International Journal of Comparative Psychology, 2007, **20**, 134-144.

Effects of Noise on Rodent Physiology

Ann Linda Baldwin
University of Arizona, U.S.A.

Experiments are described in which Sprague Dawley rats were deliberately subjected to a daily 15-min white noise regime (90 dB) for 3 or 6 weeks, to determine its effects on the cardiovascular system and intestinal mucosa. In one set of experiments cardiovascular responses were monitored by radiotelemetry. Exposure to noise increased heart rate and mean arterial pressure and reduced stimulation of the parasympathetic nervous system. In the second set of experiments, one group of rats was exposed to the noise protocol for 3 weeks and a second group was not. All the rats were then anaesthetized and the small intestines of half the animals were fixed for microscopy. The remaining rats had their mesenteric microvasculature perfused for one minute with fluorescent albumin before fixing for microscopy. The rats exposed to noise showed significantly more eosinophils and degranulated mast cells in the intestinal villi than the quiet rats. In addition, the villi were swollen and the epithelial cells had widened junctions. The noise group also showed significantly more leakage of fluorescent albumin from the mesenteric microvessels. These experiments demonstrate that 90 dB white noise reduces stimulation the parasympathetic nervous system and also induces an inflammatory response in the intestinal mucosa, resulting in structural damage. These results are consistent with a stress response.

Several studies have shown that noise in animal care facilities can reach as high as 90 – 100 dB (Pfaff & Stecker, 1976; Milligan, Sales & Khirnykh, 1993). Such levels of noise can induce physiological and behavioral responses in laboratory rodents such as increased plasma corticosterone levels, reduction in body weight, decrease in gastric secretion, changes in immune response and tumor resistance, and a decrease in reproductive function. Behavioral responses include increases in total activity, grooming themselves and their cage-mates, and rearing onto their hind legs (Clough, 1982; Gamble, 1982; Sales, Wilson, Spencer, & Milligan, 1988; Milligan et al., 1993, Baldwin, Primeau, & Johnson, 2006). These changes are similar to those seen in rodents exposed to other stressful situations (Sharp, Azar, & Lawson, 2003). In spite of the evidence that noise levels in animal facilities are often high enough to produce uncontrolled physiological and psychological responses, the acoustic levels continue to be not as monitored as other environmental factors (lighting, temperature, humidity, etc).

Although noise has deleterious effects on rodent physiology, little is known about how the autonomic nervous system (ANS) is affected. Such information would indicate the state of emotional stress of the animals (Cerutti, Bianchi, & Mainardi, 1995). It is essential that the stress status of laboratory animals is monitored and controlled because stress may alter the experimental data obtained from those animals (Poole, 1997). One way of recording changes in the ANS is to measure the beat-to-beat changes in heart rate (i.e. heart rate variability, HRV). The variability is due to the changes in the activity of the sympathetic and

Correspondence concerning this article should be addressed to Ann Baldwin, Department of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724-5051, U.S.A. (abaldwin@u.arizona.edu).

parasympathetic nerves of the ANS, resulting in an alteration of sympathovagal balance. Acute social and psychological stressors affect the ANS by increasing sympathetic activation and decreasing parasympathetic activation, and these actions are reflected in changes in HRV. This article describes experiments in which groups of rats were exposed daily to a 15-min white noise regime (90 dB) for three weeks, to determine the effects of noise on the ANS (Burwell & Baldwin, 2006). Since stress responses can exert their influence by affecting ANS and endocrine output to the viscera (Mayer, Naliboff,, & Chang, 2001) further studies are described (Baldwin et al., 2006; Baldwin & Bell, 2007), in which a similar noise protocol was used to determine effects of noise on the integrity of the intestinal mucosa and mesenteric microvessels. The experimental methods are fully described in the publications cited above and just are outlined briefly here.

Method

Effects of Noise on ANS

Animals. Six male Sprague Dawley rats weighing 375 – 400 g were obtained from Charles River Laboratories (Portage, MI). Three of the rats were implanted at Charles River with PhysioTel® C50-PXT telemetry transmitters (Data Sciences International (DSI), St. Paul, MN), allowed to recover and shipped to Tucson, AZ. Upon arrival, each implanted rat was pair-housed with a non-implanted rat. No data were collected from the non-implanted rats; they served only as cage-mates for the implanted rats. Lights were on from 06:00 until 18:00. All research procedures and animal care were reviewed and overseen by the University of Arizona's institutional animal care and use committee (IACUC).

Experimental Protocol. The same animals were used throughout the experiments and were subjected to 3 or 6 weeks of daily noise, separated by 3 weeks of quiet time. The white noise stimulus consisted of a combination of frequencies from 10 Hz to 10 kHz that were electronically generated and recorded onto a CD in a 15-minute segment played between 8:00 and 8:15 each morning. The total SPL of the white noise in the animal room was 90 dB as compared with the background noise of 50 dB. On three mornings per week, telemetry data were collected before (07:50 – 08:00), during (08:00 – 08:15) and after (08:15 – 08:25) delivery of the noise. During quiet (control) periods, no stimulus was delivered and telemetry data were collected for 15 minutes sometime between 07:50 and 08:25. For three nights per week, when the rats were in their active phase, during noise experiments and quiet periods, telemetry data were collected for 15 minutes sometime between 20:00 and 21:00. Three distinct frequency ranges were identified in the power spectrum of the data: very low frequency (VLF, 0.05 – 0.25 Hz), low frequency (LF, 0.25 – 1.00 Hz), and high frequency (HF, 1.00 – 3.00 Hz). Spectral analysis of HRV in times of emotional stress shows an increase in LF power, a decrease in HF power, and an increase in the LF/HF ratio.

Statistical Analysis. Data were compared under different conditions, within the same animal and during the same observation period, using the paired Student t-test, with $p < 0.05$ considered to be statistically significant, after checking that the data passed the tests for normality and equal variance. All data are presented as mean ± standard error of the mean (SEM).

Effects of Noise on Intestinal Mucosa and Microvascular Leakage

Animals. Male Sprague Dawley rats were housed in pairs in cages as described previously (Burwell & Baldwin, 2006) in two separate identical rooms. The one intentional difference between the environments in the two rooms was that the rats in one of the rooms received a white noise stimulus (90 dB) for 15 minutes each day at the same time every day, for 3 weeks, just before the lights were switched off at 18:00. These rats are referred to as 'noise' rats. The rats in the other room

('quiet' rats) did not receive the white noise stimulus. Both rooms were chosen so that they were remote from noise-producing equipment, such as cage washers. Apart from the investigator, the animal care technician was the only person who entered the rooms. Background noise in these rooms did not exceed 50 dB. A third group of rats were housed in the 'noise' room for 3 weeks and then moved to the 'quiet' room for a further 3 weeks to determine whether noise-induced effects on the intestinal mucosa could be reversed. These rats are referred to as 'recovery' rats.

Experimental Protocol. After three weeks the animals were anesthetized for surgery (Baldwin, Primeau, & Johnson, 2006). Half of the animals from each room had their intestinal ileum prepared for light and electron microscopy in order to evaluate degranulation of mucosal mast cells, migration of eosinophils from the blood into the lamina propria, mean width of villus lamina propria and integrity of the mucosal epithelium (8 rats per group). To prepare the ileum for microscopy, the portal vein was incised for use as a flow outlet and the intestinal microvasculature was perfused at physiological pressure with physiologically-buffered Karnovsky fixative. After one hour, an 8 cm segment from the ileum was excised and fixed for one more hour. The segment was then divided into 4 portions that were incubated in 2% diaminobenzidine, post-fixed in osmium tetroxide, dehydrated and embedded in Spurrs' resin. The tissue was thick-sectioned for light microscopy and stained with 1% toluidine blue; it was also thin-sectioned for electron microscopy and stained with uranyl acetate and lead citrate. Thick sections were observed using an Axioplan microscope (Zeiss, Germany) equipped with 20x (numerical aperture 0.6) and 40x (numerical aperture 0.75; water immersion) Zeiss objectives. Thin sections were observed for electron microscopy using a model CM12 Phillips electron microscope (FEI Company, Tacoma WA).

In later experiments the presence of reactive oxygen species (ROS) was monitored in 'noise' and in 'quiet' rats by exposing a small segment of mucosa and suffusing it with dihydrorhodamine (DHR) 123 under epi-fluorescence microscopy. Niu et al. (1996) have shown that superoxide can be detected in the tissue using DHR which only fluoresces when in contact with ROS, specifically hydrogen peroxide-derived oxidants, and intra-vital digital micro-fluorography allows for quantification of oxidant production.

For the remaining animals (6 rats per group) the superior mesenteric artery was cannulated, the animals euthanized (Baldwin & Bell, 2007) and the mesenteric microcirculation was perfused for one minute with fluorescent albumin followed by fixative. The mesenteric tissue was then observed under epi-fluorescence microscopy to determine the mean number and area of leakage spots of fluorescent albumin per unit length of venule. In later experiments some of these rats were fed a special diet with increased concentrations of the antioxidants, vitamin E (10,000 IU/kg diet) and α-lipoic acid (1.65g/kg diet).

Statistical Analysis. For each parameter the Kruskal-Wallis test was applied for comparing different animals within the same group, and the Mann-Whitney Rank Sum test for comparing pairs of groups. The n was taken as the number of rats in a group and a p-value < 0.05 indicated significance.

Results

Effects of Noise on ANS

In response to white noise all 3 rats showed significant increases in HR and MAP (8% and 15%, respectively), compared to before the stimulus, and these parameters stayed elevated during the 10 minutes after the stimulus. No consistent or significant patterns were observed regarding the sympathetic nervous system (power of the LF range) in any of the rats in response to the white noise. However, an attenuation (12-13%) of the parasympathetic nervous system (power of the HF range) during and/or after the white noise was observed in all rats. Corresponding shifts in the sympathovagal balance (LF/HF ratio) were also observed during and after the white noise compared to before the stimulus. The increases in the LF/HF

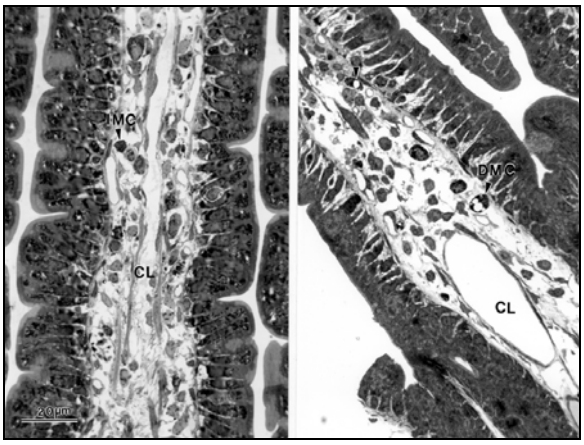
ratio were often small because the sympathetic nervous system remained relatively unchanged as the parasympathetic nervous system was attenuated.

Effects of Noise Stress on the Structure of the Intestinal Mucosa

Overall Appearance. Upon visual inspection, the small intestine of the 'noise' rats was noticeably more swollen and inflamed (hyperaemic) than seen in the 'quiet' rats. In addition, the Peyers' patches along the whole length of the jejunum and ileum were more swollen, suggesting increased activation of the immune system.

Light Microscopy. Longitudinally cut thick sections of parts of villi from a 'quiet' rat and a 'noise' rat are shown in Figures 1a and 1b, respectively. An intact mast cell (IMC), identified by its stained granules, in the lamina propria and adjacent to the central lacteal (CL) can be seen in Figure 1a. Degranulated mast cells (DMC) in the lamina propria can be seen in Figure 1b. There were significantly more degranulated mast cells per villus cross-section in the 10 villi closest to each edge of each Peyers' patch examined in 'noise' rats than in 'quiet' rats (3.95 ± 0.80 (SEM), 60 villi versus 0.35 ± 0.29, 80 villi). The Kruskal-Wallis test demonstrated that there was much greater variance between groups ($p<0.001$) than within groups ($p = 0.06$). 'Recovery' rats did not show a significant reduction in the number of degranulated mast cells, compared to the 'noise' rats (2.37 ± 0.83, 115). Similar results with degranulated mast cells were obtained when the 'noise' and 'quiet' rooms were reversed. Villi near Peyers' patches showed 2.77 ± 0.72 and 0.39 ± 0.48 for 'noise' rats and 'quiet' rats respectively. A one-way blocked ANOVA test demonstrated a significant difference between 'noise' and 'quiet' groups, but not between rooms, *per se* indicating that the data were not confounded by intrinsic differences between the rooms themselves. In villi near Peyers' patches significantly more eosinophils per villus section could be seen in the lamina propria of 'noise' rats than of "quiet" rats (9.46 ± 0.44, 60 villi versus 4.58 ± 0.38, 60 villi.)

Overall, the intestinal villi from 'noise' rats were significantly more edematous than those from 'quiet' rats, as assessed by measurements of villus lamina propria width using light microscopy. The mean villus widths of the 'noise', 'quiet' and 'recovery' groups were 57.0 ± 0.9, 39.0 ± 0.7 and 59.0 ± 0.7 μm, respectively (4 animals/group, 40 villi /animal). The distended central lymphatic vessels in villi from 'noise' rats (compare CL in Figures 1a and 1b) and the greater area of cell-free tissue indicate that the increased width of the villus lamina propria was produced by edema, rather than by increased cell growth. The villi of the 'recovery' group were just as edematous as those from the 'noise' group, consistent with the finding that the number of degranulated mast cells also remained high in this group.



a. **b.**

Figure 1. Light micrographs of longitudinally cut thick sections of parts of villi from a "quiet" rat (a) and a "noise" rat (b). The sections were stained with toluidine blue. See enlarged central lacteal (CL) in (B). Scale bar: 20 μm.

Electron Microscopy. Representative photomicrographs of the mucosal epithelium from the three groups of rats are shown in Figures 2a-c. Figure 2a demonstrates that in 'quiet' rats, the epithelial cells (E) were generally attached to each other and to the basement membrane. Very few eosinophils were evident. 'Noise' room rats, on the other hand, (Figure 2b), usually demonstrated large numbers of epithelial cells that were separating from each other and, in places, were separated from the basement membrane. Epithelial cells were considered to be separated from each other if a distinct gap could be seen between adjacent cells which extended in length from the basement membrane to the top of the cell nuclei (nearest the epithelial surface microvilli). Epithelial cells were considered to be separated from the basement if a gap appeared between the main body of the cell and the remnants of the cell adhering to the basement membrane.

Many intestinal villi contained eosinophils (EO) and partially degranulated mast cells (MC). In figure 2b an inter-epithelial leukocyte (IEL) and capillary (C) are also visible. Three weeks in the quiet room, following 3 weeks in the noise room, produced some epithelial repair (Figure 2c). Although the epithelial cells were still somewhat separated from each other, and extended long, tenuous cytoplasmic projections from their junctional aspects, the cells were rarely separated from the basement membrane.

Presence of Reactive Oxygen Species in Intestinal Mucosa

Significantly more intense DHR fluorescence was seen in the villus epithelium of 'noise' rats (58 ± 10 (SD), arbitrary units, 9 rats, 93 villi), compared to 'quiet' rats (35 ± 13, 3 rats, 55 villi), and fluorescent granules appeared in the lamina propria of 'noise' rats. These results imply that the noise-induced mucosal damage was oxidative in nature

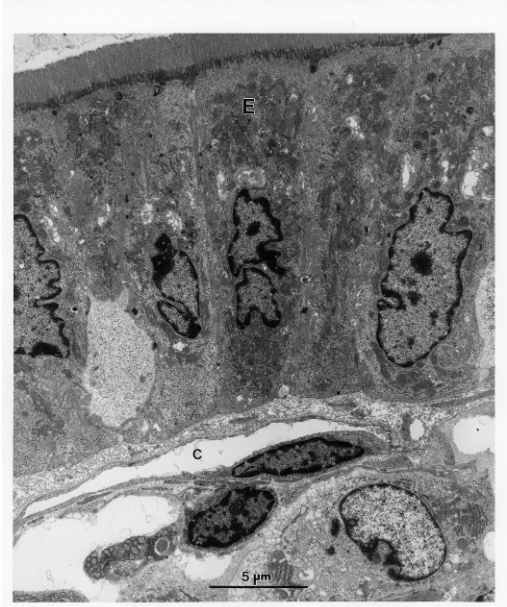


Figure 2a. Demonstrates that in "quiet" rats, the epithelial cells (E) were generally attached to each other and to the basement membrane. Very few eosinophils were evident.

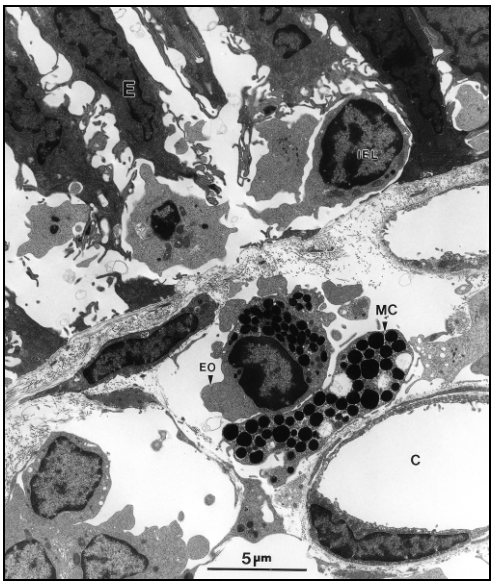


Figure 2b. "Noise" room rats usually demonstrated large numbers of epithelial cells that were separating from each other and, in places, were separated from the basement. Many intestinal villi contained eosinophils (EO) and partially degranulated mast cells (MC). In this figure an interepithelial leukocyte (IEL) and capillary (C) are also visible.

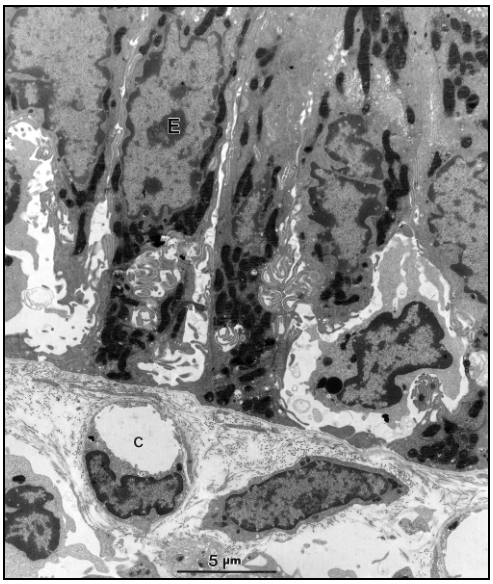


Figure 2c. Shows that three weeks in the quiet room, after 3 weeks in the noise room, resulted in some epithelial repair. Scale bars: 5 µm.

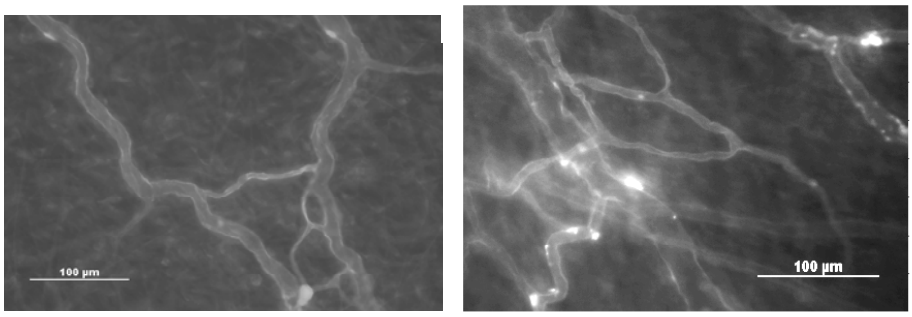
Effects of Noise Stress on Microvascular Leakage

Rats from the noise group (n=9) demonstrated significantly more leakage sites (3.84 ± 0.46 (SEM) x 10^{-3} μ^{-1}, n=95 venules) and a significantly greater leakage area per length of venule (3.20 ± 0.49 μ^2/μ), than rats from the quiet group (n=10) (1.38 ± 0.26 (SEM) x 10^{-3} $\mu^{-1}$and 0.30 ± 0.06 μ^2/μ, respectively, n=123 venules) or the recovery group (n=6) (1.40 ± 0.24 (SEM) x 10^{-3} μ^{-1} and 0.63 ± 0.16 μ^2/μ, respectively, n=108 venules). Rats from the recovery and quiet groups showed similar numbers of leaks per length of venule, but the recovery group demonstrated significantly greater leak area per venule length than the quiet group, although still significantly less than for the noise group . The percentages of venules observed that contained leaks in the noise, quiet and recovery groups were 73%, 37% and 39%, respectively. Light micrographs of typical microvascular networks from a quiet group rat and a noise group rat, after perfusion with FITC-albumin, are shown in figures 3a and 3b. Extensive fluorescent leaks are visible in the network from the noise group rat but few leaks can be seen in the network from the quiet group rat.

Mast Cell Degranulation

The mean number of degranulated mast cells per microscopic field of view (1.13 mm^2) was significantly greater for the noise group (13.75 ± 0.77) and the recovery group (12.09 ± 0.90) than for the quiet group (7.43 ± 0.36). These results

indicate that daily noise markedly increases microvascular permeability in rats, and that this change may be stimulated by mast cell degranulation.



a. **b**.

Figure 3. Light micrographs of mesenteric microvascular networks after perfusion with fluorescent (FITC)-labeled albumin. (a) Network from an animal that had not been exposed to daily noise. No leaks can be seen. (b) Network from an animal that had been exposed to daily noise. Many leaks of FITC-albumin from the venules are visible. Scale bars: 100 µm.

Antioxidants and Microvascular Leaks

Vitamin E with α-lipoic acid significantly reduced noise-induced venular leakage to fluorescent albumin although not to control levels. The quiet control animals (n=6) had a mean number of leaks per micron length of venule of 0.44 ± 0.06 (SEM) x 10^{-2} μ^{-1}, (n= 341 venules), compared to 3.05 ± 0.32 (SEM) x 10^{-2} μ^{-1}, (n= 294 venules, n=6 rats) for noise alone, and 1.04 ± 0.19 (SEM) x 10^{-2} μ^{-1}, (n= 304 venules, n=6 rats), for noise and vitamin E with α-lipoic acid. The results for leak area per micron length of venule were similar, corresponding values being 0.44 ± 0.10 (SEM) $\mu^2\mu^{-1}$, 6.60 ± 0.88 (SEM), 1.90 ± 0.51(SEM) and 2.33 ± 0.29 (SEM). Thus leak number was significantly reduced by about 66% with vitamin E and α-lipoic acid. Leak area was reduced even more, by 70% with vitamin E and α-lipoic acid.

Discussion

Exposure of rats to 90 dB white noise every day increases both HR and MAP when recorded during, and immediately after, the noise. It could be argued that the increases in HR and MAP produced by noise could have been caused by increased activity rather than by a stress response. However, that is unlikely in these experiments because apart from a startle response, lasting a second or so on the first day of the noise, very little activity was observed at this time. Thus the increased cardiovascular parameters were caused by a stress response. In this study we show that a decrease in the activation of the parasympathetic nervous system is responsible for the cardiovascular response, rather than an increased activity of the sympathetic autonomic branch. This effect is not surprising because the

parasympathetic branch is dominant when animals are asleep, as was the case when the rats were exposed to the noise. The elevations of HR and MAP seen during the daily exposure to white noise are consistent with data obtained by other investigators from rodents exposed to stressful situations, such as handling, restraint, cage-changes and injections (Sales, 1972; Kramer et al., 1993; Kramer et al., 2000; Sharp, Zammit, Azar, & Lawson, 2002; Sharp et al., 2003).

It might be argued that since the cardiovascular effects of noise only resulted in small increases in HR and BP (about 10-15% of initial values) that noise would not be a major confounding factor in rodent experiments. However, the stimuli used in these studies were only delivered once a day, at the same time every day and for short duration, unlike the audible sounds that routinely occur in animal facilities. As reported by other authors, noise levels peak many times during the day in an animal facility and contain a wide range of frequencies (Pfaff & Stecker, 1976; Sales et al., 1988; Milligan et al., 1993). Because noise levels in animal facilities tend to be poorly controlled, the cardiovascular state of the animals may also be poorly controlled and unpredictable. Although stress does not always compromise health and welfare, and in fact the stress response is necessary for survival in the wild, stress always disturbs the body's homeostasis and imposes a cost to the body, particularly when it is elicited repeatedly. This cost arises if stress-induced mediators, such as adrenal hormones, neurotransmitters, cytokines etc., are released too often.

Not only does exposure to 90 dB white noise alter cardiovascular parameters in rats; the small intestine and mesenteric microvessels become inflamed. It is not clear whether this response is mediated via the hypothalamic-pituitary-adrenal axis because accurate measures of plasma corticosterone concentrations before and during the noise could not be obtained without causing further stress to the animals. Windle et al. (1998) found that plasma corticosterone concentrations in rats varied periodically throughout the day but increased significantly in response to 114 dB noise for 10 min., if the onset of the noise coincided with the rising phase of a basal corticosterone pulse. This result suggests that the intestinal responses observed in the present study in response to noise may have been a stress response that was mediated via the hypothalamic-pituitary-adrenal axis.

The intestinal damage appeared to be oxidative in nature. Activated phagocytes, such as neutrophils, eosinophils and macrophages, are the best-recognized sources of free radicals and the intestinal mucosa of rats exposed to noise showed significantly larger numbers of eosinophils in the villi lamina propria compared to 'quiet' rats. These eosinophils were probably recruited by the presence of degranulated mast cells. Activated mast cells can release interleukin-5 (IL-5) that attracts eosinophils (28). In fact our electron micrographs often demonstrated eosinophils and degranulated mast cells in close juxtaposition (Figure 2b). The ROS and other products released by eosinophils may be partly responsible for the epithelial disruption observed near the Peyers' patches of 'noise' rats.

In summary, exposure of rodents to chronic noise appears to induce a

stress response, as demonstrated by behavioral changes and increases in HR and MAP, that is accompanied by intestinal and microvascular inflammation, possibly triggered by increased activation of the immune system.

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