Do Audible and Ultrasonic Sounds of Intensities Common in Animal Facilities Affect the Autonomic Nervous System of Rodents?

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Do Audible and Ultrasonic Sounds of Intensities Common in Animal Facilities Affect the Autonomic Nervous System of Rodents?

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In animal facilities, noises, often poorly controlled, occur over a wide range of frequencies and intensities. Evidence demonstrates that audible noise and ultrasound have deleterious effects on rodent physiology, but it is not known how they affect the autonomic nervous system (ANS). This study exposed 3 unrestrained, male, Sprague–Dawley rats daily to a 15-min white noise regime (90 dB), a quiet regime, or a 15-min ultrasound regime (90 dB at 4 frequencies in the range 20 to 40 kHz)—each for several weeks—and used radiotelemetry to monitor their cardiovascular responses. Exposure to audible noise increased heart rate and mean arterial pressure. Spectral analysis of HR variability showed diminished stimulation of the parasympathetic nervous system, shifting the sympathovagal balance. However, ultrasound, at the frequencies used, did not reproducibly affect cardiovascular parameters. The preliminary data obtained from this study indicate that audible noise, but not ultrasound (delivered using the same protocol), affects the ANS. Because the cardiovascular, respiratory, renal, and gastrointestinal systems are under autonomic control, such noise could have wide-ranging effects on animal physiology.
Noise in animal care facilities usually arises from five sources: (a) electronics (video monitors, oscilloscopes, and TV cameras), (b) cleaning machines (cage washers, floor scrubbers, vacuum hoses, and bottle washers), (c) building ventilation and air conditioning systems, (d) humans working in the facility, and (e) the animals. During periods of high personnel activity, noise levels in animal facilities can reach as high as 90 dB to 100 dB (Milligan, Sales, & Khirnykh, 1993; Pfaff & Stecker, 1976). Many of these high-intensity sounds are above the range of human hearing (greater than 20 kHz) and go unnoticed by animal care personnel (Pfaff & Stecker, 1976; Sales, Wilson, Spencer, & Milligan, 1988). Although humans may not hear these high-frequency sounds, rodents use ultrasound to communicate and are therefore highly sensitive to sounds in the ultrasonic range (Gourevitch & Hack, 1966; Sales, 1972; Sales et al., 1988; Sewell, 1967, 1970). Potential sources of ultrasound in animal care facilities include cage washers, hoses, running taps, squeaky doors, ringing telephones, and computer monitors (Sales et al., 1988).

Many animals in the laboratory are known to be sensitive to sounds (ultrasounds) beyond the nominal upper limit (20 kHz) of the human hearing range. In a previous study (Sales et al., 1988), sources of sound in laboratories and animal houses were examined to determine the extent of ambient ultrasound. Of 39 sources monitored, 24 were found to emit ultrasonic sounds. Many of these (cage washers and hoses) also produced sound in the audible range. Running taps, squeaky chairs, and rotating glass stoppers created particularly high sound pressure levels (SPLs) and contained frequencies to over 100 kHz. The oscilloscopes and visual display units investigated provided particular cause for concern, as they emitted sounds that were ultrasonic and therefore were apparently silent. Ambient ultrasound therefore appears to be common in laboratories and animal houses. Its effect on laboratory animals should be investigated and guidelines on acceptable levels should be formulated.

Despite the evidence that high-intensity audible and ultrasonic sounds are present over a wide frequency range in animal facilities, the acoustic levels continue to be less controlled than other environmental factors (lighting, temperature, and humidity). Surprisingly, a small pilot survey conducted by one of the authors (Jain & Baldwin, 2003) indicated that the majority of respondents considered their facilities quiet or very quiet, even though almost half of them identified three to five noise sources in their facilities. Multiple studies have shown that fairly high levels (90 to 110 dB) of low-frequency noise (audible to humans) can induce physiological and behavioral responses in laboratory animals. In rodents, such physiological responses include the following:

1. Peripheral vasoconstriction, changes in blood pressure (BP) and heart rate (HR).
2. Increased plasma corticosterone levels.
3. Elevated levels of cholesterol.
4. Increased atherosclerosis.
6. Decrease in gastric secretion.
8. A decrease in reproductive function (Bao, Metreveli, & Fletcher, 1999; Borg, 1978; Borg & Moller, 1978; Cheung, Hachinski, & Cechetto, 1997; Clough, 1982; Gamble, 1982; Milligan et al., 1993; Sales et al., 1988).

It has also been found that daily exposure to a short period of 90-dB white noise causes inflammation and epithelial cell disruption in the intestinal mucosa as well as an increase in microvascular permeability (Baldwin, Primeau, & Johnson, 2006; Baldwin & Wilson, 1999). Similar responses are found in rodents exposed to the sounds of routine personnel activity in a large animal facility (Wilson & Baldwin, 1998, 1999).

Behavioral responses to audible sound in laboratory rodents include audiogenic seizures, changes in eating and drinking patterns, and increases in total activity. It also includes grooming themselves and their cagemates and rearing onto their hind legs (Baldwin et al., 2006; Clough, 1982; Gamble, 1982; Krebs, Weyers, Macht, Weijers, & Janke, 1997; Milligan et al., 1993; Sales et al., 1988). These changes are similar to those seen in rodents exposed to other stressful situations (Duke, Sammit, & Lawson, 2001; Moyaho & Valencia, 2002; Sharp, Azar, & Lawson, 2003; Sharp, Zammit, Azar, & Lawson, 2002).

Because humans cannot hear ultrasound, few studies have been performed to demonstrate the effects of ultrasound on rodent physiology. Sales et al. (1988) suggested that exposure to ultrasound can have consequences similar to those observed during exposure to audible sound. In one study, diuresis and increased excretion of sodium were reported in rats exposed to short periods of 100-dB ultrasound (Lockett, 1970). Behavioral effects of exposure to ultrasound in rodents, including audiogenic convulsions, increased rearing behavior, and changes in total activity levels have been documented in two studies (Clough, 1982; Sales et al., 1988). Although noise and perhaps ultrasound appear to have deleterious effects on rodent physiology, it is not known how these stimuli affect the autonomic nervous system (ANS). Monitoring the effects of noise and ultrasound on the ANS is important because it would provide information concerning the state of emotional stress of the animals (Cerutti, Bianchi, & Mainardi, 1995; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). 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 HR (HR variability [HRV]). The variability is due to the changes in the activity of the sympathetic and parasympa-
thetic 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.

Many researchers have used radiotelemetry systems to investigate the cardiovascular effects of routine procedures (restraint, handling, weighing, cage changing, and subcutaneous and tail-vein injections) on laboratory rodents (Duke et al., 2001; Kramer et al., 1993; Kramer et al., 2000; Sharp et al., 2003; Sharp et al., 2002). This process involves the use of a radio transmitter, implanted in the abdomen of the rat, that produces a signal from which HR, BP, and HRV can be obtained. The primary advantage of using a telemetry system is that it allows investigators to measure physiological data in conscious, unrestrained, laboratory animals and thus provides a more efficient, accurate, and humane alternative to other techniques in which the animals are sedated or tethered. Although telemetry has shown that audible noise increases HR in rodents (Schlatter & Zbinden, 1982), no measurements of HRV were performed, and the effects of ultrasound were not addressed. In this study, experiments were performed using telemetry to test the hypothesis that noise and ultrasound at intensities often present in animal facilities (Milligan et al., 1993; Pfaff & Stecker, 1976; Sales et al., 1988) will affect the ANS of rodents, producing an increase in HR, mean arterial pressure (MAP), and in the ratio of sympathetic to parasympathetic stimulation. Because no other studies of the effects of ultrasound on cardiovascular parameters in rodents have been published, we started with the simplest possible protocol with the minimum number of variables; that is, continuous delivery of ultrasound for a defined period. Audible noise was delivered similarly for comparison. Three conscious, unrestrained, male Sprague–Dawley rats were subjected daily to a 15-min white noise regime (90 dB), a quiet regime, or a 15-min ultrasound regime (90 dB at four distinct frequencies [20 to 40 kHz]), each for periods ranging from 1 to 6 weeks. The 6-week period was used to determine whether the rats would acclimatize to the stimulus.

**METHOD**

**Animals**

Six male Sprague–Dawley rats weighing 375 g to 400 g were obtained from Charles River Laboratories (Portage, MI). Three of the rats were implanted with PhysioTel® C50–PXT telemetry transmitters (Data Sciences International [DSI], St. Paul, MN). After giving the implanted animals 8 days to recover from surgery, all 6 animals were shipped to Tucson, AZ. On arrival, each implanted rat was pair-housed with a nonimplanted rat in wire mesh cages (16 in. × 12 in. × 12 in.) with plastic bottoms. No data were collected from the 3 rats who were
not implanted with telemetry transmitters; they served only as cagemates for the implanted rats. Each cage contained a ramp leading to a wire mesh shelf (16 in. × 4 in.) and a piece of polyvinylchloride (PVC) tubing (length = 8 in., diameter = 4.5 in.) for enrichment. The rat diet consisted of Harlan Teklad 7001 rat chow (Harlan Teklad, Madison, WI) and water that was deionized and chlorinated to 10 parts per million. Fresh food and water were available ad libitum. The same investigator performed all measurements throughout the complete experimental procedure, and the only other person who entered the room was the animal caretaker, who was instructed to perform his duties gently and quietly. All research procedures and animal care were reviewed and overseen by the University of Arizona’s institutional animal care and use committee.

Transmitter Implantation

All surgical procedures were performed at Charles River Laboratories (Portage, MI). Briefly, the animals were anesthetized using a combination of Ketamine and Xylazine. A pressure catheter was inserted into the abdominal aorta in a direction opposing blood flow and was fixed in place using tissue adhesive. The body of the transmitter was sutured in place in the abdominal cavity, and two electrocardiogram (ECG) leads were sutured subcutaneously over the chest muscles in a Lead II position (Data Sciences International, 2001). The incision was then carefully closed, and the animals were allowed to recover. The rats were provided with an analgesic (Buprenex) for 8 days to prevent the onset of pain.

Experimental Protocol

A timeline of the conducted experiments is shown in Figure 1. The same animals were used in all quiet and noise (white noise or ultrasound) experiments; thus, the animals served as their own experimental controls. During periods of daily noise delivery, while the animals were in their inactive phase, they were exposed to the stimulus (either white noise or ultrasound) from 8:00 to 8:15 every morning. On these mornings, telemetry data were collected before (7:50 to 8:00), during (8:00 to 8:15), and after (8:15 to 8:25) delivery of the stimulus. During quiet (control) periods, no stimulus was delivered, and telemetry data were collected for 15 min sometime between 7:50 and 8:25 a.m. At night, when the rats were in their active phase during noise or ultrasound experiments and quiet periods, telemetry data were collected for 15 min sometime between 8:00 and 9:00 p.m. Inactive and active phase data were recorded on the same day on the same animals.

Because this was a preliminary study, we made an estimate at the beginning of the study of the optimal frequency of data collection and sampling schedule. After performing the first phase of the study (Quiet 1, Noise 1, Quiet 2), we discovered
that a 10-sec sampling period was inadequate for analysis of HRV, so we increased it to 30 sec. For that reason, there are no HRV data for the first phase of the study.

During the quiet period (Quiet 2) between the 3-week noise study (Noise 1) and the 3-week ultrasound study (Ultrasound 1), the animals had to be moved to a different facility. The rats were given 1 week to adjust to the new facility before the ultrasound study was started. After the move, we decided to increase the frequency of data collection to gain a more accurate assessment of the status of the animals. This change was reflected in the data analysis in the \( n \) values. Later analysis showed that the mean resting HR and MAP (before morning stimulus) were higher in 2 of the rats during the Ultrasound 1 study (after the move) than during the Noise 1 study (Figures 2 to 7). For this reason, the rats were exposed to an additional week of daily ultrasound stimulus (Ultrasound 2) 13 weeks after completion of the 6-week noise (Noise 2) study.

The specific protocols for each experiment are shown in Table 1. Telemetric data were acquired using a radiotelemetry system from DSI (St. Paul, MN). The system consisted of (a) three implantable transmitters (C50–PXT), (b) three receivers (RPC–1), (c) an ambient pressure reference (APR–1), (d) a data exchange matrix, and (e) a computer (Dell Optiplex GX270). The transmitters were switched on and off by passing a small magnet over the abdomen of each implanted animal and listening to the emitted tones with an AM radio. Transmitters were switched on and off each time data were collected to preserve battery life; handling of the animals was not required. During data collection, a receiver was placed underneath or along the back wall of each cage. Data were transferred from the transmitters to the receivers using radio waves at a frequency of 455 kHz. The analog signal was transmitted from the receivers to the data exchange matrix, where it was digitized and transferred to the computer.

HR and BP data were acquired using Dataquest A.R.T. 3.0 Acquisition software (DSI, St. Paul, MN). The specific sampling schedules for each experiment
are shown in Table 1. Sampling frequencies remained constant throughout all experiments at 1,000 Hz for ECG data and 500 Hz for BP data.

Generation of Stimuli

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-min segment. The CD was played in a continuous loop on a CD player that was attached to one standard stereo speaker and was delivered only between 8:00 and 8:15 every morning. The total SPL of the white noise in the animal room was 90 dB. The ultrasound stimulus was generated by a device containing a piezoelec-
The resonator produced a frequency of 40 kHz, and the oscillators generated the remaining frequencies—fifth-octave intervals between the frequencies of 20 and 40 kHz. The ultrasound stimulus contained frequencies of 23.0, 26.4, 30.3, 34.8, and 40.0 kHz and also had a total SPL of 90 dB. These frequencies and intensities are similar to those that have been recorded 1 m from sources normally present in animal facilities (Sales et al., 1988). Delivery of the ultrasound was controlled using a timer that activated the speakers only between 8:00 and 8:15 every morning. Noise and ultrasonic systems were obtained from ELC Audio Engineering (Prescott, AZ).
Measurement of Ambient and Experimental Noise and Ultrasound

Before arrival of the animals, ambient and experimental noise pressure levels in the animal room were measured using a calibrated Bruel and Kjaer Type 4133 microphone, Type 4230 sound level calibrator, and Krohn-Hite Corp. Model 3202 variable filter. This equipment enables the measurement of SPLs for frequencies from below 20 Hz to above 20 kHz. The microphone was mounted on a tripod to avoid effects (acoustical shadows) of people within the room and was rotated in both horizontal and vertical planes to give an average reading. The mi-

![Image](attachment:image_url)

**FIGURE 6** Changes in mean arterial pressure (MAP) in response to white noise and ultrasound (u/s) for Rat 474. *Significantly increased; # significantly decreased compared to the relevant “before” data. \( p < .05 \).

**FIGURE 7** Changes in mean arterial pressure in response to white noise and ultrasound for Rat 475. *Significantly increased. \( p < .05 \).
### TABLE 1
Experimental Protocol

<table>
<thead>
<tr>
<th></th>
<th>3 Weeks of White Noise 1</th>
<th>3 Weeks of Ultrasound 1</th>
<th>6 Weeks of White Noise 2</th>
<th>1 Week of Ultrasound 2</th>
</tr>
</thead>
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<tr>
<td><strong>Telemetry data</strong></td>
<td>2 mornings 2 nights</td>
<td>2 mornings 2 nights</td>
<td>3 mornings 3 nights</td>
<td>5 mornings 3 nights</td>
</tr>
<tr>
<td><strong>Behavioral data</strong></td>
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<td>3 mornings 3 nights</td>
<td>3 mornings 3 nights</td>
<td>3 mornings</td>
</tr>
<tr>
<td><strong>Sampling schedule</strong></td>
<td>10 sec each animal every 60 sec</td>
<td>30 sec each animal every 90 sec</td>
<td>35 sec each animal every 90 sec</td>
<td>35 sec each animal every 90 sec</td>
</tr>
<tr>
<td><strong>Light cycle (light:dark)</strong></td>
<td>12:12</td>
<td>14:10</td>
<td>12:12</td>
<td>12:12</td>
</tr>
</tbody>
</table>

*Light 6:00 a.m. to 6:00 p.m.*

*Light 7:00 a.m. to 9:00 p.m.*
Microphone orientation had little effect on the readings of ambient and experimental noise, suggesting a homogeneous sound field within the room. The total SPL of the ambient noise was ~50 dB.

Ambient and experimental ultrasound levels in the frequency range of 18 kHz to 50 kHz were measured using a Bruel and Kjaer 4939 calibrated microphone. The total SPL of the ambient noise was found to be of low amplitude (38 dB). The SPL of the ultrasound noise stimulus was measured by positioning the microphone within the cages at various points in the room where the rats would be located and was found to be 90 dB. A bat detector, positioned, in turn, within each cage before the rats arrived from Charles River, was also used as a second means to check that the ultrasonic source, when switched on, was delivering ultrasound that was easily detectable in the cages.

Behavioral Data Collection and Analysis

The rats’ behavior was videotaped in 2-min segments while telemetry data were being collected, according to the schedules shown in Table 1. Note that the light regime for the animals was set such that their active period was at night (Table 1). During periods of daily sound (white noise or ultrasound), each rat was taped in the morning for 2 min before, during, and after delivery of the noise stimulus. During quiet periods, each rat was taped in the morning for 2 min as telemetric data were being collected. At night (active period), whether the animals were exposed to noise during the day, each rat was taped for 2 min during collection of telemetric data.

The tapes were later analyzed, and the duration of inactive (sleeping, sitting still) and active (eating, drinking, moving, climbing, rearing, grooming, and fighting with cagemate) behaviors were recorded. Percentages of time spent in all active behaviors (presented as total activity level) were calculated.

Heart Rate Variability Calculation

HRV was calculated using Dataquest A.R.T. 3.0 Analysis software (DSI, St. Paul, MN). The interbeat interval (IBI) was extracted from the BP waveform. Because it provides more accurate and reliable IBI data, the BP waveform was chosen over the ECG waveform for HRV analysis. IBI was defined as the time between the maximum +dP/dt of the BP waveform between diastole and systole of each cardiac cycle. The IBI data were carefully checked against the BP waveform for accuracy, and erroneous data points were deleted. Next, the IBI data were interpolated at a frequency of 50 Hz (0.02 sec). A power spectrum of the interpolated IBI data was obtained by performing a fast Fourier transform on the autocorrelation of the data.
Three distinct frequency ranges were identified in the power spectrum of the IBI data. The very low frequency (VLF) range was defined as 0.05 Hz to 0.25 Hz; the low frequency (LF) range was defined as 0.25 Hz to 1.00 Hz; and the high frequency (HF) range was defined as 1.00 Hz to 3.00 Hz. Because no physiological significance has been identified for the VLF range, only the LF, HF, and LF/HF values are reported as markers of sympathetic activity, parasympathetic activity, and sympathovagal balance, respectively (Cerutti et al., 1995; Rubini, Porta, Baselli, Cerutti, & Paro, 1993; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). 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 (Farah, Joaquim, Bernatova, & Morris, 2004; Inagaki, Kuwahara, & Tsubone, 2004; Sgoifo et al., 1997; Sgoifo, Stilli, deBoer, Koolhaas, & Musso, 1998).

Statistical Analysis

Because this was a pilot study and data were obtained from only 3 rats, it was not possible to perform the usual statistical analysis in which the $n$ value corresponds to the number of animals. However, because each animal was used in a variety of procedures and could act as his or her own control, comparison was made for each animal between different procedures, using as $n$ the number of data sampling intervals recorded over a 3-week period for a given procedure, inactive phase or active phase ($n$ was in the range 20 to 30).

The statistics demonstrate the consistency of response of each rat to a given stimulus. The data were also analyzed in 1-week blocks, rather than 3-week or 6-week blocks. Because no habituation to the stimuli was found, the 3-week and 6-week block data are presented. HR, MAP, behavioral parameters, and HRV parameters (LF, HF, and LF/HF) were averaged for each animal over each condition (Noise 1)—inactive phase readings treated separately from active phase readings—and all data are presented as mean ± standard error of the mean (SEM). Data were compared under different conditions, within the same animal and during the same observation period, using the paired Student $t$ test, after checking to make sure that the data passed the tests for normality and equal variance. For conditions monitored during different observation periods (noise vs. quiet or inactive phase vs. active phase), data were compared using the Student $t$ test (or analysis of variance followed by the Holm–Sidak method for comparison of more than two groups), with $p < .05$ considered to be statistically significant. Data from Noise 1 were compared with Quiet 1 and Quiet 2, Ultrasound 1 with Quiet 3, Noise 2 with the first 2 weeks of Quiet 4, and Ultrasound 2 with the last week of Quiet 4. In all cases, separate analyses were performed for inactive phase and active phase data. All statistical analysis was performed using SigmaStat 3.1 software (Systat Software, Inc., Point Richmond, CA).
RESULTS

White Noise

Inactive phase data pertaining to the four quiet periods are presented in Figures 8 and 9. Figures 2 through 7 show the changes in HR and MAP produced in each individual rat in response to each of the protocols.

In response to white noise for 6 weeks (Noise 2), all 3 rats showed significant increases in HR and MAP, compared to before the stimulus; these parameters stayed elevated during the 10 min after the stimulus. When the data from Weeks 1 through 3 were analyzed separately from Weeks 4 through 6 (not shown), these changes were more marked in both cases compared to the Noise 1 study. In the active phase (night), both HR and MAP were similar to those values recorded during the 15-min noise phases during the inactive phase. However, when the HR and MAP, measured during the active phase, were compared between noise and quiet periods, no difference was observed. Thus, the increased cardiovascular parameters measured at night, compared to the inactive period during the day, were just a result of the increased nocturnal activity and were not connected to the noise stimulation.

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 of the parasympathetic nervous system (power of the HF range) during or after the white noise was observed in all rats (Figures 10 to 12). Corresponding shifts in the sympathovagal balance (LF/HF ratio) were also observed during and after the white noise compared to before the stimulus (Figures 13 to 15). 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.

FIGURE 8  Mean heart rate for individual rats during quiet periods 1 to 4. u/s = ultrasound.
With regard to cardiovascular parameters measured at night, no significant differences were observed when comparing Noise 1 with Quiet 1 or Quiet 2 and Noise 2 with Quiet 4.

Ultrasound

Figures 2 through 7 show the effects of the ultrasound on HR and MAP of the rats before, during, and after the stimulus. No consistent changes in HR or MAP were observed in any of the rats in response to either of the ultrasound stimuli (before vs. during, during vs. after). No nocturnal changes were seen in any of the cardiovascular parameters in response to 3 weeks of daily exposure to 90 dB of ultrasound (data not shown). For both ultrasound periods, the HR and MAP during the active phase (night) were significantly greater than during the 15-min daily exposure to ultrasound, once again reflecting the increased nocturnal activity. When the HR and MAP measured during the active phase were compared between ultrasound and quiet periods, no difference was observed.

The effects of ultrasound on HRV (LF/HF and HF) are shown in Figures 10 through 15. Data are not presented for LF because no significant differences were found in response to either noise or ultrasound. Ultrasound 1 did not produce any change in LF/HF; Ultrasound 2 caused a reduction in only one rat (473), with a corresponding increase in HF.

Behavior

Behavioral data were collected in the mornings (inactive phase) and at night (active phase) during the Noise 1, Ultrasound 1, Quiet 2, and Quiet 3 studies. Data from the two quiet periods were reported together. No significant differences in
FIGURE 10 Changes in high frequency (HF) power in response to white noise and ultrasound (u/s) for Rat 473. *Significantly increased; # significantly decreased compared to the relevant “before” data. $p < .05$.

FIGURE 11 Changes in high frequency (HF) power in response to white noise and ultrasound (u/s) for Rat 474. #Significantly decreased compared to the relevant “before” data. $p < .05$.

FIGURE 12 Changes in high frequency (HF) power in response to white noise and ultrasound (u/s) for Rat 475. #Significantly decreased compared to the relevant “before” data. $p < .05$. 
FIGURE 13 Changes in low frequency/high frequency (LF/HF) ratio in response to white noise and ultrasound (u/s) for Rat 473. #Significantly decreased compared to the relevant “before” data. $p < .05$.

FIGURE 14 Changes in low frequency/high frequency (LF/HF) ratio in response to white noise and ultrasound (u/s) for Rat 474. *Significantly increased. $p < .05$.

FIGURE 15 Changes in low frequency/high frequency (LF/HF) ratio in response to white noise and ultrasound (u/s) for Rat 475. *Significantly increased. $p < .05$. 
the percentage of time spent in all active behaviors were seen when comparing data recorded before, during, or after the white noise or ultrasound stimuli. In all cases, the mean percentage of time spent in active behavior was low, ranging from 0.6% to 12.8%. Behavioral activity monitored at night was significantly higher than that monitored during the day—whatever treatment the rats were receiving during the day. During the Noise 1 period, the mean percentages of active behavior for the 3 rats were 65%, 52%, and 56%. Corresponding values for Ultrasound 1 were 59%, 72%, and 52%; for Quiet 2 and Quiet 3, the values were 37%, 26%, 52% and 27%, 58%, 35%. Nocturnal behavioral activity during the Noise 1 study was only significantly higher than during the corresponding quiet period for one rat (474), whereas Ultrasound 1 caused an increase in nocturnal behavior in 2 rats (474 and 475). Because no behavioral changes were common among all 3 rats, the data are not presented here.

DISCUSSION

Exposure to white noise produced increases in 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. Possibly, if the rats were exposed to noise during their active phase, the cardiovascular response would be triggered by an increase in the sympathetic branch.

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 (Kramer et al., 1993; Kramer et al., 2000; Sales, 1972; Sharp et al., 2003; Sharp et al., 2002). In this study, white noise also produced an attenuation of the parasympathetic nervous system (HF power), resulting in a slight shift in the sympathovagal balance (LF/HF ratio). However, it should be noted that respiration is a powerful modulator of HRV, and changes in breathing frequency could confound the respective predominance of LF and HF oscillations in HRV. No measurements of respiration were performed in this pilot study, and this complication might be addressed in future studies.
Contrary to our hypothesis, exposure to 15 min of ultrasound (90 dB) did not cause elevations in HR and MAP. One possible explanation for the differences in cardiovascular responses to white noise and ultrasound observed in this study could be the frequencies contained in the ultrasound stimulus (23.0, 26.4, 30.3, 34.8, and 40.0 kHz). According to the literature, rats use two distinct ultrasonic frequencies to communicate (Brudzynski, 2005; Brudzynski & Pniak, 2002; Sales, 1991; Sewell, 1970). Rats have been shown to produce short pulses (3 to 60 msec) of ultrasound at a frequency of about 50 kHz in aggressive or stressful (when being handled) situations (Sales, 1972) and in anticipation of a social contact (Brudzynski & Pniak, 2002). Longer pulses (lasting up to 3,400 msec) at frequencies of 22 kHz to 30 kHz are produced by submissive rats or when rats are feeding, grooming, or exploring their environment (Sales, 1972, 1991; Sewell, 1967, 1970).

One reason that the ultrasound stimulus used in this study did not have the same cardiovascular, stress-like effects on the rats as the white noise stimulus could be that it did not contain a 50-kHz frequency. In fact, exposure of rats to a 22-kHz frequency is accompanied by a reduced aggression of dominant rats (Sales, 1972). Thus, the ultrasonic frequencies used in this study might have calmed the rats. Another possible explanation is that the ultrasound stimulus was continuous, whereas ultrasonic calls made by rats are less than a second in duration, and there is a remarkable variation in call duration. These differences in ultrasound delivery will be explored in future studies.

It might be argued that because the cardiovascular effects of noise resulted in only small increases in HR and BP (about 20% of initial values), that noise would not be a major confounding factor in rodent experiments. However, unlike the audible sounds and ultrasounds that routinely occur in animal facilities, the stimuli used in these studies were only delivered once a day, at the same time every day, and for a short duration. As reported by other authors, noise levels peak many times during the day in an animal facility and contain a wide range of frequencies (Milligan et al., 1993; Pfaff & Stecker, 1976; Sales et al., 1988). Because noise and ultrasound levels in animal facilities tend to be poorly controlled, the cardiovascular state of the animals may also be poorly controlled and unpredictable. Further experiments are required in which noise and ultrasound are delivered to the animals several times a day, at random intervals, to determine whether their cardiovascular systems are affected to a significantly greater degree than observed in this experiment. If so, then it would be possible that experimental data from animals housed in a noisy environment would be confounded by a variable stress response.

CONCLUSIONS

One surprising result from this study was the lack of significant behavioral responses to the noise and ultrasound. All 3 rats showed a trend of increased noc-
turnal activity during periods of daily noise or ultrasound, but the standard deviations were relatively high, indicating a wide variability in the response within each individual rat—as well as between individuals. There is evidence that individual rodents, even those from the same strain, cope with stress in different ways. Our preliminary results indicate that coping mechanisms may vary even within the same rat. Variation in coping mechanisms is important for the following reason. Previous results indicate that active copers show more active behavior—driven by the “fight or flight” response—whereas passive copers respond by hiding or—if that is not possible—by freezing (Cannon, 1915; Korte et al., 1992; Korte, DeBoer, De Kloet, & Bohus, 1995). In the first case, this behavior is accompanied by high stimulation of the sympathetic ANS and consequent release of epinephrine and norepinephrine. In the second case, the parasympathetic nervous system is activated; in addition, the hypothalamus–adrenal–pituitary axis is stimulated, leading to greater release of corticosterone.

Under baseline conditions, there is no difference between active and passive copers regarding stimulation of the sympathetic and parasympathetic nervous systems; the difference occurs only when the animals are stressed. Because these hormones together affect (a) HR, (b) BP, (c) constriction or dilation of blood vessels and airways, (d) activity of the immune system, (e) metabolic rate, and (f) lipolysis, these bodily processes will also show a greater variation between animals when the animals are inadvertently exposed to stress. This consequence of stress will greatly diminish the quality of the scientific data obtained from research animals and lead to the sacrifice of unnecessarily large numbers of animals to achieve statistical significance.

Although it is true that stress does not always compromise health and welfare and that the stress response is necessary for survival in the wild, stress—particularly when elicited repeatedly—disturbs the body’s homeostasis and imposes a cost to the body. This cost arises if stress-induced mediators such as adrenal hormones, neurotransmitters, and cytokines are released too often. For this reason, uncontrolled stress is a confounding factor in experiments on animals. Further experiments are necessary to determine whether randomly delivered audible noise and ultrasound may be producing an unknown and variable stress in caged rodents.

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