in ROS levels (as indicated by DHE staining intensity) was observed in the vascular wall of cerebral arterioles, which persisted after 4d of noise cessation. Two-way ANOVA with Bonferroni's multiple comparison test was used to compare concentration-response curves, and 1-way ANOVA with Tukey's multiple comparisons test was used to compare staining intensities. For all experiments $n = 8$ per group; **p < 0.01, ***p < 0.001; ****p < 0.0001; Scale bar = 20 μm.

180 min of noise cessation and precedes the reduction of cochlear blood flow (Lamm and Arnold, 1996).

Dwellers in highly trafficked urban areas are more exposed to higher noise levels than recommended by the WHO and, as such, may be at higher cardiovascular risk. Much evidence that excessive noise exposure can be detrimental to health has become available, including our translational scientific evidence in humans (Herzog et al., 2019; Schmidt et al., 2015; Schmidt et al., 2013) and murine models (Frenis et al., 2021b; Kroller-Schon et al., 2018; Munzel et al., 2017), as well as work by others (Eze et al., 2017; Recio et al., 2016) including meta-analyses (Clark and Paunovic, 2018; Guski et al., 2017; Kempen et al., 2018). In sum, noise exposure is associated with a variety of cardiovascular diseases, including arterial hypertension, stroke, heart failure, arrhythmia, and mental impairment (Munzel et al., 2014; Munzel et al., 2018a; Stansfeld et al., 2005; Sygna et al., 2014) through oxidative, inflammatory, and hormonal pathways that have recently been elucidated, all of which may have prompted the WHO to update their noise guidelines for the European Region (WHO/EU, 2018). This topic is becoming even more critical considering the additive adverse health effects of transportation noise with other environmental stressors that usually show colocalization in urban areas, e.g., as recently proven for additive cardiometabolic risk by noise and particulate matter and NO2 exposure in animals (Kuntic et al., 2023) and humans (Huang et al., 2023; Munzel et al., 2023; Sorensen et al., 2022).

4.1. Limitations of the study

Of note, the blood pressure in the 2d noise cessation group was consistently slightly higher than the 1d noise cessation values (Fig. 2A). We also see a more efficient improvement of endothelial function by 1d of noise cessation as compared to 2 and 4 days of noise cessation (Fig. 2B). For both observations we have no explanation so far. A further limitation of our study may be that our results cannot be transferred up to 100 % to the human setting, where coping and resilience mechanisms may come into play.

5. Conclusions and clinical implications

Transportation noise is becoming increasingly a significant health risk factor for cardiovascular and cerebral diseases (Hahad et al., 2022; Munzel et al., 2021). With the present studies, we demonstrate for the first time that the adverse effects of acute noise exposure are largely but not completely reversible in the noise-off observation period. Noise-triggered antioxidant stress response via Nrf2/Keap1-dependent induction of heme oxygenase-1 protective pathways (Bayo Jimenez et al., 2021) may still be active upon noise cessation and are likely responsible for the fast recovery of large conductance vessels such as the aorta. The lack of recovery of the cerebral arteriolar function is likely due to the persistent oxidative stress observed in this vessel region without any improvement, even 4d, after cessation of noise. Thus it appears that the cerebral microvasculature is particularly sensitive to transportation noise exhibiting a persistent vascular dysfunction and increased oxidative stress, which may explain at least in part the development of neurodegenerative diseases such as dementia, in particular Alzheimer's disease, being observed in response to transportation noise. Using more extended noise-off periods, we will answer this question with future studies.

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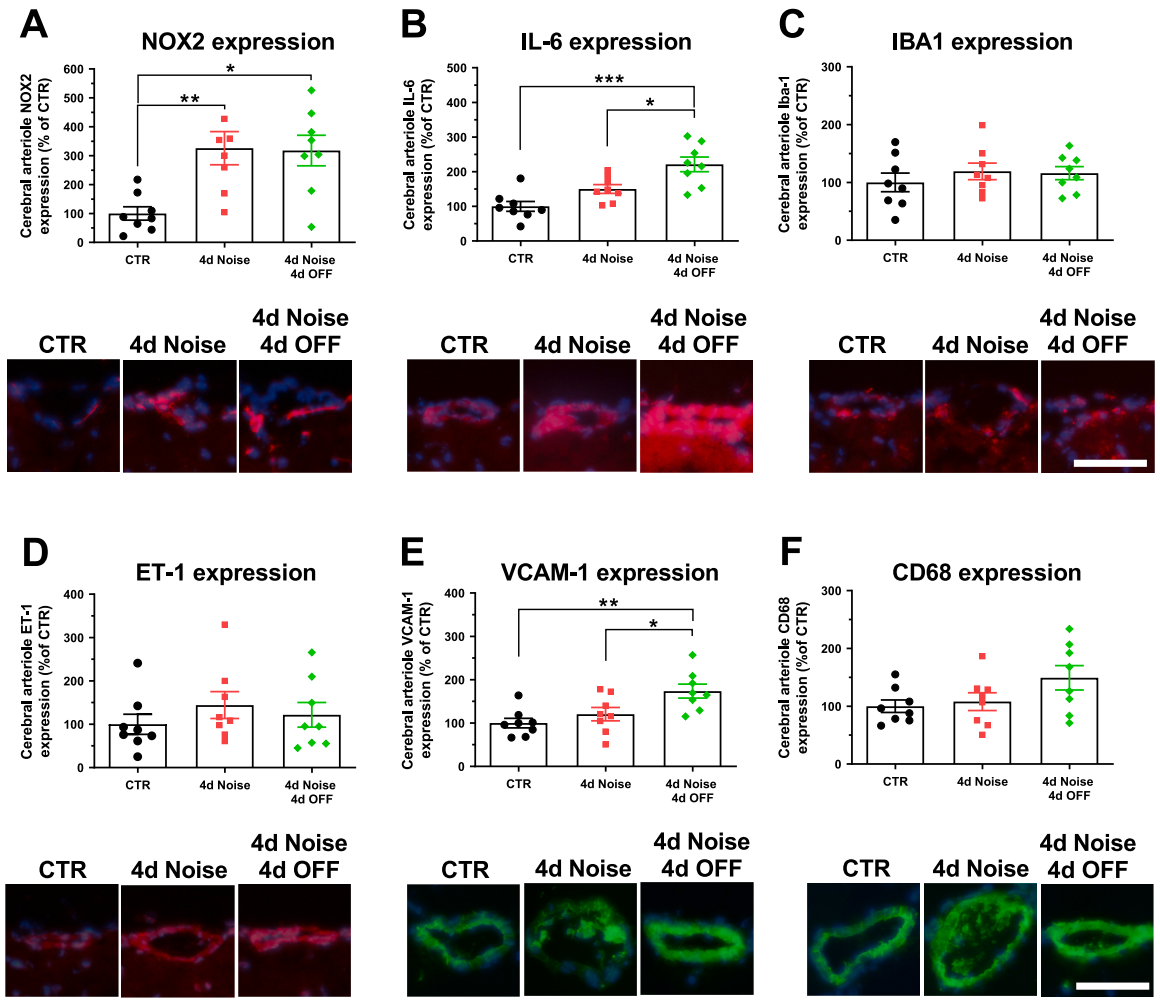

Fig. 6. Oxidative stress, inflammation, and cell adhesion markers after 4d aircraft noise exposure and 4d noise cessation. Remarkably, NOX2 (A) expression was elevated after 4d of noise exposure and did not markedly decrease after 4d of noise cessation. Moreover, expression of the inflammatory cytokine, IL-6 (B), and of the cell adhesion molecule, VCAM-1 (E), was highest after 4d of noise cessation. In contrast, no noise-dependent differences were seen in IBA1 (C), ET-1 (D), and CD68 (F) protein expression. Data points are measurements from individual samples. One-way ANOVA with Tukey's multiple comparisons test was used to compare staining intensities. For all experiments n = 8 per group; *p < 0.05, **p < 0.01, ***p < 0.001; Scale bar = 20 μm.

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CRediT authorship contribution statement

Conceptualization (A.G., A.D., T.M.); Formal analysis (M.T.B.J., A. G., M.O., M.Kun., A.D., T.M.); Funding acquisition (A.G., G.D.D., A.D., T. M.); Investigation (M.T.B.J., K.F., S.R., M.Kva., S.K.-S., M.O., M.Kun., I. K., D.M., Q.T., S.J., Y.R.); Project administration (A.D., T.M.); Supervision (A.G., G.D.D., A.D., T.M.); Roles/Writing – original draft (M.T.B.J., A.G., M.O., A.D., T.M.); Writing – review & editing (M.Kun., G.D.D., S.S., M.J.S., O.H., H.L., A.D., T.M.).

Declaration of competing interest

The authors declare that they have no conflicts of interest with the contents of this article.

Data availability

Data will be made available on request.

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