



Exposure to Intermittent Noise Exacerbates the Cardiovascular Response of Wistar–Kyoto Rats to Ozone Inhalation and Arrhythmogenic Challenge

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Abstract

Noise has become a prevalent public health problem across the world. Although there is a significant amount of data demonstrating the harmful effects of noise on the body, very little is known about how it impacts subsequent responses to other environmental stressors like air pollution, which tend to colocalize in urban centers. Therefore, this study was conducted to determine the effect of intermittent noise on cardiovascular function and subsequent responses to ozone (O₃). Male Wistar–Kyoto rats implanted with radiotelemeters to non-invasively measure heart rate (HR) and blood pressure (BP), and assess heart rate variability (HRV) and baroreflex sensitivity (BRS) were kept in the quiet or exposed to intermittent white noise (85–90 dB) for one week and then exposed to either O₃ (0.8 ppm) or filtered air. Left ventricular function and arrhythmia sensitivity were measured 24 h after exposure. Intermittent noise caused an initial increase in HR and BP, which decreased significantly later in the regimen and coincided with an increase in HRV and BRS. Noise caused HR and BP to be significantly elevated early during O₃ and lower at the end when compared to animals kept in the quiet while the increased HRV and BRS persisted during the 24 h after. Lastly, noise increased arrhythmogenesis and may predispose the heart to mechanical function changes after O₃. This is the first study to demonstrate that intermittent noise worsens the cardiovascular response to inhaled O₃. These effects may occur due to autonomic changes and dysregulation of homeostatic controls, which persist one day after exposure to noise. Hence, co-exposure to noise should be taken into account when assessing the health effects of urban air pollution.

Keywords Noise · Cardiovascular · Ozone · Heart rate variability · Arrhythmia

Introduction

Numerous epidemiological studies indicate that exposure to air pollution causes cardiovascular morbidity and mortality. However, this paradigm is not solely characterized by the intensity of exposure because numerous factors, both

intrinsic (e.g., underlying disease) and extrinsic (e.g. co-exposures) to the host, clearly play a modifying role in the severity of the response. Indeed, modifying factors alter host physiology and even increase incidence and progression of underlying disease. For instance, environmental noise pollution has increased significantly over several decades, particularly due to vehicular traffic, and its observed effects on the cardiovascular system are both notable and concerning [1]. Yet, when examining the health effects of increased vehicular traffic, relatively little is known about the combined impact of noise and air pollution. This is particularly relevant given the growing urbanization of the world and persistent exposure to both of these environmental pollutants in urban centers. Thus, this study was conducted to determine the impact of intermittent noise, which occurs over a period of time with small gaps, on cardiovascular function and subsequent responses to ozone (O₃).

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The World Health Organization (WHO) declared noise to be a pollutant in 1972 [2] and this measure was adopted by the United States Environmental Protection Agency (Noise Control Act) in the same year. Noise in this context is the unwanted or disturbing environmental (e.g. vehicular) or residential sounds above 70 dB in a given community that are not from occupational sources [3]. The major sources of urban noise are road vehicles, airplanes, industry and construction located near residential areas. The health effects of noise are a growing global concern with the WHO indicating that the resulting environmental burden of disease is thousands of disability-adjusted life-years (DALYs—an estimate of the potential years of life lost due to premature death and the equivalent years of healthy living lost due to disease, WHO [4]. Exposure to noise is known to cause annoyance, sleep disturbance, and cognitive impairment in children [5]. Moreover, it has been shown to contribute to cardiovascular disease like hypertension, ischemic coronary artery disease, and stroke through oxidative stress, autonomic imbalance [6] or altered endocrine responses [7, 8]. The latter two mechanisms are likely modulated due to episodic stress, but some studies show that depression and anxiety, which also alter autonomic and endocrine function, can often result from noise exposure [9].

Animal models have been used to examine the impacts of noise, demonstrating changes in blood pressure, immune reactions, and other systemic issues [1]. In rats, 85 to 100 dB of sporadic (i.e., without a regular pattern) white noise impairs vasodilation, increases lipid peroxidation and has even been shown to cause histological changes in the heart [10, 11], suggesting an acute stress-induced response. On the other hand, studies indicate that intermittent exposures to noise at unpredictable times minimize acute stress and instead cause a mild chronic stress that is marked by depression and anxiety-like signs like social withdrawal and changes in blood pressure [12], and also autonomic dysregulation [13]. However, the persistence of these effects and their ability to modify the body's subsequent response to other environmental stressors remains largely uncharacterized. Therefore, in this study, we examined the impact of random intermittent noise on

the cardiovascular physiological function of Wistar–Kyoto rats and its effect on a subsequent exposure to ozone.

Materials and Methods

Animals

Twelve-week old male Wistar–Kyoto (WKY) rats (Charles River, Wilmington, MA) weighing 300–400 g were used in the study. Upon arrival, animals were housed one per cage and maintained on a 12-h light/dark cycle at approximately 22 °C and 50% relative humidity with food and water available ad libitum in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved facility; all animals were treated humanely and with regard to alleviation of suffering. All experimental protocols were approved by and in accordance with the guidelines of the Institutional Animal Care and Use Committee of the United States Environmental Protection Agency, Research Triangle Park, North Carolina.

Experimental Design

Rats were randomly assigned and kept in either quiet/noise housing (Q) or exposed to intermittent noise (N) for varying durations, day or night, over a one-week period and thereafter exposed to either filtered air (FA) or 0.8 ppm ozone (O₃) for 4 h (Fig. 1). Separate cohorts of rats were used for (1) radiotelemetry, (2) intraventricular function, and (3) aconitine challenge testing. Group size determination was performed using power analysis in Sigmaplot 13.0 (Systat Software, San Jose, CA). Sample size analysis was based on the number of experimental groups (Q-FA, Q-O₃, N-FA, N-O₃), a significance level = 0.05, a power = 0.8, and the effect size index, which is derived by multiplying the expected effect size by the standard deviation. An $n = 6–7$ was selected based on these calculations.

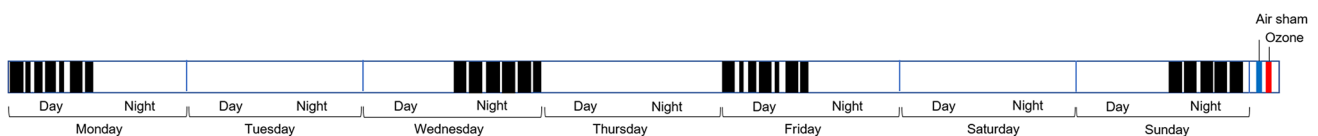


Fig. 1 Experimental design. Rats were either kept in quiet housing (Q) or exposed to intermittent noise (N) over the course of one week; the dark bars indicate noise exposure periods ranging in duration from 5 to 15 minutes, as indicated by the thickness. All the rats in cohort 1 (radiotelemetry) were exposed to a sham FA exposure

approximately 4 h after the last quiet/noise episode followed by exposure to O₃ 24 h later. Rats in cohort 2 (intraventricular function) and cohort 3 (aconitine challenge) were exposed to either FA or O₃ 24 h after the last quiet/noise episode. Animals were acclimated to the exposure chamber twice during the prior week

Radiotelemeter Implantation

Animals were anesthetized with ketamine/xylazine (80 mg/ml ketamine HCL and 12 mg/ml xylazine HCL; 1 ml/kg i.p.; Sigma Chemical Co., St. Louis, MO) and implanted with radiotelemeters transmitting electrocardiogram (ECG), aortic blood pressure (BP) and core body temperature (T_{co}) (9 weeks old, telemeter Model TL11M2-C50-PXT, Data Sciences International) at Charles River as described previously [14]. Rats implanted with radiotelemeters were either kept in quiet or exposed to intermittent noise for a one-week period. After the end of the one-week period, all animals were exposed first to FA (i.e. sham) and then O₃ 1 day later. Thus, each animal served as its own control and provided within subject physiological change from FA to O₃, which improves comparability of parameters like BP and heart rate (HR).

Radiotelemetry Data Acquisition

Radiotelemetry methodology was used to track changes in cardiovascular function by monitoring ECG, HR, BP and T_{co} . This methodology provided continuous monitoring and collection of physiologic data from unrestrained, un-anesthetized rats. Data signals were transmitted from surgically implanted radiotelemeters to a remote receiver (DataART2.1: Data Sciences International, Inc., St. Paul, MN) located under the animal's cage. HR was obtained from the ECG waveform, which were sampled at 1000 Hz and recorded for five-minute segments every five minutes while the animal was in its home cage and continuously during exposure. ECGAuto software (EMKA technologies USA, Falls Church, VA) was used to visualize individual ECG signals, measure HR, analyze ECG segments for artifacts, calculate heart rate variability time-domain and frequency-domain measures, and identify cardiac arrhythmias.

Heart Rate Variability and Baroreflex Analysis

ECG waveforms were used to determine HRV with computer software (ECGauto 2.8.1.26; EMKA Technologies, Falls Church, VA) as previously described [15]. HRV analysis generated HR and time-domain measures, including mean time between adjacent QRS-complex peaks (RR interval), standard deviation of the RR interval (SDNN), coefficient of variation or SDNN normalized to rate (CV) and square root of the mean of squared differences of adjacent RR intervals (RMSSD). SDNN represents overall HRV, whereas RMSSD represents parasympathetic influence over HR [16]. HRV analysis also provided frequency-domain parameters, including low-frequency (LF: 0.200–0.750 Hz, representing a combination of sympathetic and parasympathetic tone) and high frequency (HF: 0.75–3.50 Hz, indicating

parasympathetic tone) and the ratio of these two (LF/HF, indicating sympathovagal balance) analyzed with a Hanning window for segment lengths of 512 samples with 50% overlapping. Arrhythmia identification and exclusion from HRV and ECG morphology analyses were performed blind to treatment and according to previously described criteria [15]. Baroreflex sensitivity (BRS) was calculated using the sequence method [17, 18] (HemoLab). The sequence method identifies sequences of four or more heart beats during which BP and pulse interval (PI) change in the same direction. Linear regression lines were calculated for all individual sequences of BP and PI, and the average of the slopes of all lines was then used as an index of BRS. The data showed not only the overall BRS gain, but also the up gain or the sequences in which the BP and PI increased and the down gain in which the BP and PI decreased. Irregular sequences were manually removed. The units for BRS are ms/mmHg.

Noise Exposure

Noise was produced using intermittent white noise generated (Type 1405; Bruel & Kjaer, Naerum, Denmark), amplified (Amp100; AudioSource, Portland, OR), and delivered through 6.25 inch diameter speakers (#TS-A1676R; Pioneer Electronics, USA) with a 32 Hz–40 kHz (– 20 dB) frequency response. The system was

connected to a programmable outlet strip (#94450-10; Cole Parmer, Vernon Hills, IL). Each speaker was mounted 1.25 inches above the middle of each cage and the programmable timer was used to generate variable noise durations ranging from 5 to 15 min, with 2 to 12 h between each presentation. Rats were exposed to an intermittent noise regimen before the air or ozone exposure during both light and dark cycles (Fig. 1).

Noise levels were tested and optimized for each cage prior to the start of the experiment. We have previously determined that noise levels in the four corners of the cage vary from 80 to 85 dB SPL (re: 20 μ Pa), while levels at the top middle of the cage (nearest the speaker) vary from 90 to 95 dB. Thus, depending on the movement and location of the rat during noise exposure, rats will experience a range of noise intensity of ~80 to 95 dB while the speakers are active. These levels are similar to what is experienced from a dishwasher or food blender. Noise at these levels have not been shown to cause hearing damage in rats [19].

Ozone Exposure

Animals were exposed via whole-body inhalation to FA or 0.8 ppm O₃ once for 4 h after the last noise exposure. All rats implanted with radiotelemeters served as their own controls and were exposed to FA sham approximately 4 h after the last quiet/noise episode and then to O₃ 24 h later. ECG, HR,

body temperature, activity and BP were monitored before, during, and after exposure in that cohort. A second cohort of un-telemetered rats from each exposure group was used for intraventricular function assessments. A third cohort of rats in each exposure group was challenged with aconitine to assess sensitivity to arrhythmogenic challenge. O₃ was generated by passing extra dry oxygen past an arcing transformer in a model V5-0 ozone generator (Ozone Research & Equipment Corp., Phoenix, AZ). The chamber concentration (0.8 ppm) was controlled by the computer program DASY-Lab (version 9.0; DasyTec USA, Amherst, NH), which controlled the opening and closing of a mass flow controller at each chamber. The actual concentration was then read by an O₃ analyzer (model 400; Teledyne-Advanced Pollution Instruments, Inc., Thousand Oaks, CA), which fed a signal to a proportional, integral, derivative loop control, which then either opened or closed the mass flow controller to maintain the O₃ concentration in the chamber at the desired level. Rats were acclimated to the whole-body chamber for 1 h/day for 2 days before exposure to FA or O₃.

Intraventricular Functional Assessments

Twenty-four hours after exposure to FA or O₃, rats were anesthetized with urethane (1.5 mg/kg i.p., Sigma) and then prepared for left ventricular pressure (LVP) measurement by right carotid arterial catheterization with a 2-French transducer (SPR-320, Millar Instruments). The LV probe was connected via a Pressure Control Unit (Model 2000, Millar Instruments) to a receiver (Powerlab, ADInstruments) and a computer acquiring data at 1000 Hz. The left jugular vein was cannulated for cardiac stress test by sympathomimetic infusion (dobutamine). The transducer was advanced into the left ventricle for a 4-min baseline, and freshly diluted dobutamine hydrochloride (dissolved in 0.9% NaCl saline at 640 µg/ml) was infused for 2 mins at a dose of 320 µg/kg/min. Software (LabChart Pro 7.3.2, ADInstruments) generated HR, LVP (i.e., left ventricular end systolic pressure), and the maximum and minimum pressure slopes (dP/dt_{max} and dP/dt_{min} , respectively) per beat, indicative of contractility and relaxation rate (lusitropy), respectively. All experiments were performed blinded.

Aconitine Arrhythmia Challenge

Twenty-four hours after the FA/O₃ exposure, a separate cohort of animals were anesthetized with urethane (1.5 g/kg, *ip*) and underwent aconitine challenge; supplemental doses of the anesthetic were administered intravenously when necessary to abolish pain reflex. Animal body temperature was maintained at ~ 36 °C with a heating pad. The left jugular vein was cannulated with P.E. 50 polyethylene tubing for the administration of aconitine. About 10 µg/ml

aconitine was continuously infused at a speed of 0.2 ml/min, while ECG was continuously monitored and timed. Sensitivity to arrhythmia was measured as the threshold dose of aconitine required to produce ventricular premature beats (VPBs), ventricular tachycardia (VT) and ventricular fibrillation (VF), and was calculated using the following formula:

Threshold dose (mg/kg) for arrhythmia

$$= 10\mu\text{g/ml} \times 0.2\text{ ml/min} \times \text{time required to induce arrhythmia (min)}/\text{body weight (kg)}$$

All experiments were performed blinded.

Statistics

All data are expressed as means ± SEM. Statistical analyses for HR, BP, HRV, BRS, intraventricular function parameters and arrhythmia sensitivity were performed using Sigmaplot 13.0 (Systat Software, San Jose, CA) software. We performed tests of normality for all continuous variables and used parametric methods of analysis. A linear mixed model with least squares means and repeated measures ANOVA was used to determine which interactions (i.e., time × treatment, treatment × exposure) were statistically significant. Multiple comparison adjustment for the *p* values and confidence limits for the differences between the least squares means was done using a *post hoc* test. The statistical significance was set at *p* < 0.05.

Results

Body Weight

Animals were randomly assigned to the Q (290.9 ± 8.7 g) or N (274.0 ± 7.3 g) groups at the start of the study. There was no significant difference in the body weights of the groups after the one-week period of quiet/noise regimen or after exposure to either FA or O₃ (Q = 312.1 ± 5.6 g; N = 302.2 ± 1.0 g).

Noise Effects on Heart Rate and Blood Pressure

Heart rate did not differ significantly between the Q and N groups prior to beginning the noise regimen (Fig. 2—Pre-noise). The HR of rats was increased during the first noise exposure when compared to the Q group during the same period (*p* = 0.031). Although HR fluctuated for both Q and N groups during the noise regimen, there were no significant differences. Similarly, although BP was not different between Q and N animals prior to the start of the regimen (*p* = 0.296), it was increased significantly in the latter during

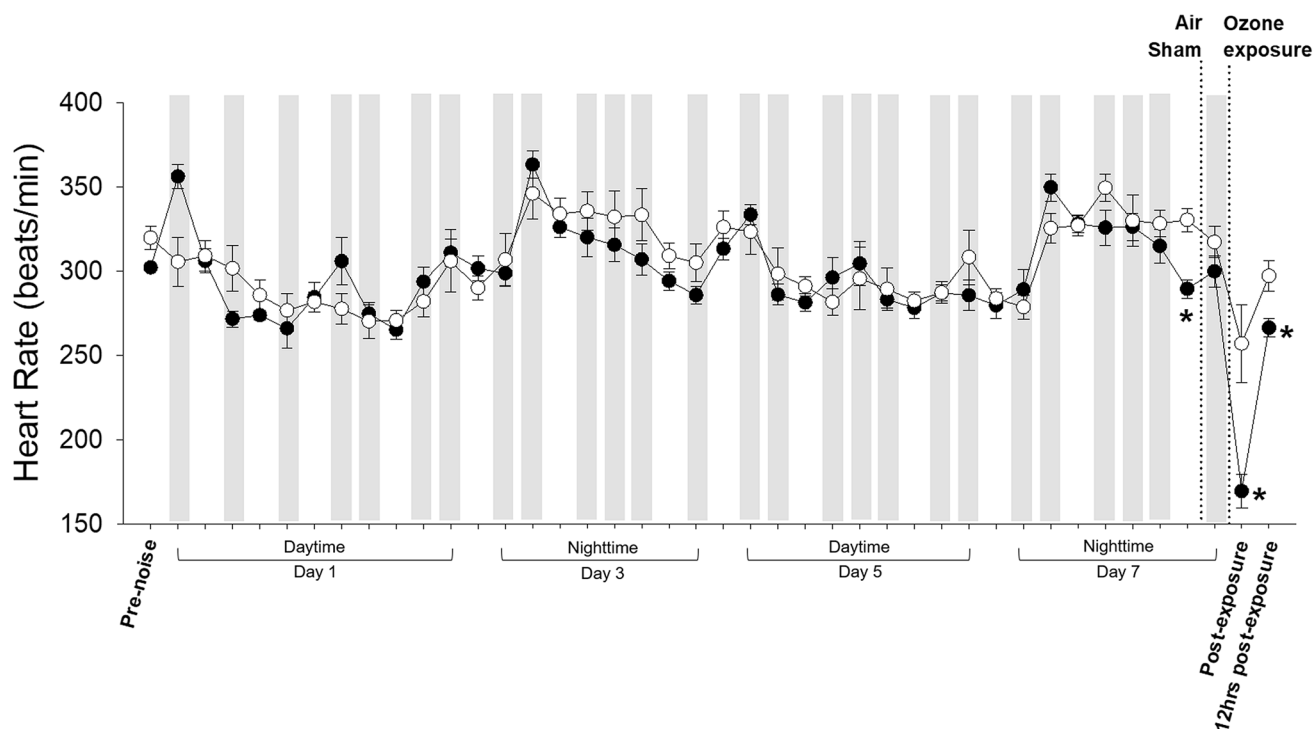


Fig. 2 Heart rate during intermittent noise. Heart rate fluctuated in all animals during the day and nighttime over the course of the week. Although noise caused HR to increase during the first episode, it did not differ significantly from Q over the remainder of the regimen and

was significantly lower after the regimen and following exposure to O₃. Gray bars represent noise episodes; open circles=no noise; filled circles=noise. * significantly different from Q, values are mean ± SEM, *n* = 6–7; *p* < 0.05

the first noise exposure (*p* = 0.007). Thereafter, BP in N animals returned to levels like in Q but was significantly lower at certain points during the noise regimen and particularly at the end (Fig. 3). Data points in Figs. 2 and 3 represent averages of the five-minute segments acquired during the noise episodes and during the entire quiet period.

Noise Effects on Heart Rate Variability and Baroreflex Sensitivity

Time and frequency domain measure of HRV are presented in Table 1. There was no significant difference in SDNN, RMSSD or LF/HF between the groups before starting the noise regimen. There were fluctuations in these parameters during the noise regimen, which are presented as an average of those periods, but these were not different from the Q group. On the last day of the regimen (Day 4), all HRV parameters were significantly increased due to noise (*p* < 0.001); whereas only SDNN and CV were increased afterwards (post-noise: SDNN—*p* = 0.046; CV—*p* = 0.007). On the other hand, noise caused LF/HF to be significantly higher than Q before O₃ (*p* = 0.046).

There was no significant difference in the BRS gain of animals in the Q and N groups before the start of the noise regimen (Table 2). Although there was no effect

of intermittent noise on BRS during the regimen (Days 1–4), total, up and down BRS gain were significantly increased in N rats after the regimen when compared to Q (Post-noise—*p* < 0.001).

Air Sham and Ozone Effects on Heart Rate and Blood Pressure

When compared to Q, noise-exposed animals had a significantly lower HR at the beginning of the air sham (*p* = 0.003) and an increased HR during the first half of the O₃ exposure (*p* = 0.03), which then showed a decreasing trend during the second half (*p* = 0.08) (Fig. 4a). Heart rate remained significantly decreased in the N group immediately after O₃ (*p* < 0.001) and even 12 h later (*p* < 0.001). Moreover, like HR, BP was initially lower in the N group during the air sham (*p* = 0.003) and was increased during the first half of the O₃ exposure and showed a decreasing trend during the second half (Fig. 4b) when compared to Q (*p* = 0.054). Blood pressure was decreased in the N group immediately after exposure (*p* < 0.001) and was back to normal 12 h thereafter.

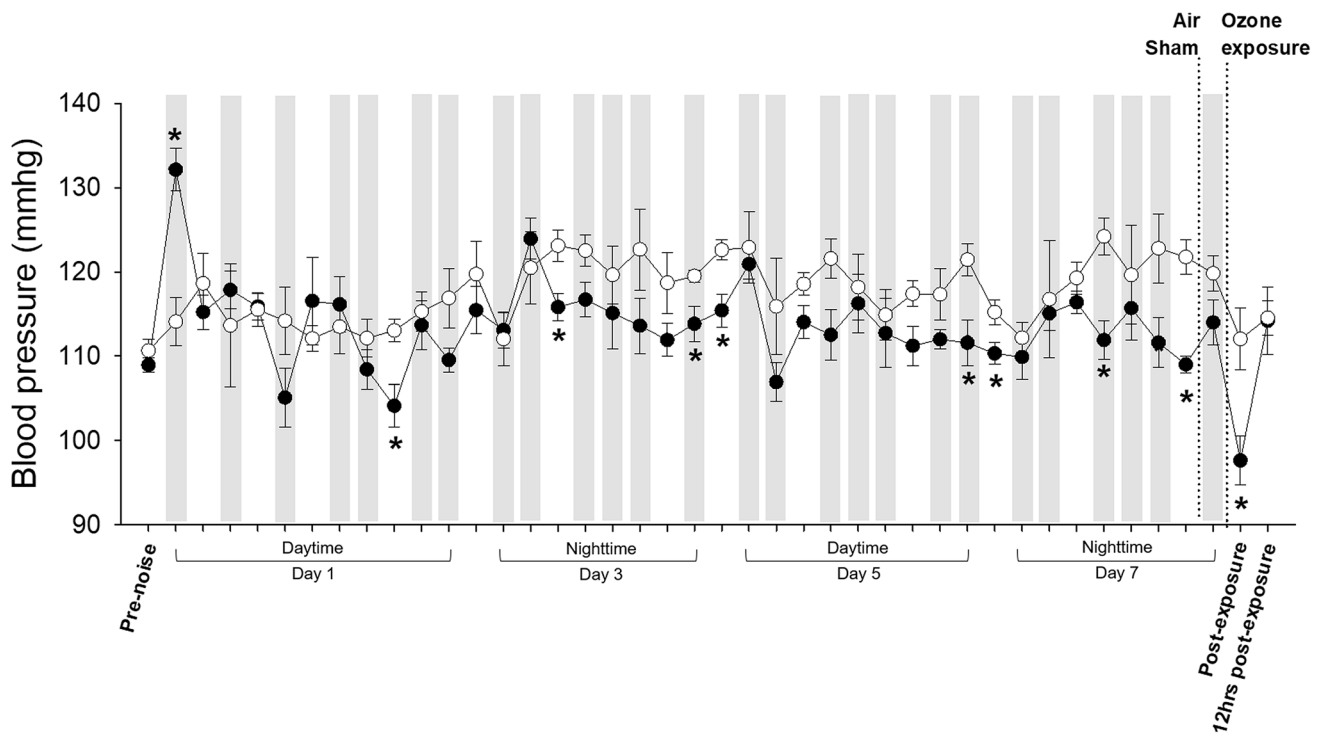


Fig. 3 Blood pressure during intermittent noise. Blood pressure fluctuated in all animals during the day and nighttime over the course of the week. Although noise caused BP to increase during the first episode, it was significantly lower than Q over the remainder of the regi-

men, after the regimen and immediately following exposure to O₃. Gray bars represent noise episodes; open circles = no noise; filled circles = noise. * significantly different from Q, values are mean ± SEM, $n = 6-7$; $p < 0.05$

Air Sham and Ozone Effects on Heart Rate Variability and Baroreflex Sensitivity

Exposure to O₃ caused an increase in all HRV parameters for both Q and N animals, particularly during the second, third and fourth hours. Although there was no significant difference in SDNN between Q and N animals during exposure, there was a trend of decreased CV in the last hour of exposure ($p = 0.09$), which suggested that a concurrent decrease in HR may have masked a decrease in SDNN. The elevated values remained significantly increased for both groups after exposure and were higher in the N group (SDNN— $p = 0.017$; RMSSD— $p = 0.007$; LF/HF— $p = 0.016$). On the other hand, the increase in BRS post-noise persisted during the air sham exposure but was not observed during O₃ exposure or afterwards because BRS gain was increased in the Q group as well (Exposure Hours 1–4 and Post-exposure). Lastly, we measured PR interval from the ECG waveforms to assess if there was any additional indication of autonomic shift. There was no difference in PR interval between Q and N rats except during the last hour of ozone exposure when the latter was significantly decreased ($Q = 54.4 \pm 3.2$ ms vs. $N = 49.5 \pm 1.2$ ms, $p = 0.048$).

Intraventricular Functional Assessments

Intraventricular function was measured in Q and N rats exposed to either FA or O₃ one day after the exposure. Although there were no statistically significant differences between any of the groups, noise followed by O₃ exposure showed a trend towards an increase in LVP ($p = 0.069$) and dP/dT_{\max} ($p = 0.073$), which indicate the static pressure in the left ventricle and contractility, respectively (Fig. 5a and b). We also examined left ventricular end diastolic pressure (LVEDP) but did not observe any significant results (data not shown). Dobutamine caused LVP and dP/dT_{\max} to increase in all groups but there were no significant differences. Similarly, there were no significant differences in dP/dT_{\min} or the measure of lusitropy or rate of myocardial relaxation between any groups (Fig. 5c).

Aconitine Arrhythmia Challenge

Continuous infusion of aconitine caused progressively worsening arrhythmia in all rats. Significantly lower threshold doses of aconitine caused arrhythmias in rats exposed to both noise and O₃ together when compared to noise or O₃ alone ($p < 0.001$) (Fig. 6).

Table 1 Heart rate variability during noise regimen, air sham and ozone exposure

	Before	Noise regimen				Post-noise	Pre-exposure	Exposure			Post-exposure	
		Day 1	Day 2	Day 3	Day 4			Hour 1	Hour 2	Hour 3		Hour 4
Quiet												
SDNN	8.0±0.4	8.7±0.3	8.4±0.7	8.3±0.4	7.6±0.7	12.5±0.7*	8.4±0.8	10.1±1.3	22.2±6.2	36.3±13.6	53.6±11.5	15.3±1.3#
CV	4.0±0.2	4.1±0.2	4.2±0.4	4.0±0.2	3.8±0.3	5.4±0.2	4.2±0.4	4.8±0.6	6.8±1.2	9.2±3.2	13.4±2.8	6.5±0.6
RMSSD	5.6±0.5	6.5±0.4	6.8±0.8	6.4±0.5	6.3±0.6	9.9±0.5*	6.4±0.7	7.7±1.4	23.9±8.7	46.3±17.7	68.9±15.4	13.5±1.1#
LF/HF	1.5±0.4	1.1±0.3	1.8±0.6	0.8±0.1	0.6±0.0	1.7±0.3	0.7±0.1	4.7±0.6	2.2±0.7	2.6±0.5	4.8±2.1	2.4±0.7#
Noise												
SDNN	8.1±0.9	8.2±0.5	7.4±0.6	7.9±0.2	14.0±1.0 [◆]	14.4±0.9** [◆]	7.7±0.4	7.6±0.9	11.7±2.7	26.8±3.9	47.8±4.3	19.7±1.7 [◆]
CV	4.0±0.3	4.0±0.3	3.8±0.3	3.8±0.1	6.2±0.3 [◆]	6.5±0.2 [◆]	3.9±0.2	3.9±0.5	5.1±0.8	7.3±0.6	9.7±0.7	7.9±0.6
RMSSD	6.2±0.5	6.7±0.5	6.2±0.8	5.9±0.2	11.1±0.7 [◆]	11.4±0.8*	6.1±0.6	5.9±1.1	11.4±3.5	30.6±3.9	57.2±4.9	18.3±1.5 [◆]
LF/HF	0.7±0.1	0.7±0.1	1.0±0.1	1.5±0.6	2.6±0.3 [◆]	2.6±0.5*	2.1±0.7** [◆]	1.0±0.2	1.5±0.2	3.5±0.6	6.8±1.2	3.5±0.4 [◆]

*Significantly different from before, *p* < 0.05

#Significantly different from pre-exposure, *p* < 0.05

◆Significantly different from Q, *p* < 0.05

Discussion

The findings of this study demonstrate that intermittent noise alters cardiovascular physiological function in conscious and unrestrained rats. Previous studies have measured various biochemical indicators of cardiovascular health including heart rate and blood pressure under anesthesia after noise exposure [11, 20], but none have assessed real-time changes that occur during a noise event. Moreover, this work was also undertaken to determine the broader implications of such an exposure on air pollution health effects given the prevalence of both noise and air pollution, particularly in urban centers. To that end, changes in autonomic function and baroreflex sensitivity (BRS) were examined throughout the study to determine if noise has an effect on homeostatic control and renders the body susceptible to a subsequent environmental stressor like air pollution. The data presented here are the first to show that intermittent noise exposure causes autonomic and BRS imbalance, which can result in altered heart rate and blood pressure regulation, and also increases the risk of arrhythmias and change in cardiac mechanical function following exposure to ozone.

Epidemiological studies indicate that exposure to traffic-related noise alone results in cardiovascular morbidity, particularly due to increased hypertension, in addition to other health and non-health consequences [21, 22]. Regarding traffic noise and air pollution, the few epidemiological studies that have examined the effect of air pollution on noise-induced cardiovascular dysfunction suggest that each may be an independent contributor to the observed responses, or that any increase in risk from simultaneous air pollution inhalation is only marginal [23, 24]. Despite these findings, studies like this one are important because epidemiological studies cannot uncover exposure-induced latent physiological changes or clarify the underlying mechanisms. Our group previously demonstrated that sequential ozone and noise exposure has differential effects on uterine blood flow in pregnant dams; however, there were minimal effects in the offspring [25]. In addition, we were unable to find any toxicological studies examining the cardiovascular physiological effects of both noise and air pollution in adult rodents. Although simultaneous exposure would have been ideal, the technical hurdles for achieving this were insurmountable so we performed sequential exposures to determine whether noise primes the cardiovascular system to a subsequent air pollution insult.

The increase in heart rate and blood pressure observed during the first day of noise was similar to increases in other rodent studies [11] and typical of the acute stress response seen in humans [26], which is marked by an increase in corticosterone and markers of oxidative stress [20]. Although we did not measure these biochemical

Table 2 Baroreflex sensitivity during no noise/noise regimen, air sham and ozone exposure

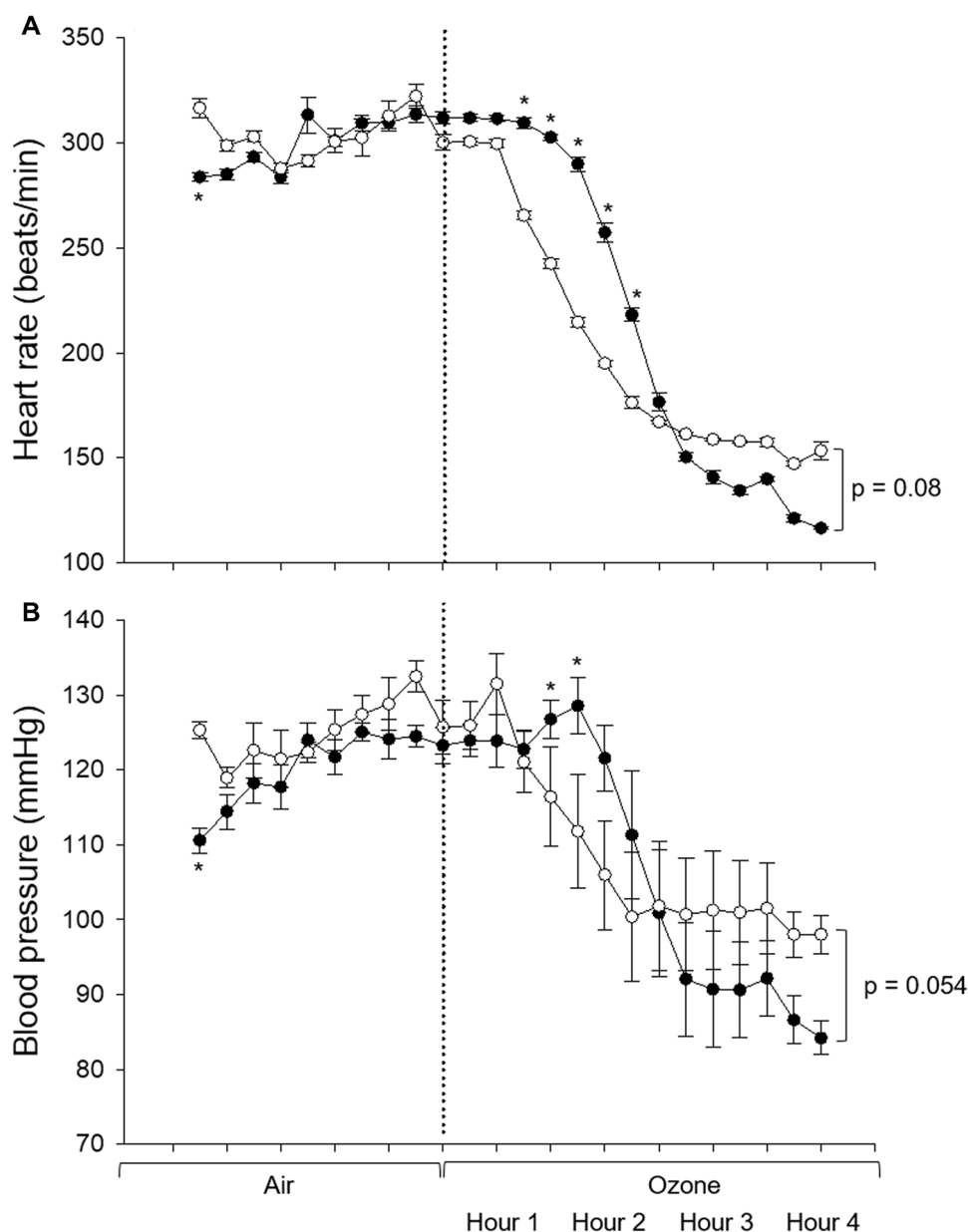
	Before	Noise regimen				Post-noise				Exposure				Post-exposure
		Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4	Hour 1 (% change)	Hour 2 (% change)	Hour 3 (% change)	Hour 4 (% change)	
Quiet														
Overall gain	2.9±0.2	1.9±0.1	2.3±0.4	2.7±0.2	2.1±0.1	4.5±1.0	2.7±0.2	2.9±0.7 (4.1)	8.1±2.5 (185.5)	13.7±3.7 (398.2)	14.2±5.6 (446.4)	10.7±2.8		
Up	3.3±0.2	1.8±0.1	2.5±0.7	3.1±0.5	1.8±0.1	2.9±0.6	2.6±0.2	3.1±0.9 (11.3)	5.9±2.7 (103.5)	10.3±2.3 (193.2)	10.5±3.6 (285.8)	8.1±2.3		
Down	2.6±0.2	1.9±0.2	2.0±0.1	2.4±0.1	2.3±0.2	5.2±1.3	2.7±0.2	2.7±0.7 (-5.7)	8.0±2.6 (185.7)	13.3±3.9 (392.3)	15.3±6.0 (509.9)	11.0±3.0		
Noise														
Overall gain	3.1±0.3	3.3±0.3	2.5±0.2	2.9±0.3	2.7±0.1	10.8±1.3*♦	4.8±0.5*♦	2.8±0.4 (-40.6)	6.3±1.3 (41.2)	11.9±2.1 (179.2)	17.2±3.5 (278.4)	11.2±1.9		
Up	3.3±0.4	3.1±0.4	2.5±0.2	3.4±0.4	2.7±0.3	9.1±2.7*♦	5.5±0.9*♦	2.9±0.6 (-46.0)	5.2±1.3 (10.0)	11.3±2.0 (157.4)	19.8±4.0 (287.9)	9.0±1.4		
Down	2.9±0.3	3.5±0.4	2.5±0.4	2.6±0.5	2.7±0.3	12.3±1.6*♦	4.1±0.6*♦	2.7±0.4 (-22.7)	7.3±1.3 (119.7)	12.3±2.1 (255.9)	16.8±3.6 (427.6)	12.3±2.1		

Units for BRS are ms/mmHg

*Significantly different from before, $p < 0.05$

♦Significantly different from Q, $p < 0.05$

Fig. 4 Cardiovascular response to O_3 is altered in rats exposed to noise. Noise-exposed rats had significantly lower HR (a) and BP (b) before starting the sham exposure when compared to Q, this steadily increased until there was no significant difference between the two groups. HR and BP in Q rats steadily decreased during O_3 exposure. Although these endpoints remained elevated in N rats during the first and second hours of O_3 , they quickly decreased thereafter and showed a decreased trend to Q. Open circles = no noise; filled circles = noise. *Significantly different from Q, values are mean \pm SEM, $n=6-7$; $p<0.05$



indicators, the physiological results are consistent with previously published data on the cardiovascular response to acute stress and importantly represent changes acquired without anesthesia or restraint. To prevent adaptation, we used a noise regimen that was intermittent and irregular, both during the day and night. Noting that the first noise exposure (Day 1) in our study was in the daytime, or when rats are normally asleep, the increased heart rate and blood pressure seem to confirm that noise likely caused sleep disturbance/deprivation and annoyance, in addition to stress [27]. On the other hand, we were surprised to see that subsequent noise episodes either had no effect or caused a decrease in blood pressure, especially during nighttime

when rats are awake. Noise causes both direct and indirect effects and its impact on physiological and psychological function depends on its intensity, duration and frequency [28]. In addition, there is some evidence that suggests hypotensive responses to noise occur more commonly than previously thought [29], and that the association between noise and cardiovascular disease (i.e. hypertension) is only stronger in certain groups (e.g. diabetics) [30].

Noise-induced hypotension does not receive a lot of attention because stress and hypertension are global primary risk factors known to increase cardiovascular morbidity and mortality. Yet, a handful of studies have documented that people can experience a decrease in blood pressure during noise

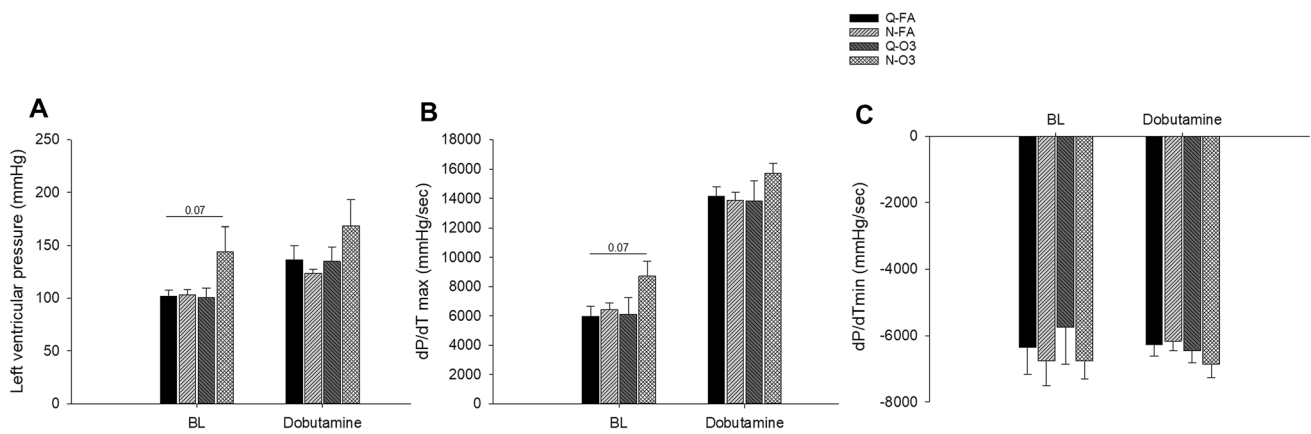


Fig. 5 Intraventricular function. Although there were no significant differences in LVP (a), dP/dT_{max} (b) or dP/dT_{min} (c) between any of the groups, there was a trend towards increased LVP and dP/dT_{max}

in rats exposed to noise and O_3 . Similarly, dobutamine caused an increase in LVP and dP/dT_{max} in all groups, but there were no significant differences. Values are mean \pm SEM, $n=7$

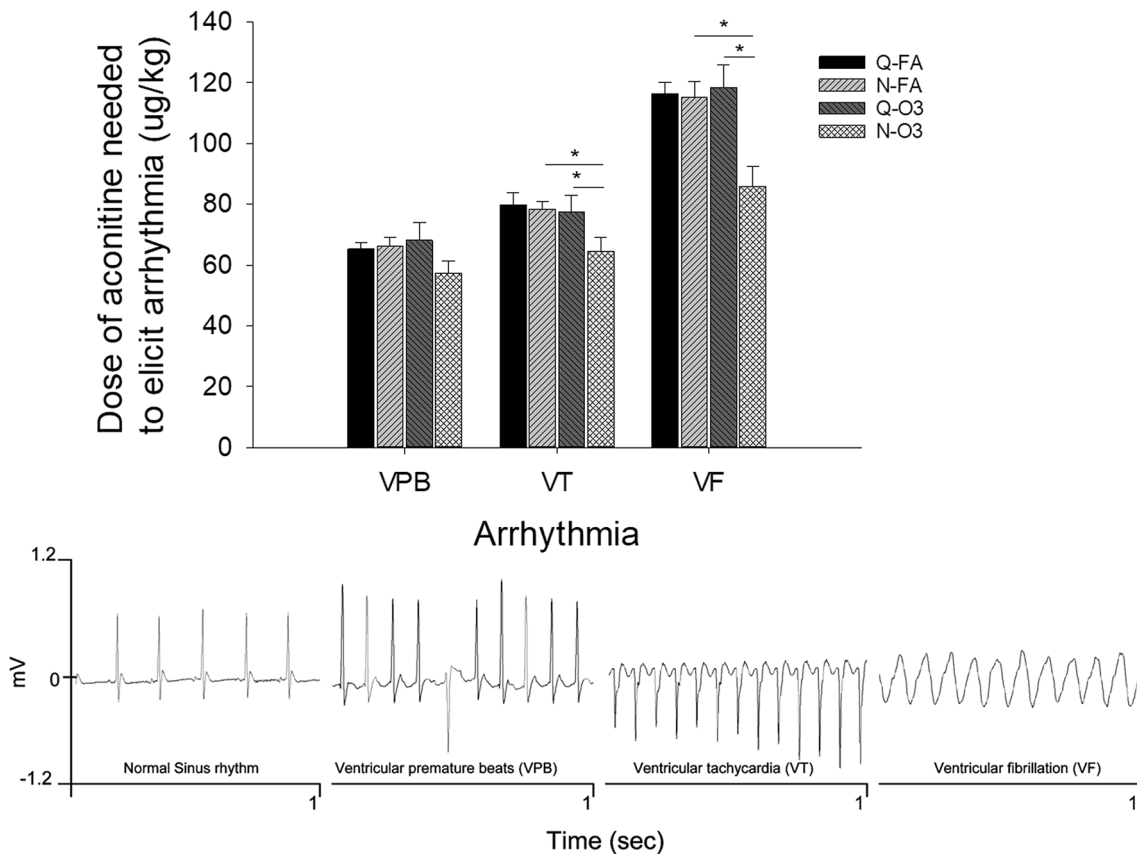


Fig. 6 Prior exposure to noise increases sensitivity to cardiac arrhythmia after O_3 . Continuous infusion of aconitine progressively worsened arrhythmia in all rats (VPB \rightarrow VT \rightarrow VF). The dose of aconitine needed to elicit VT and VF in noise-exposed rats was sig-

nificantly lower after O_3 when compared to N-FA and Q- O_3 . Representative ECG traces of normal sinus rhythm, VPB, VT and VF. * significantly different, values are mean \pm SEM, $n=6-7$, $p<0.05$

episodes, especially when it is encountered intermittently and continuously [29, 31]. Unfortunately, there is no data on this effect in rodents as far as we can tell. Although it is

likely that there were brief increases in blood pressure when the noise episode first started, the overall response was either no change or a decrease over the entire period (i.e., 4–6 noise

episodes on each of Days 3, 5 and 7). This is particularly the case on the last day of the regimen (Day 7) and immediately afterwards when noise-exposed rats had increased HRV and BRS. Epidemiological studies have shown that noise is positively associated with SDNN and HF or parasympathetic influence, but also concurrent increases in HR, which suggests effects other than “fight-or-flight” [32, 33]. These investigators suggested that the relative ramping up or withdrawal of the parasympathetic and sympathetic influences depended on the intensity of the noise and the activation of several biological pathways. Consequently, increased BRS has been found to coincide with decreased blood pressure, which is presumed to occur because of overcompensation and stabilization of blood pressure at a lower level [34, 35]. This overcompensation can occur due to spikes in blood pressure and overstimulation of the baroreflex resulting in sustained hypotension [36]. Whether this is occurring in our noise-exposed rats is not clear; however, the persistently lower blood pressure, even during the air sham, suggests a homeostatic shift that continued despite cessation of the noise regimen.

These effects might be due to the development of anxiety and depression, which sometimes occur with repeated and intermittent noise exposure. For example, noise annoyance was found to be strongly associated with anxiety and depression [9, 37]. These noise-induced psychological responses can occur in humans [38, 39] and with chronic unpredictable mild stress in rats [40, 41]. Even then, the degree to which we observed decreases in blood pressure was still unexpected. Indeed, a different noise regimen, maybe one that was more acute or with longer intervals in between episodes, might have produced the typical stress-related cardiovascular changes (e.g. sustained high blood pressure, decreased HRV). However, these results underscore the modifying effect of noise on cardiovascular control and subsequent responsiveness to stressors.

We decided to use ozone because it is a ubiquitous air pollutant and has been thoroughly characterized, both by us and other researchers [42–44]. Moreover, we previously showed that a prior exposure to NO₂ sensitizes rats to be more responsive to ozone, which possibly points to the mechanism by which low levels of ozone cause tangible cardiovascular effects [45]. As such, others have demonstrated that ozone may not have direct cardiovascular effects in the absence of co-pollutants [46]. The HR, BP and HRV changes in Q animals were like what we previously showed at this concentration of ozone [43] and noise resulted in increased heart rate and blood pressure during the first half of ozone and a significant decrease thereafter. Moreover, a noise-induced decrease in PR-interval late during the ozone exposure suggesting increase sympathetic modulation despite the decreases in HR and BP. Thus, it seems that prior exposure to noise sensitizes the cardiovascular system

to ozone, possibly due to increases in baroreflex gain and autonomic imbalance.

The increase in LF/HF, which has been shown previously to occur with white noise in humans [47], might indicate why there were noise-induced heart rate and blood pressure differences during ozone, especially during the extreme hypotension at the very end of exposure. In a different model, Hanss et al. showed that an increased LF/HF predicted a high risk of hypotension [48]. Indeed, a high LF/HF suggests overall autonomic imbalance given LF represents sympathetic influence, vagal and respiratory inputs, and baroreflex contributions, whereas HF represents only the parasympathetic. Yet, it is worth noting that the increased BRS likely contributed to the shift in LF/HF after noise, particularly given the LF fluctuations are associated with baroreceptor activity [49]. Regardless, to our knowledge, this is the first study to demonstrate that prior exposure to noise not only causes persistent autonomic imbalance, but also increases cardiac arrhythmogenesis. However, a lack of diastolic dysfunction (i.e., changes in LVEDP), as might be observed with chronic hypertension, and only a trend towards increased contractile function suggests that although intermittent noise exposure causes homeostatic shifts that persist for a day or two after exposure, it does not necessarily result in noticeable functional changes in the heart. A more prolonged noise regimen may have worsened it.

In conclusion, these data suggest for the first time that prior exposure to noise sensitizes the cardiovascular system to a subsequent air pollution stressor. Although the findings appear to parallel human responses and likely represent similar physiological changes that point to heightened risk, we must point out that humans and rodents differ in the control of cardiovascular function, in particular autonomic regulation. Thus, rather than viewing these results as evidence of a conclusive mechanism of how noise renders the body susceptible to the effects of air pollution, we suggest that the current study provides tangible data on the modifying effects of noise. Moreover, even short-term exposure has the ability to disrupt homeostatic balance and decrease the body’s ability to compensate when faced with stressors. Future studies will need to measure the effects of both noise and air pollution simultaneously, not to mention investigate the mechanistic basis for the responses observed in this study, and determine if the presence of underlying disease (e.g. hypertension) disproportionately impacts the response.

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Compliance with Ethical Standards

Conflict of interest There are no conflicts of interest or competing interests to disclose.

Ethical Approval The study protocol received approval from Institutional Animal Care and Use Committee of the United States Environmental Protection Agency, as well as quality assurance clearance. All animals were treated humanely and with regard to alleviation of suffering

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