Chronic Noise Exposure in the Spontaneously Hypertensive Rat

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Abstract

Introduction: Epidemiological studies have suggested an association between the relative risk for developing cardiovascular disease (CVD) and long-term exposure to elevated levels of transportation noise. The contention is that this association is largely owing to an increase in stress-related biomarkers that are thought to be associated with CVD. Animal models have demonstrated that acute noise exposure is capable of triggering a stress response; however, similar studies using chronic noise models are less common. Materials and Methods: The current study assessed the effects of intermittent daily exposure to broadband 80 kHz bandwidth noise of 87.3 dBA for a period of 21 consecutive days in spontaneously hypertensive rats. Results: Twenty-one days of exposure to noise significantly reduced body weight relative to the sham and unhandled control groups; however, noise had no statistically significant impact on plasma adrenocorticotropic hormone (or adrenal gland weights). Noise was associated with a significant, albeit modest, increase in both corticosterone and aldosterone concentrations following the 21 days of exposure. Interleukin 1 and interleukin 6 levels were unchanged in the noise group, whereas both tumour necrosis factor alpha and C-reactive protein were significantly reduced in noise exposed rats. Tail blood sampling for corticosterone throughout the exposure period showed no appreciable difference between the noise and sham exposed animals, largely due to the sizeable variation for each group as well as the observed fluctuations over time. Discussion: The current pilot study provides only modest support that chronic noise may promote stress-related biological and/or developmental effects. More research is required to verify the current findings and resolve some of the unexpected observations.

Keywords: Cardiovascular disease, hypertension, noise stress, stress hormones

Introduction

Stressor exposure activates several physiological pathways that may be implicated in some of the purported health effects observed in individuals who are exposed to relatively high levels of environmental noise for several years, especially those that involve the cardiovascular system.¹⁻³ These pathways include alterations in the hypothalamic pituitary adrenal (HPA) axis with a consequent increase in adrenocorticotropic hormone (ACTH) and cortisol (corticosterone in rodents);⁴⁻⁶ others have shown rats habituate to loud noises as evidenced by a gradual return to baseline in corticosterone levels by the fourth day of exposure.⁷ Similarly, pro-inflammatory cytokines, such as interleukin 1 (IL-1), tumour necrosis factor (TNF)-alpha and interleukin 6 (IL-6) have been implicated in the stress response.⁴⁻⁸⁹ Circulating aldosterone levels, which influence blood pressure, are also influenced by exposure to stress/anxiety.¹⁰⁻¹¹ More recently, chronic up-regulation of the inflammatory marker C-reactive protein (CRP) has been positively associated with cardiovascular disease (CVD)¹²⁻¹³ and concentrations of plasma CRP have been reported to increase following chronic unpredictable stress in apolipoprotein deficient mice.⁹ It is, therefore plausible that exposure to stressors, such as noise, may increase the chance for development of CVD in people, and this may be more apparent in those with a genetic predisposition.¹⁴

Animal strains that are genetically predisposed to developing CVD, such as the spontaneously hypertensive rat (SHR),

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can be useful in the identification of molecular/biological underpinnings for CVD. Several years ago, McCarty and Kopin\[15\] reported that the SHR had an exaggerated catecholamine response to an acute stressor, which was put forward as a mechanism underlying the maintenance of hypertension in these rats. While Gomez et al\[16\] found no basal differences in corticosterone or ACTH levels among five rat strains, including SHR rats, Djordjevic et al\[17,18\] found that compared to normotensive controls, the SHR had some slightly elevated basal levels of both ACTH and corticosterone. Furthermore, SHR rats had a much greater ACTH (but not corticosterone) response to both cold and immobilisation stressors\[17,18\] ACTH was relatively unchanged compared to Wystar Kyoto normotensive rats following 15 or 30 min foot shock stress, though it was significantly greater than the levels measured in the Lewis strain following 30 min of foot shock.\[16\] Corticosterone levels did not significantly differ between SHR and Wystar Kyoto normotensive rats following 15 or 30 min of foot shock, though they were significantly elevated when compared to rats of the Lewis strain.\[16\] An absent corticosterone reactive response when compared to the matched normotensive strain may be an issue of timing, endogenous negative feedback and/or stressor type. These specificities are highlighted by the findings of McCarty and Kopin\[15\] described above, and the more recent divergent observations by Roman et al.,\[19\] wherein 2 min of mild handling stress was associated with a significant increase in both plasma ACTH and corticosterone in SHR, but not normotensive controls.

The effects of long-term noise exposure in the SHR model have been assessed by Herrmann et al\[20\]. They exposed the SHR to 65 dB of continuous noise (4 and 250 Hz tone) for 52 weeks and found a number of changes in cardiovascular parameters, including an increase in microvessel wall area, cardiac fibrosis and ischaemic myocardial lesions. Although these authors did not examine HPA or inflammatory responses, they suggest that the changes they observed are likely to be the consequence of noise stress-induced increase in sympathetic tone and a consequent increase in the release of hormones that mediate vasoconstriction. Given the role of the HPA axis and circulating pro-inflammatory cytokines in response to stress, the aim of the current work is to assess whether these are altered in this animal model of hypertension; to our knowledge such a characterisation has never been performed among SHR rats exposed to long-term intermittent noise stress. We hypothesised that intermittent exposure to long-term noise stress should be associated with an increase in parameters that have been shown to be implicated in the development or maintenance of hypertension in the SHR.

To test this hypothesis, we assessed the impact of chronic noise exposure in SHR on (1) adrenal gland weight, plasma aldosterone and classic stress biomarkers, including corticosterone and ACTH; (2) other inflammatory biomarkers that have been positively associated with CVD, including pro-inflammatory cytokines and CRP; and (3) general development as measured by body weight gain.

### MATERIALS AND METHODS

#### Animals

Male SHR 7 weeks of age – already considered to exhibit prehypertensive tachycardia\[21\] – with initial body weights of approximately 125–150 g obtained from Charles River (Canada) were used. The animals were housed in pairs in standard rodent cages (36 cm × 31 cm × 17 cm) with a 12/12 h light – dark cycle (lights on at 07:00 h) and temperature maintained at 22±2°C with 45–55% relative humidity. Animals had ad libitum access to PurinaTM rat chow and tap water that was monitored daily. All animals were weighed daily (about 07:15 h) using an electronic balance by animal care personnel. The weighing times were selected to avoid overlapping with any noise exposures (see below). All experimental procedures met the guidelines on the ethical treatment of animal subjects in experimental research set forth by Health Canada, and all procedures were conducted in accordance with Health Canada’s Animal Care Committee’s approved research protocol.

The rats were randomly divided into three groups, each consisting of 14 animals. Chronic noise exposed rats were exposed eight times a day to 15 min of white noise (see below). Periodic tail blood samples were taken on seven different occasions for corticosterone profiling over the course of the 21-day noise exposure. Tail blood samples were also taken from a group of sham controls with no noise exposure (sham control). This group was used to dissociate the potential effects of periodic tail bleeds from noise on stress biomarkers. Finally, a third group (control) served as a tail blood control group was exposed to minimal handling (body weight measurements, normal cage cleaning) to assess the potential stress that could result from tail blood sampling.

#### Noise treatment

Noise exposures were equally split between the dark phase (18:00–6:00) and the light phase of the light cycle (6:00–18:00). The total daily noise exposure duration was set to 5.5 h. The exposures consisted of 15 min of noise with an unweighted level of 91.5 dBJ (when band limited 50 Hz to 80 kHz) or 87.3 A-weighted (dBA). Eleven exposures were distributed randomly throughout the light phase starting on the quarter hour, and eleven exposures were similarly distributed randomly during the dark phase. This resulted in continuous noise for durations ranging from 15 min to 1 h and quiet periods that lasted from 15 min to 5 h (i.e., from a minimum of 1 × 15 min noise exposure, prior to a quiet period, to a maximum of 4 × 15 min adjacent noise exposures prior to a quiet period). The quiet periods, which were also multiples of 15 min, were as short as 15 min and as long as 5 h. During quiet periods, the background level inside the cages was 56 dBZ unweighted, primarily due to heating and air conditioning. On Tuesday and Friday mornings at 06:00 AM the noise exposures in the light phase were rearranged to allow a 4.5 h period for animal facility staff to service the animals.
The target unweighted sound level was 90 dBZ. At the end of the experiment, depending on position within the cages, the unweighted overall sound level varied from 90.4 to 93.6 dBZ. This estimate was based on measurements over nine positions in seven cages, with bedding and full food trays, but no rats. Noise levels averaged 76.3 dBZ over the 33 individual 1/3 octave bands between 50 and 80 kHz. Within each cage, these levels varied as a function of position between 65.3 and 86 dBZ, with an overall standard deviation of 2.3 dB (over 33 frequencies, nine positions and seven cages). The 1/3 octave frequency band with the sound level furthest from the average was 800 Hz where, at one position in one cage, the level was as low as 65.3 dBZ. In this frequency band, the standard deviation was 3.3 dB (based on nine positions in seven cages). At 40 kHz, the levels in one position in one cage were as high as 86 dBZ, the second furthest value from the average, and the standard deviation was 3.2 dB (based on nine positions in seven cages).

Noise exposures were made in the top cages of two separate cage racks touching back to back forming two rows of four cages, with one cage empty. Exposures were provided by four Paradigm Signature S1 P-Be loudspeakers (Paradigm Electronics Inc., Mississauga, ON, Canada). The speakers were mounted in two rows of two speakers 58 cm above the floor of the cages. Each speaker primarily exposed two cages.

To verify the noise exposure, sound levels were continuously monitored using two Brüel & Kjær type 4939 ¼ in. microphones and a similar 4135 ¼ in. microphone (Brüel & Kjær, Point Claire, QC, Canada) suspended in a line 48 cm above the cage floors. Each of the first and the last microphones at the ends of the line were centred over two end cages.

Cages and microphones were arranged similarly in the sham and unhandled control rooms. There were three Avisoft CMPA-P48/CM16 monitoring microphones in the sham control room, and there was one Avisoft microphone and one Brüel & Kjaer type 4135 microphone in the unhandled room mounted 48 cm above the cage floors. Brüel & Kjær microphone calibrations were checked using a Brüel & Kjær type 4226 multi function calibrator (Brüel & Kjær, Point Claire, QC, Canada) before and after measurements.

Exposures and monitoring (source of the stimulus) were controlled with 204.8 kHz 24 bit National Instruments PXI 4461 four channel input module and two PXI 4461 2 input 2 output modules (National Instruments, Mississauga, ON, Canada). Each speaker used one output channel, and the monitoring microphones required eight input channels. Hardware was controlled via National Instruments Labview (Version 2009) software running on a laptop computer.

Additional equipment included two Bryston 4B-ST amplifiers (Bryston Ltd, Peterborough, ON, Canada) to drive the speakers. The Brüel & Kjær microphones also used Brüel & Kjær type 2670 and type 2639 preamplifiers and a type 2829 power supply.

Tail blood sampling

Sampling occurred on seven different days over the course of the 21-day noise exposure. Sampling included a baseline day and six additional days (2 samples per week). Outside of noise stressor exposure periods (see below) and between 08:30–10:30, rats from the noise and sham control groups were removed from their home cage one at a time and gently wrapped in a clean surgical towel in the animal holding room. They were then brought to the procedure room where the tail was inserted in warm water (40°C) for approximately 1 min to allow for rapid sampling of blood, particularly in stressed animals. A second technician placed the tail on a hard surface to puncture the tip with a lancet. The blood (~250 µL) was collected with a Pasteur pipette and transferred to 903® protein saver cards (Whatman Inc., NJ, USA). Following collecting, gentle pressure was applied to the incision, and the rat was returned to the animal room in the towel. The entire procedure took approximately 4 min for each rat. Sampling was counterbalanced between the two groups. Collected blood was left to air dry at room temperature (~1.5 h) before being stored at ~80°C where it was stored with desiccant for approximately 2 months before corticosterone assays were performed. Rats were acclimatized to the tail blood handling procedures for several days prior to the initial baseline sample.

Trunk blood sampling

Counterbalanced across groups, decapitation without anaesthesia took place between the hours of 08:00–10:00 approximately 24 h following the 21st day of noise exposure. Trunk blood was collected from 14 rats per day for 3 days into 10 mL K2EDTA coated Vacutainer® tubes via disposable Na2EDTA coated funnels. Samples were gently mixed by inversion 8 times, left at room temperature (~30 min) and centrifuged at 500xg for 12 min at 4°C. Equal volume of Plasma in aliquots was poured into 5 × 1.5 mL cryogenic tubes and left temporarily on dry ice, until they were stored at ~80°C until assays were performed.

Trunk blood hormone and immune factor determination

IL-6, TNF-alpha and IL-1-alpha assays were performed using the LEGENDplex™ custom rat multi-plex cytokine panel (Biolegend, Inc. San Diego, USA). Detailed procedures for these assays can be found at the Biolegend website (http://www. biolegend.com). A little or negligible cross-reactivity was found with these and multiple recombinant proteins tested (see Biolegend documentation for an extensive list). The intra-assay precision for these cytokines ranges from 6 to 24% with an inter-assay precision ranged from 10 to 15%. ACTH, corticosterone, CRP and aldosterone concentrations were determined by enzyme-linked immunosorbent assay (ELISA) method using MD Bioproducts (St. Paul, MN, USA), Arbor Assays LLC (Ann Arbor, MI, USA), ALPCO...
method. In all cases, blood biomarker endpoints were sampling were based on previous studies having used this assay were halved. Procedures for tail blood corticosterone sample and standard volume requirements specified in the kit were then followed with the one exception being that temperature. Assay procedures from the radioimmunoassay morning, tubes were re-shaken at 50 rpm for 1 h at room temperature and placed at 4°C overnight. The following morning, tubes were re-shaken at 50 rpm for 1 h at room temperature. Assay procedures from the radioimmunoassay kit were then followed with the one exception being that sample and standard volume requirements specified in the assay were halved. Procedures for tail blood corticosterone sampling were based on previous studies having used this method. In all cases, blood biomarker endpoints were determined in triplicates.

Data analysis

All statistical analyses were performed using the IBM Statistics 19 software package. Change in body weights was analysed using a mixed two-factor analysis of covariance (ANCOVA) design. Treatment group (3 levels: noise, sham, control) was the independent factor while time was the repeated one with three levels; baseline body weight served as a fixed covariate. Note that although body weight was recorded daily, providing similar minimal manipulation to all animals, only the data at one, two, and three weeks of noise exposure were included in the analysis. Analysis results are similar when the 21 days are instead used in the analysis but for simplicity, we present the analysis on the 7, 14, and 21 days of treatment that can be more easily seen in the accompanying graph. Adrenal weights were analysed using a similar design, with treatment group as the independent factor, adrenal side as the repeated factor (right, left) and body weight prior to sacrifice as the fixed covariate.

Plasma hormone levels were analysed differently depending on the source of blood. Tail blood corticosterone levels were analysed as above using a mixed two-factor ANCOVA design with group (2 levels: noise and sham) as the independent factor, time as the repeated factor (6 levels) and baseline corticosterone levels as the fixed covariate. Note that the repeated measures factor was corrected for violations to the assumption of sphericity by the Huynh–Feldt procedure. Trunk blood levels of IL-6 were analysed using a simple analysis of variance (ANOVA) design to look for group differences; due to heterogeneous variances in the case of ACTH and corticosterone, the analysis was run on Log transformed data. In the case of aldosterone, CRP and TNF-alpha, given that data transformation did not improve variance heterogeneity, the data were instead analysed using the nonparametric Kruskal–Wallis test. Posthoc pair-wise comparisons were conducted, where appropriate, using a Bonferroni correction in the case of the parametric analyses.

Results

Figure 1 shows the change in body weight for each group across the three weeks of noise exposure. The only significant group difference observed was that overall body weights were lowest in noise exposed animals ($F_{2,38} = 17.18; P < 0.0001$). Baseline weights did not differ between groups. No significant effects or interaction between treatment and laterality was detected for the adrenal gland weights after adjusting for body weight using basal body weight as a covariate [Table 1]; adrenal gland weight is reduced in stressed animals, thus, this is used as a gross measure of stress exposure.

The corticosterone results from repeated measurements of tail blood are shown in Figure 2. The concentrations were not statistically effected by noise exposure, nor were the groups

| Treatment Group | Left Adrenal Gland Mean Weight (g) ± SEM | Right Adrenal Gland Mean Weight (g) ± SEM |
|-----------------|-------------------------------------------|-------------------------------------------|
| Noise           | 0.021 ± 0.0007                             | 0.019 ± 0.0004                             |
| Sham            | 0.022 ± 0.0007                             | 0.021 ± 0.0009                             |
| Unhandled Control | 0.022 ± 0.0008                            | 0.020 ± 0.0004                             |

No statistically significant differences were observed between left and right adrenal gland weights. Likewise, there were no significant differences between treatment groups. Mean left and right adrenal gland weight, in grams (g) are shown with standard error of the mean (SEM).

Figure 1: Change in body weight over time. Results are mean body weight ± SEM. After adjusting for baseline body weight using an analysis of covariance, the only significant observation was that rats exposed to chronic noise had lower overall adjusted body weight, *$P < 0.0001$. base = baseline, g = grams
different over time; note that no group differences were discernable at baseline (day-3).

Hormone analysis from trunk blood showed no statistically significant effect of noise on ACTH [Figure 3A], but there was a significant group difference observed in corticosterone levels collected at the time of sacrifice ($F_{2,39} = 5.786; P < 0.01$) [Figure 3B]. Corticosterone levels were significantly higher in noise-exposed rats compared to sham and unhandled control groups separately ($P < 0.05$ for both), while the two control groups did not differ from each other.

In all animals, plasma IL-1 levels were below the level of detection (i.e., 1.5 pg/mL, data not shown) and there were no treatment effects on IL-6 levels [Figure 4A]. A significant treatment effect in CRP levels ($\chi^2 = 24.601; P < 0.001$) with Control > Sham > Noise ($P < 0.05$ for each pairwise comparison) was observed [Figure 4B]. A significant treatment effect was also found for aldosterone ($\chi^2 = 6.846; P < 0.05$) and TNF-alpha ($\chi^2 = 10.726; P < 0.01$) with only the Control and Noise groups significantly different from each other ($P < 0.05$); Noise > Control in the case of aldosterone and Control > Noise in the case of TNF-alpha [Figure 4C and D, respectively].

**DISCUSSION**

The current study found that 21 days of consecutive intermittent noise exposure produced slight elevations in plasma corticosterone concentrations when sampled 24 h following the final exposure period. This suggests a sustained activation of the HPA axis resulting from chronic noise, but this finding should not be interpreted in isolation. Even though the mean ACTH levels appeared to be highest among the noise group, this could not be confirmed statistically due to the high variability in this endpoint. Furthermore, corticosterone concentrations measured from tail blood sampled twice per week over 3 weeks of noise was too variable to reveal any consistent differences between the noise and sham exposed rats. This fluctuation occurred despite efforts to acclimatise rats to the procedure and keep the sampling period the same across sampling days and within a 90-min window to minimise the circadian influence on plasma corticosterone levels. Chronic activation of the HPA axis with chronic variable stress is known to cause adrenal hypertrophy;[26,27] however, in the current study, treatment was not associated with any group differences in adrenal gland weight. Therefore, our findings only provide tentative support for the notion that chronic noise appears to be associated with a modest increase in basal corticosterone levels in the SHR.

In this study, pro-inflammatory cytokines were assessed because they have been shown to be related to the progression of CVDs.[28-32] To our knowledge, this is the first study to examine changes in circulating IL-1 alpha, IL-6 and TNF-alpha in the SHR following 21-days of intermittent noise exposure. IL-1 alpha concentrations were mostly below the level of detection in all animals, and we found no remarkable group differences in plasma IL-6 concentrations, which is not in line with the observation that IL-6 levels increased in a time-dependent fashion in rats exposed to an open field stressor.[33] This difference could be owing to the differences in cytokine production.
In acute\cite{33} compared to chronic stress in the current study and/or differences in stressor type.

In our study, TNF-alpha was modestly reduced in the rats exposed to the noise stressor, relative to their unhandled control counterparts. The observed direction of change is inconsistent with most studies that show an increase in plasma TNF-alpha in response to chronic stress in rats.\cite{4,34,35} However, our results are in line with other findings that lipopolysaccharide (LPS)-stimulated TNF-alpha secretion from whole blood taken shortly after stressor exposure in mice can be reduced relative to controls.\cite{36,37} These authors demonstrated that elevated corticosterone may have been responsible for the blunted TNF-alpha response following stress, which is consistent with the result in the current study that long term noise stress was associated with elevated corticosterone levels in trunk blood. However, psychological stress, which is known to increase cortisol levels in humans,\cite{38,39} has also been shown to increase pro-inflammatory cytokines, including TNF-alpha.\cite{40} Therefore, it is possible that a blunted TNF-alpha response in the current study is simply a chance finding.

In the recent past, there has been growing interest in CRP because of its association with CVD\cite{12,13,41} Gouin \textit{et al.}\cite{42} found that chronic daily stressors were associated with elevated serum CRP levels and CRP has been reported to increase among adolescents with high interpersonal stress scores.\cite{43} Additionally, chronic unpredictable stress has been shown to increase inflammatory responses thought to play a role in the development of atherosclerosis.\cite{9} CRP and IL-6 were among the biomarkers significantly increased in mice exposed to stressors.\cite{9}

To our knowledge, the current study represents the first animal model to assess CRP levels in response to chronic noise exposure. For this reason, we are reluctant to over-interpret the significance of the observation that plasma CRP concentrations were lowest among the noise-exposed animals. To date, there is currently no similar finding in the published literature that can be used to account for this direction of change. One possibility might be that chronic noise exposure increased circulating anti-inflammatory mediators to levels that could inhibit inflammatory responses, thereby reducing the levels of circulating CRP. Our observation that TNF-alpha concentrations were lowest among the noise group is consistent with a self-limited inflammatory response.\cite{44} It is of interest that in a small (unpublished) pilot study among Sprague–Dawley rats ($n = 4–5$/group) we found the same direction of change in average plasma CRP levels among the rats exposed to noise (31.7 ng/mL) compared to sham rats (41.6 ng/mL) and
unhandled controls (42.8 ng/mL), suggesting that this may not be a phenomenon specific to the SHR animals.

Relative to the unhandled control group, rats exposed to noise had higher levels of plasma aldosterone. The link between aldosterone and hypertension has been well-established and the finding in our study provides some biological support for some of the epidemiological observations that chronic noise exposure may be associated with hypertension. The group differences are not dramatic and should, therefore, be interpreted with caution. When it comes to interpreting changes (or lack thereof) among the systems assessed in the current study there should be consideration of the study design. There is uncertainty in how endogenous systems that are known to be sensitive to stressors will react in the absence of a stressor that has been removed after several days of exposure. In our case, sampling was conducted 24 h following the 21st day of noise exposure. The absence of a noise stressor during this time could have influenced the systems assessed in this study. This delay was introduced to avoid the possibility that endocrine changes could otherwise reflect a transient reaction to the last day of noise stress and not necessarily the chronic exposure. Future studies in this area may benefit by exposing a subgroup of the sham and noise exposed rats to a transient stressor prior to sampling.

Because our noise exposure paradigm did not provide rats with a reprieve from noise during the light cycle when they would be expected to sleep it is worth considering that some of the changes observed in the current study may be owing to disrupted sleep. Some studies, but not all have found that sleep disruption in rats leads to an increase in circulating and cerebral levels and expression, respectively, of IL-6, IL-1 and TNF-alpha. Although not objectively assessed, any sleep disruption that may have occurred in our study failed to produce any measured increases in these endpoints. On the other hand, noise exposed rats had lower body weights and most studies do report that sleep disruption leads to a reduction in body weight in rats. [51] see also review [52] Corticosterone concentrations have also been reported to increase with sleep deprivation. [53-55] CRP has not been assessed in rat models of sleep disturbance, but human studies suggest that elevated CRP levels are associated with several types of sleep disturbance. [56-58] Therefore, it is possible that the observed increase in corticosterone and decrease in weight among noise exposed rats could reflect a response to disrupted sleep. Taken together, our results show that in the SHR, 21 days of exposure to loud noise had no appreciable impact on adrenal weights, ACTH levels, IL-1 or IL-6. However, noise exposure was associated with lower body weight and modest increases in both corticosterone and aldosterone. The magnitude of increase may be owing to the timing of assessment in not only the diurnal variation, but may be reflective of a recovery following the 24 h period since the last noise exposure. The modest, at best, responses that we observed may be particular to the SHR animals; perhaps they already show an activated HPA axis, if compared to normotensive rats, with further stress stimulation not able to elicit an additional response. However, normotensive rats exposed to a similar paradigm showed very little changes in comparable measures. [6] Until more research works come out to disentangle some of these possibilities, our results need to be considered tentative and interpreted with caution. Our finding of lower plasma TNF-alpha is consistent with the effects that stressor exposure can have on this endpoint, but further research is required to determine the basis for reduced CRP levels among the noise-exposed animals. Additional research looking at multiple time points and different molecular markers throughout the system, including in key regions of the brain, may help elucidate the significance that this may have on the overall response to stressors in general and noise stress in particular among the SHR.

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Conflicts of interest
There are no conflicts of interest.

References
1. Babisch W. The noise/stress concept, risk assessment and research needs. Noise Health 2002;4:1-11. pmid: 12537836.
2. Babisch W. Stress hormones in the research on cardiovascular effects of noise. Noise Health 2003;5:1-11. pmid: 12631430.
3. Babisch W, Kamp J. Exposure–response relationship of the association between aircraft noise and the risk of hypertension. Noise Health 2009;11:161-8. pmid: 19602770. doi: 10.4103/1463-1741.53363.
4. Black PH, Garbutt LD. Stress, inflammation and cardiovascular disease. J Psychosom Res 2002;52:1-23. pmid: 11801260.
5. Michaud DS, McLean J, Keith SE, Ferrarotto C, Hayley S, Khan SA, et al. Differential impact of audiogenic stressors on Lewis and Fischer rats: Behavioral, neurochemical, and endocrine variations. Neuropsychopharmacology 2003;28:1068-81. pmid: 12700709.
6. Michaud D, Miller S, Ferrarotto C, Keith S, Bowers W, Kumarathasan P, et al. Exposure to chronic noise and fractionated X-ray radiation elicits biochemical changes and disrupts body weight gain in rat. Int J Radiat Biol 2005;81:299-307. pmid: 16019939. doi: 10.1080/09553000500084795.
7. Campeau S, Dolan D, Akil H, Watson SJ. c-fos mRNA induction in acute and chronic audiogenic stress: possible role of the orbitofrontal cortex in habituation. Stress 2002;5:121-30. pmid: 12186690. doi: 10.1080/1025890290027895.
8. Anisman H, Merali Z. Cytokines, stress, and depressive illness. Brain Behav Immun 2002;16:513-24. pmid: 12401465.
9. Zhang T, Chen Y, Liu H, Zhou Z, Zhai Y, Yang J. Chronic unpredictable stress accelerates atherosclerosis through promoting inflammation in apolipoprotein E knockout mice. Thromb Res 2010;116:386-92. pmid: 20800268. doi: 10.1016/j.thromres.2010.07.022.
10. Hlavacova N, Jezova D. Chronic treatment with the mineralocorticoid hormone aldosterone results in increased anxiety-like behavior. Horm Behav 2008;54:90-7. pmid: 18377905. doi: 10.1016/j.yhbeh.2008.02.004.
11. Raff H, Bruder ED, Cullinan WE, Ziegler DR, Cohen EP. Effect of animal facility construction on basal hypothalamic-pituitary-adrenal and renin-aldosterone activity in the rat. Endocrinology 2011;152:1218-21. pmid: 21248141. doi: 10.1210/en.2010.1432.
12. Genest J. C-reactive protein: Risk factor, biomarker and/or therapeutic target? Can J Cardiol 2010;26(Suppl A):41A-4A. pmid: 20386760.
The genetics of coronary artery disease.

Curr Opin Cardiol 2012;27:221-7. doi: 10.1097/HCC.0b013e3283515b4b.

Behavior during acute stress in spontaneously hypertensive and Wistar-Kyoto normotensive rats. Life Sci 1978;22:997-1005. pmid: 642710.

Kaptoge S, Di AE, Lowe G, Pepys MB, Thompson SG, Collins R, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: An individual participant meta-analysis. Lancet 2010;375: 132-40. pmid: 20331199. doi: 10.1016/S0140-6736(09)61717-7.

Roberts R, Stewart AF. The genetics of coronary artery disease. Curr Opin Cardiol 2012;27:221-7. doi: 10.1097/HCC.0b013e3283515b4b.

McCarty R, Kopin IJ. Alterations in plasma catecholamines and behavior during acute stress in spontaneously hypertensive and Wistar–Kyoto normotensive rats. Life Sci 1978;22:997-1005. pmid: 642710.

Gómez F, De Kloet ER, Armanio A, Glucocorticoid negative feedback on the HPA axis in five inbred rat strains. Am J Physiol 1998;274: R420-7. doi: 10.1152/ajpregu.000108.2007.

Djordjevic J, Cvijic G, Davidovic V. Different activation of ACTH and corticosterone release in response to various stressors in rats. Physiol Res 2003;52:67-72. pmid: 1265809.

Djordjevic J, Vuckovic T, Jasnic N, Cvijic G. Effect of various stressors on the blood ACTH and corticosterone concentration in normotensive Wistar and spontaneously hypertensive Wistar-Kyoto rats. Gen Comp Endocrinol 2007;153:217-20. pmid: 17383653. doi: 10.1016/j.ygene.2007.02.004.

Roman O, Seres J, Pometaova M, Jurcovicova J. Neuroendocrine or behavioral effects of acute or chronic emotional stress in Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Endocr Regul 2004;38:151-5. pmid: 1584794.

Herrmann HJ, Rohde HG, Schulze W, Eichhorn C, Luft FC. Effect of noise stress and ethanol intake on hearts of spontaneously hypertensive rats. Basic Res Cardiol 1994;89:510-23. pmid: 7702540.

Dickhout JG, Lee RM. Blood pressure and heart rate development in young spontaneously hypertensive rats. Am J Physiol 1998;274:H794-800. pmid: 9530190.

Lee G, Goosens KA. Sampling blood from the lateral tail vein of the rat. J Vis Exp 2015:e52766. doi: 10.3791/52766.

Konkle AT, Baker SL, Kentner AC, Barbagallo LS, Merali Z, Bialajew C. Evaluation of the effects of chronic mild stressors on hedonic and physiological responses: Sex and strain compared. Brain Res 2003;992:227-38. pmid: 14625061.

Worthman CM, Stallings JF. Hormone measures in finger-prick blood spot samples: New field methods for reproductive endocrinology. Am J Phys Anthropol 1997;104:1-21. pmid: 9331450.

SPSS Inc. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.

Rosenbrock H, Koros E, Bloching A, Podhorna J, Borsini F. Effect of angiotensin II on mRNA expression of angiotensin II receptors and its subtypes in rat vascular smooth muscle cells. Hypertension 1999;34:118-25. pmid: 10406834.

Lee DL, Leite R, Fleming C, Pollock JS, Webb RC, Brands MW. Hypertensive response to acute stress is attenuated in interleukin-6 knockout mice. Hypertension 2004;44:259-63. pmid: 15289466. doi: 10.1161/01.HYP.0000139913.56461.fb.
51. Everson CA, Szabo A. Recurrent restriction of sleep and inadequate recuperation induce both adaptive changes and pathological outcomes. Am J Physiol Regul Integr Comp Physiol 2009;297:R1430-40. pmid: 19692662. doi: 10.1152/ajpregu.00230.2009.

52. Rechtschaffen A, Bergmann BM. Sleep deprivation in the rat: An update of the 1989 paper. Sleep 2002;25:18-24. pmid: 11833856.

53. Dattilo M, Antunes HK, Medeiros A, Monico-Neto M, Souza HS, Lee KS, et al. Paradoxical sleep deprivation induces muscle atrophy. Muscle Nerve 2012;45:431-3. pmid: 22334180. doi: 10.1002/mus.22322.

54. Sgoifo A, Buwalda B, Roos M, Costoli T, Merati G, Meerlo P. Effects of sleep deprivation on cardiac autonomic and pituitary-adrenocortical stress reactivity in rats. Psychoneuroendocrinology 2006;31:197-208. pmid: 16154708. doi: 10.1016/j.psyneuen.2005.06.009.

55. Wu JL, Wu RS, Yang JG, Huang CC, Chen KB, Fang KH, et al. Effects of sleep deprivation on serum testosterone concentrations in the rat. Neurosci Lett 2011;494:124-9. pmid: 21376782. doi: 10.1016/j.neulet.2011.02.073.

56. Li F, Huang H, Song L, Hao H, Ying M. Effects of obstructive sleep apnea hypopnea syndrome on blood pressure and C-reactive protein in male hypertension patients. J Clin Med Res 2016;8:220-4. pmid: 26858795. doi: 10.14740/jocmr2409w.

57. Razeghi E, Sahraian MA, Heidari R, Bagherzadeh M. Association of inflammatory biomarkers with sleep disorders in hemodialysis patients. Acta Neurol Belg 2012;112:45-9. pmid: 22427289. doi: 10.1007/s13760-012-0003-7.

58. Tie YX, Fu YY, Xu Z, Peng Y. Relationship between C-reactive protein levels and obstructive sleep apnea syndrome. Genet Mol Res 2016, 15. pmid: 27323094. doi: 10.4238/gmr.15027808.