



An assessment of ambient noise and other environmental variables in a nonhuman primate housing facility

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Acoustic noise and other environmental variables represent potential confounds for animal research. Of relevance to auditory research, sustained high levels of ambient noise may modify hearing sensitivity and decrease well-being among laboratory animals. The present study was conducted to assess environmental conditions in an animal facility that houses nonhuman primates used for auditory research at the Vanderbilt University Medical Center. Sound levels, vibration, temperature, humidity and luminance were recorded using an environmental monitoring device placed inside of an empty cage in a macaque housing room. Recordings lasted 1 week each, at three different locations within the room. Vibration, temperature, humidity and luminance all varied within recommended levels for nonhuman primates, with one exception of low luminance levels in the bottom cage location. Sound levels at each cage location were characterized by a low baseline of 58–62 dB sound pressure level, with transient peaks up to 109 dB sound pressure level. Sound levels differed significantly across locations, but only by about 1.5 dB. The transient peaks beyond recommended sound levels reflected a very low noise dose, but exceeded startle-inducing levels, which could elicit stress responses. Based on these findings, ambient noise levels in the housing rooms in this primate facility are within acceptable levels and unlikely to contribute to hearing deficits in the nonhuman primates. Our results establish normative values for environmental conditions in a primate facility, can be used to inform best practices for nonhuman primate research and care, and form a baseline for future studies of aging and chronic noise exposure.

Hearing loss is the fourth highest cause of disability worldwide, with an estimated 466 million people affected by disabling hearing loss¹. In humans, acute and chronic noise exposure may result in a multitude of auditory and non-auditory effects, including noise-induced hearing loss (NIHL)², sleep disturbance³, increased risk of cardiovascular disease⁴ and poorer patient health outcomes in hospitals^{5,6}. NIHL, distinct from age-related hearing loss, accounts for a large portion of hearing loss despite being largely preventable². Noise exposure is also one of the most common occupational hazards, with an estimated contribution of up to 16% of all disabling hearing loss globally⁷.

The effects of noise exposure are highly relevant to research involving laboratory animals. The Guide for the Care and Use of Laboratory Animals describes the importance of tracking and assessing noise, which may serve as a major stressor for animals in laboratory facilities⁸. When unmonitored, noise can act as an unexpected confounding variable to animal research, disrupting studies and creating animal welfare concerns⁹. Furthermore, despite being outside of the human audible range, ultrasonic signals (sound above 20 kHz) are audible to many research animal species¹⁰, including nonhuman primates (NHPs)^{11,12} and other species such as mice, rats, guinea pigs, rabbits, dogs, cats and pigs^{13,14}. Common sources of ultrasonic noise in the laboratory environment may include lighting, running taps, computers, humidity and temperature meters, compressed gas lines and test equipment^{9,14}. These ultrasonic sounds audible to animals may go unnoticed by researchers unless specifically tested. Most prior studies of ambient noise levels in animal facilities have involved rodent housing rooms^{15–18}, with few, if any, studies of NHP facilities. This may be due to greater prevalence of rodent species in research, and the susceptibility of rodents to

NIHL at lower levels compared with primates^{19,20}. Nonetheless, primates are affected by ambient noise: both acute noise blasts²¹ and continuous noise²² can induce a substantial stress response in NHPs. NHPs are one of the most limited and highly valuable resources for scientific research²³; therefore, to maximize resources and improve reproducibility, it is of utmost importance to monitor and control external variables like noise in NHP studies.

In auditory research, NHPs serve as an invaluable bridge between research conducted in rodent models and in humans, given their phylogenetic similarity to humans and their ability to complete complex listening tasks^{20,24–26}. In auditory NHP research facilities, assessment of environmental noise is of particular relevance; not only may noise serve as a stressor for these NHPs, but noise may also directly affect the data that are collected by unintentionally inducing auditory sensitivity changes in NHP subjects. Noise exposure can induce temporary or permanent shifts in hearing sensitivity^{27–30}. These shifts occur secondary to reversible or permanent damage to sensory components of the cochlea. The specific mechanisms of damage may include mechanical damage to hair cell stereocilia, glutamate excitotoxicity at ribbon synapses, and oxidative stress-induced cell death^{30–33}. Both temporary and permanent acoustic injury caused by ambient noise levels would be confounding to experiments assessing auditory function under normal and pathologic states. The present study was conducted to assess environmental conditions, particularly ambient noise levels, in an animal facility that houses rhesus macaques used for auditory research at the Vanderbilt University Medical Center.

Owing to the various research and animal husbandry protocols employed in the primate housing rooms, the facility can be quite noisy at times. Much of this noise is associated with the rattling of

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metal and plastic parts of primate chairs used to transport monkeys from housing rooms to experimental rooms, the transport of metal housing cages to and from the wash, the opening and closing of cage doors, and noises made by the monkeys themselves as they interact with their cages and enrichment devices. Informal measurements of sound levels in the facility indicated that noise during routine events such as these could reach levels greater than 100 decibels sound pressure level (dB SPL), a level that is potentially problematic depending on the duration and frequency of these epochs of high noise levels^{19,20}. Furthermore, NHPs have an auditory range of approximately 50 Hz to 45 kHz (refs. ^{11,12}), making them one of the many research animal models that can hear ultrasonic frequencies, which are inaudible to humans. As ultrasonic sounds have the potential to go unnoticed by researchers unless specifically measured, it has been recommended that they be monitored routinely to ensure that they do not reach unsafe levels³⁴. Together, these concerns provided the motivation for this study.

Other environmental variables such as temperature, humidity, lighting and vibration must also be monitored carefully in facilities that house research animals. The Guide for the Care and Use of Laboratory Animals recommends that indoor temperatures in primate facilities be maintained between 64 °F and 84 °F (at least 74 °F being preferred for rhesus macaques), humidity levels be maintained between 30% and 70%, and lighting be maintained to provide the regular 12–14 h of light⁸. Additionally, while species-specific guidelines for luminance levels in primate cages have not been established, the Guide for the Care and Use of Laboratory Animals states that light at the cage level should be maintained between 130 and 325 lux for animals that are susceptible to phototoxic retinopathy⁸, such as rhesus monkeys³⁵. These environmental variables are particularly relevant to auditory NHP research considering that some studies have indicated that variations in both body temperature³⁶ and light exposure³⁷ can affect the severity of NIHL. Vibration levels are recommended to not exceed 0.025g in rodent housing conditions⁹. As there are no published guidelines for acceptable vibration limits in primate facilities, we used this value as a point of reference for our measured values. In the future, NHP vibration thresholds of concern should be determined in order to ensure proper animal care.

By carefully monitoring environmental variables such as noise levels, vibration, temperature, humidity and lighting in the primate housing facility over a 3-week period, we characterized in detail the living environment of a group of research NHPs that were subjects in studies of auditory function. This characterization describes the environmental context under which we previously measured normal macaque auditory perception^{12,25,38,39} and physiology^{40–42}, as well as changes following acute noise exposures^{19,43–45}. By measuring and accounting for each of these variables, we gain critical insight into the baseline controls for the housing environment of our NHPs and therefore may acknowledge the potential confounding effects of the laboratory environment in our ongoing and future NHP studies. Our findings may also be used to characterize environmental conditions in similar primate facilities and help inform best practices for NHP research and care. Additionally, the facility noise levels established here may prove useful to future studies of chronic noise exposure, for which it is necessary to first understand the noise dose received by NHPs in normative conditions to then evaluate the effects of chronic overexposure conditions.

Results

Trends in primate housing room environmental conditions.

Using a Sensory Sentinel environmental monitoring device (Fig. 1), temperature, humidity, lighting, vibration and noise were measured once per second for 1 week in three different recording locations within a specific macaque housing room: a top compartment of a cage in the middle of the room (designated as TM), a bottom

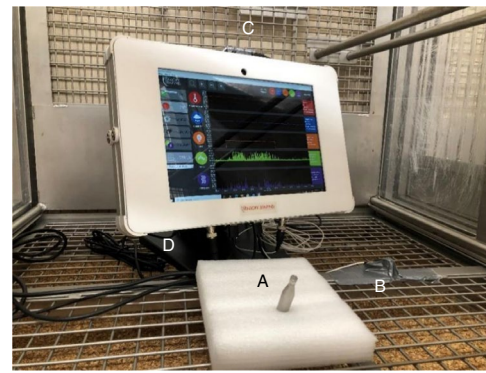


Fig. 1 | Sensory Sentinel device setup shown inside of a modular primate cage unit. Microphone (PCB 426A11 ICP) mounted in an upright, outward-facing direction using styrofoam (A). Accelerometer (PCB 353 C33) taped to floor of cage (B). Light sensor mounted on top of device angled in direction of light source (C). Temperature and humidity sensor (not visible) mounted on the base of the device (D).

compartment of a cage in the middle of the room (designated as BM) and a top compartment of a cage on the edge of the room (designated as TE) (Table 1). Temperature and humidity were found to remain within the recommended levels for NHPs. Luminance remained at a normal intensity level, with the only exception of the luminance levels in the bottom cage location. While a recommended light intensity range specifically for NHPs was not found in the literature, the value of 10.8 lux for the bottom cages is well below the 130 lux lower bound described by the Guide for the Care and Use of Laboratory Animals. Thus, light levels may be a concern for monkeys that only have access to a bottom cage space, but since most macaques in the animal facility have access to both a top and bottom cage space, environmental modifications based on this finding were not pursued. Also of note, a previous study found that NHPs preferred top cage spaces, but this preference was more determined by a desire for elevation than the increased light⁴⁶. There is little information available regarding acceptable vibration levels in NHP facilities. While average vibration levels in the housing room were below the aforementioned 0.025g standard for rodents, there were transient peaks that did exceed 0.025g. However, peak vibration levels greater than 0.025g are to be expected given that macaques are a more active species and have greater levels of vibration associated with cage movement. It may be valuable for future studies of vibration to consider the resonance frequencies of research animals, which is important in determining how strongly the vibrations are perceived⁴⁷. The remainder of the results and discussion sections of this report focus on noise, which was the primary environmental variable of interest.

Across all days and locations, baseline noise levels in the facility fluctuated between 58 and 62 dB SPL, with transient peaks associated with various noise events occurring throughout the day. Figure 2 shows an example of how sound level fluctuated over time during one day at the TM recording location. An increase in noise levels is readily apparent during the period from about 5.5 to 17.5 h, which represents the waking hour period from 5:00 to 17:00 during which the lights were typically on in the facility. Note that Fig. 2 consists of 86,000 datapoints, and as a result, the durations of the waking hour noise spikes are overrepresented by the thickness of the line that was necessary to create the plot. We show two inset plots to highlight the fluctuations in noise levels. The left inset panel of Fig. 2 provides a closer look at baseline noise levels over a typical 2-min period, which shows that noise levels varied between 58 and 62 dB SPL. The right inset panel of Fig. 2 highlights noise levels during a 2-min period that contained several noise events, reaching sound levels as high as 97 dB SPL.

Table 1 | Mean or median values for vibration, temperature, humidity and light for each recording location

	Vibration, mg median (IQR) ^a	Temperature, °F mean ± s.d.	Relative humidity, % mean ± s.d.	Light, lux mean ± s.d.
Week 1 (TM)	1.445 (0.118)	75.313 ± 0.463	38.384 ± 4.470	190.175 ± 28.209
Week 2 (BM)	1.400 (0.084)	76.343 ± 0.368	44.375 ± 1.826	10.759 ± 3.619
Week 3 (TE)	1.419 (0.128)	75.741 ± 0.539	45.428 ± 1.982	204.543 ± 3.460

^aMedian (IQR) values provided instead of mean ± standard deviation (s.d.) owing to non-Gaussian distributions.

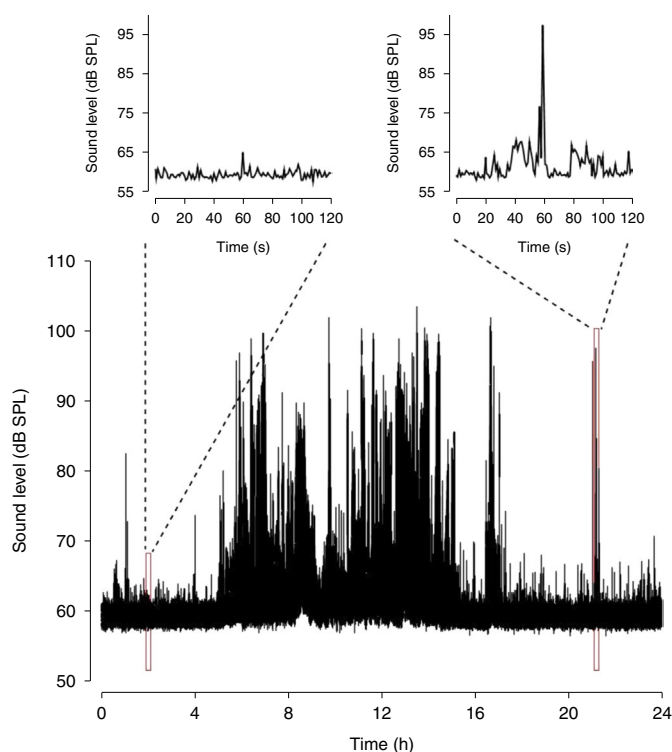


Fig. 2 | Example of noise level (dB SPL) versus time (h) plot from March 22 2021, recorded at the top-middle location. Waking hours took place from 5 h to 17 h on the x axis (that is, 5:00–17:00), and a noise peak of 103.36 dB SPL was observed for the day. Left inset panel shows the baseline fluctuation in noise levels between 58 and 62 dB SPL over a 2-min period. Right inset panel shows a 2-min period during which several loud noise events occurred in the facility. Plots were generated for all days of recording.

Descriptive statistics including mean, median, interquartile range (IQR), skewness and kurtosis were calculated for each environmental variable. These measures were computed for each day of recording and for each recording location using the full 24-h dataset (Table 2). Mean and median environmental noise levels were greatest for the TM location, followed by the TE location and were lowest for the BM location (Table 2). Across all days and recording locations, the distribution of noise levels was positively skewed and leptokurtic (that is, kurtosis value of greater than 3). The noise level distributions for each location were examined and confirmed to be non-Gaussian through visual methods (histograms in Fig. 3 and boxplots in Fig. 4a) and through statistical testing using Kolmogorov–Smirnov tests ($P < 0.001$ for all locations).

Descriptive statistics including mean, median, IQR, skewness and kurtosis were also calculated for each day of recording and for each recording location using the waking hours dataset (Table 3). Compared with the full 24-h period, mean dB SPL values during the waking hours were greater for all three recording locations by

Table 2 | Sound level mean, median, IQR, skewness and kurtosis values for each week of recording, calculated using the full 24-h period

	Sound level using the full 24-h period					
	Mean	Median	IQR	Skewness	Kurtosis	Excess kurtosis
TM (week 1)	60.775	59.68	1.86	4.225	22.971	19.971
BM (week 2)	59.630	59.34	1.51	4.666	28.410	25.410
TE (week 3)	60.434	59.53	1.35	5.015	32.665	29.665
Average	60.250					

an average of 1.3 dB SPL, and these differences were statistically significant as confirmed by a Kruskal–Wallis H test followed by post-hoc Mann–Whitney U tests for each location ($H = 395,434$, $df = 5$, $P < 0.001$, Kruskal–Wallis H test; $P < 0.001$ for all locations, TM location $Z = 195.0$, BM location $Z = 238.3$, TE location $Z = 196.2$, Mann–Whitney U tests). The waking hour period also exhibited an increased IQR, decreased skewness and decreased kurtosis compared with the full 24-h period, suggesting fewer extreme values (in this case, at the lower extreme of the distribution). These changes are consistent with the observation that there were increased noise levels and transient noise peaks during the waking hours.

The weekly total amount of time that noise in the room was above specific sound levels (in dB SPL) was also calculated for each recording location (Table 4). Noise levels were fairly low, only reaching levels above 70 dB SPL for an average of 39 min and 35 s per week. One consideration is the The National Institute for Occupational Safety and Health (NIOSH) guidelines for noise exposure, which suggest that noise levels should not exceed an average of 85 dBA during an 8-h work day for healthy hearing⁴⁸. We calculated the length of exposure to sound levels 85 dB SPL and higher, which were less than 10 min each week (Table 4).

Comparison of peak noise levels and sound level distributions between locations. Noise level peaks were obtained for each day of recording and used to assess levels across each location in the room (Table 5). The largest peak values were consistently observed for the BM location, followed by the TE and finally TM locations, and ranged from 92.6 to 109.7 dB SPL.

Histograms were generated for all days of recording to describe the distribution of the data. Figure 3 shows a representative distribution for a single day of each recording location (left column) and the overall distributions for each of the recording locations (right column). As the distributions were non-Gaussian, Kruskal–Wallis H tests were conducted to determine if sound levels varied significantly between days within a single recording location. The results indicated that there were small but significant variations in sound levels between days for the same recording location (TM location: $H = 10,203$, $df = 6$, $P < 0.001$, Kruskal–Wallis H test; BM location: $H = 15,228$, $df = 7$, $P < 0.001$, Kruskal–Wallis H test; TE

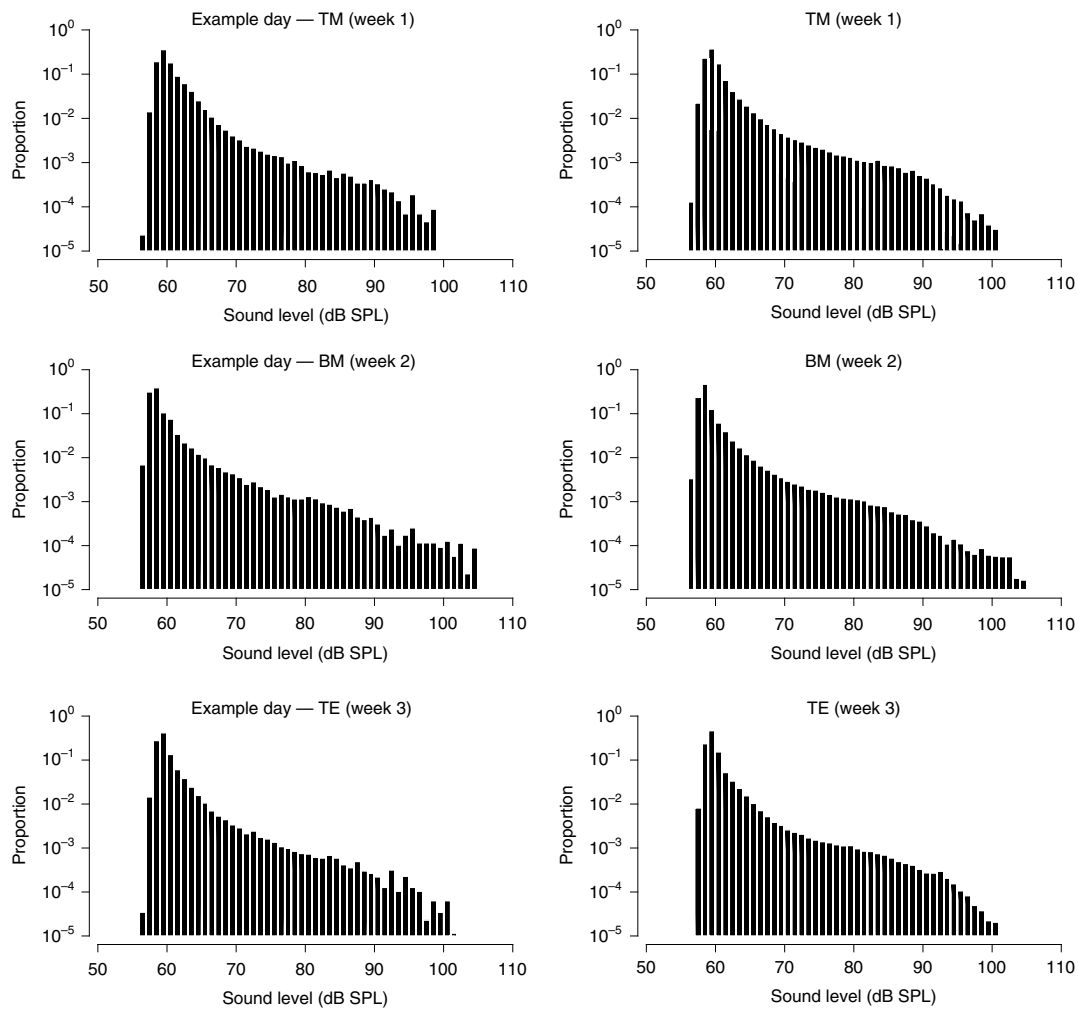


Fig. 3 | Distribution of sound levels for each recording location. Left: example percentage histogram for an individual day of each recording location. Right: percentage histograms for the entire week of each recording location. A log y axis is used to better highlight the right tail of the distributions.

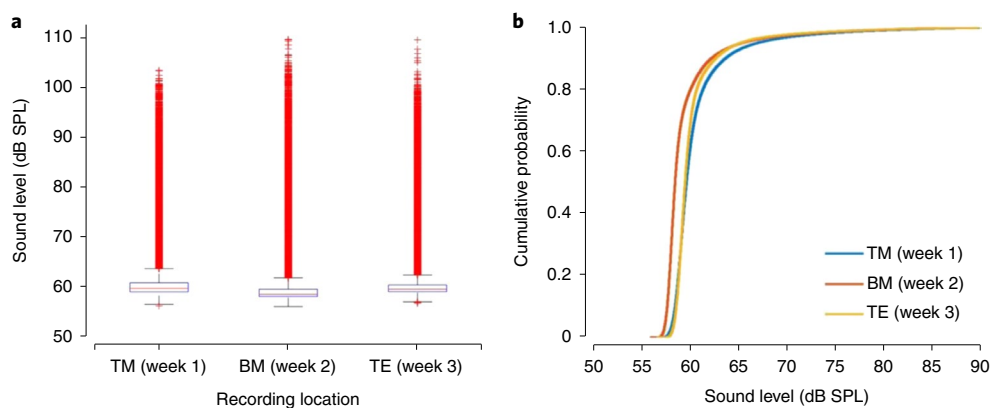


Fig. 4 | Distribution of sound levels across different recording locations. **a, b** Box plot (**a**) and CDFs (**b**) describing the distribution of data across different recording locations.

location: $H = 10,639$, $df = 7$, $P < 0.001$, Kruskal–Wallis H test), which is to be expected given the day-to-day variability in noise events in the facility. A separate Kruskal–Wallis H test was also conducted to determine if sound levels varied significantly between weeks of recording for different locations. The results of this test indicated that there were also significant differences between recording

locations across weeks ($H = 256,320$, $df = 2$, $P < 0.001$, Kruskal–Wallis H test), and the magnitudes of these differences were larger than the magnitude of the day-to-day variability within a single location (as is made evident by comparing the H values). Post-hoc Mann–Whitney U tests comparing each recording location with each other confirmed that these significant differences existed

Table 3 | Sound level mean, median, IQR, skewness and kurtosis values for each week of recording, calculated using the 12-h waking hours period (5:00–17:00)

	Sound level using the 12-h waking hours period					
	Mean	Median	IQR	Skewness	Kurtosis	Excess kurtosis
TM (week 1)	62.208	60.57	3.34	3.074	11.630	8.630
BM (week 2)	60.994	59.34	2.96	3.417	14.755	11.755
TE (week 3)	61.634	60.14	2.70	3.589	16.094	13.094
Average	61.612					

Table 4 | Weekly total amount of time above various noise levels for each recording location

	Total amount of time above various noise levels for each recording location (h:mm:ss)			
	70 dB SPL	80 dB SPL	85 dB SPL	100 dB SPL
TM (week 1)	0:45:21	0:14:23	0:07:18	0:00:04
BM (week 2)	0:38:41	0:12:13	0:05:25	0:00:19
TE (week 3)	0:34:43	0:11:48	0:05:44	0:00:04
Average	0:39:35	0:12:48	0:06:09	0:00:09

between all possible pairs of recording locations ($P < 0.001$ for all comparisons, $Z = 426.0$ between TM and BM, $Z = 46.8$ between TM and TE, $Z = -441.2$ between BM and TE, Mann–Whitney U tests). A mixed-effects model containing top versus bottom cage location, middle versus edge cage location, and weekday versus weekend as fixed effects and day as a random effect confirmed that the vertical aspect of cage location ($t = -111.0$, $df = 1.7 \times 10^6$, $P < 0.001$) and the horizontal aspect of location ($t = -108.4$, $df = 1.7 \times 10^6$, $P < 0.001$) were significantly predictive of sound level, whereas weekday versus weekend was not ($t = 0.81$, $df = 1.7 \times 10^6$, $P = 0.417$). Box plots and cumulative distribution functions (CDFs) were generated to visualize differences in the data between the three recording locations (Fig. 4).

Noise spectrum captures. Noise spectra were captured for common noise events that were hypothesized to be contributing to the elevated noise levels in the facility. These data are summarized in Fig. 5a, which also shows a mean audiogram for male macaques collected from subjects in the authors' laboratory²⁰ in red and the baseline noise levels in the room in black. The blue line in Fig. 5a shows the spectrum for the sound made by the monkeys interacting with the polycarbonate busy box enrichment devices that hang on their cages. The spectrum indicates that the resulting noise has a relatively wide bandwidth with peaks in the 200–300 Hz range. The green line in Fig. 5a shows the noise spectrum for the sound made by a monkey shaking its cage, producing a loud noise capable of eliciting a startle response in NHPs⁴⁹. Among the sounds plotted, this spectrum for cage shaking is noticeably the loudest over a wide range of frequencies, consistent with the observation that cage shaking and cage movements are one of the largest contributors to elevated noise levels during waking hours in the facility. The spectrum for cage shaking did not reach the maximum noise levels reported in Table 5 at any single frequency. However, rough estimates indicate that the overall noise level for cage shaking across

all frequencies indeed could have reached overall levels of approximately 110 dB SPL.

The dark-red line of Fig. 5a shows the spectrum recorded while the monkeys made a chorus of various cooing and grunting sounds. Several peaks are noticeable in the 100–2,000 Hz range, with the highest spectrum level of 81.4 dB SPL occurring at a frequency of 1,567.4 Hz. Figure 5b shows spectrum captures for individual monkey vocalizations, including a chirp (dark blue) and a grunt (purple). The chirp is apparent in the narrow spike at approximately 10 kHz, whereas the grunt is spread out over a wider range of lower frequencies.

Discussion

By assessing ambient noise conditions in the primate housing facility over a 3-week period, it was determined that noise levels in this NHP facility are typically characterized by a baseline level of 58–62 dB SPL, with transient noise peaks occurring throughout the day owing to specific noise events. Across all locations, a mean noise level of 60.3 dB SPL and median noise level of 59.3 dB SPL were observed, with an IQR of 1.8 dB SPL, a highly positive skew and a highly leptokurtic distribution. Small but significant differences in the mean and outlier regions of the sound level distributions were found across days and recording locations (Fig. 4a). The distributions from the TM and TE locations are the most similar, with the BM location having noticeably quieter baseline noise levels (Figs. 3 and 4b). Taken together, these findings suggest that the NHPs do receive slightly different levels of noise exposure dependent on their location in the housing room. However, the differences in mean noise levels across locations were quite small (1–1.5 dB). Furthermore, across all cage locations, mean noise levels still remained well below the 70 dB SPL threshold of concern, meaning it is unlikely that the effect of cage location contributes to any meaningful variation in NIHL or other hearing differences between monkeys. The results of this study are expected to be generalizable to most indoor NHP facilities, especially those that use similar cage setups. However, some variations may occur because of differences in housing room sizes, housing room materials, cage wash schedules, enrichment programs and other factors.

To our knowledge, this is the first study to evaluate sound levels in an NHP facility. Previous studies have been conducted in facilities housing other animal species, but they vary in their use of frequency weightings (for example, dBA and dBZ) making it difficult to draw generalized conclusions. Milligan et al. measured sound levels in a number of different animal facilities and found that background levels were generally below 50 dB SPL (ref. ⁵⁰). Lauer et al. found baseline levels of 80 dBZ in rodent housing rooms¹⁸, whereas Reynolds et al. reported an ambient noise level of 52 dBA in their rodent facility¹⁵. Given the different weighting systems, the baseline sound level of 58–62 dB SPL found in this study appears to be consistent with findings in other animal facilities. Furthermore, as has been noted in rodent facilities^{18,50}, noise levels in the primate housing facility also increased and had greater variation during the waking hours. In other species, this increase in noise during the daytime can be primarily attributed to human activity. However, NHPs are unique in their ability to contribute to a large amount of this noise increase themselves. Thus, in many cases, it may be particularly difficult to mitigate noise in primate facilities.

Guidelines for humans in terms of noise levels in the workplace are established by NIOSH and the Occupational Safety and Health Administration (OSHA). The NIOSH recommended exposure limit for noise is 85 dBA with an 8-h time-weighted average and a 3-dB exchange rate⁴⁸. Similarly, OSHA's permissible exposure limit is 90 dBA with an 8-h time-weighted average and a 5 dB exchange rate³¹. It is important to note that these NIOSH and OSHA thresholds of concern are designed to assess an 8-h workday for humans, not the full 24-h period of exposure that is present in animal facilities.

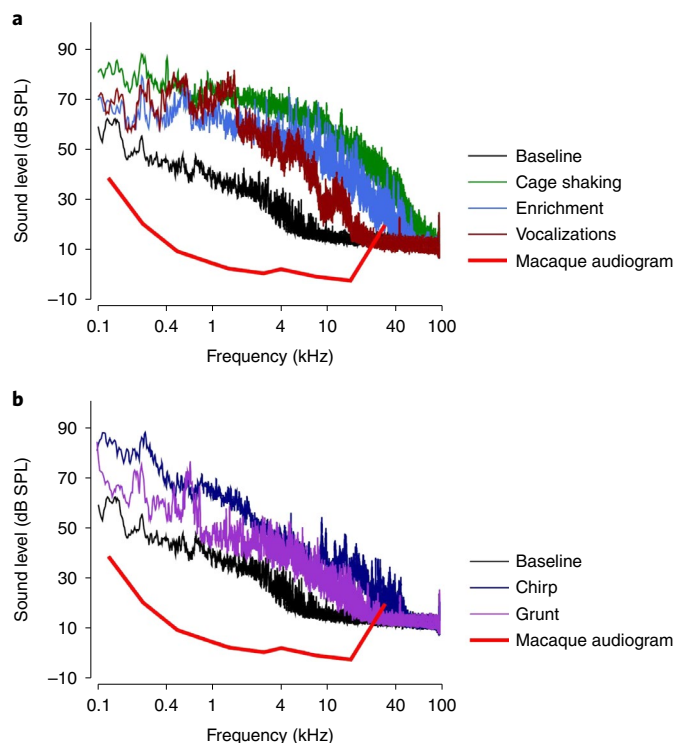


Fig. 5 | Spectra of sounds in the housing room. a, Noise spectra for various noise events, including monkey interacting with enrichment device (blue), monkey shaking metal modular primate cage (green) and monkeys making various cooing and grunting vocalizations (dark red). **b**, Individual spectra for chirp (dark blue) and grunt (purple) vocalizations. Baseline noise levels of the room are indicated (black) along with the standard macaque audiogram²⁰ (red).

The US Environmental Protection Agency and the World Health Organization, on the other hand, recommend that noise exposure levels remain at an average of less than 70 dB SPL for a full 24-h day to avoid hearing loss in humans^{52,53}. This 70 dB SPL threshold of concern is cited as an effective standard for laboratory animals in general, with some species requiring lower levels⁹. It has recently been shown that lower-level noise exposures that were previously considered benign cause permanent damage to the peripheral auditory system following temporary threshold shifts in rodents^{29,54}, NHPs¹⁹ and humans⁵⁵. In the primate housing rooms at Vanderbilt, noise levels only exceeded 70 dB SPL for an average of about 40 min per week, 85 dB SPL for about 6 min per week and 100 dB SPL for only about 9 s per week (Table 4). Taking these data into consideration with studies of threshold shifts in NHPs and known species differences in susceptibility to NIHL, NHPs are probably at low risk of incurring temporary threshold shifts of large enough magnitude to induce peripheral damage within their typical housing environments^{19,20,56}. However, further investigations are needed to ascertain the range of damaging levels as well as interactions between noise level and duration to inducing cochlear damage in various animal models and humans⁵⁷.

Overall, because mean noise levels in the facility were well below the recommended standard of a 70 dB SPL average, our assessment indicates that noise levels in the primate housing facility at Vanderbilt are unlikely to be contributing substantially to any hearing deficits in the NHPs. Establishing the baseline controls for the housing environment of our macaques is an essential component of animal research to understand the potential influence on research outcomes¹⁰. The remaining concerns raised by the sound level data

Table 5 | Peak noise levels observed for each day of recording in each recording location

Day of recording	Peak noise level (dB SPL)		
	TM	BM	TE
Day 1	100.74 ^a	102.70 ^a	92.58 ^a
Day 2	98.99	104.96	101.59
Day 3	103.48	106.61	107.73
Day 4	100.57	109.60	106.84
Day 5	103.36	109.68	105.98
Day 6	101.46	107.78	109.58
Day 7	100.53 ^a	108.57	105.04
Day 8	NA	103.58 ^a	99.27 ^a

^aPartial day of recording (that is, less than 24 h). NA, not available

are primarily related to the transient noise peaks, which at a maximum reached a value of 109.7 dB SPL. Center for Disease Control and Prevention guidelines for humans state that noise in the range of 105–110 dB SPL can potentially cause hearing loss within minutes⁵⁸. As noise in the facility was only above 100 dB SPL for an average of 9 s per week, it is likely that such high levels of noise occur for too short of a duration to induce permanent threshold shifts. A greater concern is that the noise peaks may contribute to elevated stress and decreased well-being among NHPs. Macaques consistently show a startle response for stimuli of at least 90 dB SPL (ref. ⁴⁹), indicating that the noise peaks observed in the primate housing rooms may act as startle-inducing noise for the NHPs. This source of distress represents an uncontrolled variable that, if unaddressed, may have a confounding effect on scientific studies. Additionally, consistent with the principles of the 3Rs (replacement, reduction and refinement) of humane animal research, it is important to consider and minimize sources of distress when caring for laboratory animals⁵⁹. The provision of environmental enrichment 5 days a week at the primate housing rooms may play a role in mitigating any such stress.

The trend observed for the magnitude of noise peaks across the different recording locations (Table 5) did not correlate with the trend observed for the mean noise levels across locations (Table 2). One explanation for this is that the peak values may have been primarily influenced by the behavior and temperament of the monkey that was in the cage compartment adjacent to the recording equipment at each location. The monkeys that have louder movements and cage noises will consistently generate larger peak values, but because of the transient nature of these peaks, they have little effect on the overall mean noise levels. In contrast, recording location is the most plausible explanation for the small differences in mean sound levels between cages since its effect is present throughout the entire recording period.

The baseline spectra (Fig. 5), as recorded from the animals' microenvironment within the Wilson Hall Animal Facility (WHAF) cage units, did not indicate any unusual noise peaks in the ultrasonic range. Additionally, a more detailed assessment of ultrasound in the WHAF was previously conducted by Turner Scientific in December of 2018. The report did not find any sources of ultrasound at the cage level that exceeded 45 dB SPL, their threshold of concern. It is recommended for researchers to periodically monitor and replace electrical ballasts for fluorescent light fixtures, which may emit louder ultrasonic noise as they age. Furthermore, enrichment devices such as DVD players, televisions and/or radios should be regularly measured for ultrasonic noise and placed as far away from housing cages as possible to reduce potential exposures.

Results of spectrum captures showed that cage movement and shaking, as well as monkeys interacting with their enrichment

devices, tended to produce the most intense and broadest bandwidth noise of the common noise events in the facility. To reduce startle-inducing noise in the primate housing facilities and promote NHP well-being, it may be advisable to target these specific noise sources. Stainless steel is the standard material for NHP cages because it is easy to sanitize and highly durable. Unfortunately, this makes it difficult to fully eliminate noise in NHP housing rooms as metal is a poor absorber of sound. However, materials such as Trespa and polypropylene may be used for cage partitions, platforms, shelves and perches to help mitigate noise and provide a more comfortable environment for NHPs^{60,61}. Manzanita wood is also a suitable material for NHP enrichments as it can be effectively sanitized using standard cage-wash procedures⁶². Although not studied by the authors, different caging systems may result in deviations from the findings reported here. For example, the one-over-one cage units used in the WHAF are interlocking and thus allow for vibrations caused by one monkey to spread to all connected cage units. If cage units in other facilities are isolated to prevent this, then it may be reasonable to expect lower vibration levels. Additionally, enrichment device noises could be limited by fitting the busy boxes with rubber pads to help absorb the impact of the device against the cage.

Future directions in this field could involve establishing thresholds of concern that are specific to individual species such as NHPs. As much of the existing research on environmental noise focuses on rodent models, there is a dearth of information regarding NHPs and how they respond to common research housing environments. Further elucidation of these areas will help ensure that potential confounding effects of noise in primate facilities are limited as much as possible. Additionally, the results of this study form a baseline for future studies of aging and chronic noise exposure. Using the normative exposure levels established here, chronic noise studies can titrate baseline facility levels up to achieve precise long-term noise doses.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41684-022-01017-9>.

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Methods

Data collection. For the purposes of this study, a Sensory Sentinel environmental monitoring device capable of measuring noise, vibration, temperature, humidity and luminance was loaned from Turner Scientific. The device (Fig. 1) utilizes a Microsoft Surface Pro Tablet equipped with a PCB 377C01 ICP microphone, a PCB 352C33 accelerometer, a Yocto-Meteo-V2 temperature and humidity sensor, and a Yocto-Light-V4 light sensor, all of which were used to record an environmental variable value every second for 24 h per day. The microphone and accelerometer both utilized a sampling rate of 192 kHz to generate an averaged sound level that was recorded each second, whereas the temperature, humidity and luminance sensors simply sampled once per second. Each of these environmental variables were measured in a primate housing room of dimensions 8' × 35' × 8.5'. The housing room had resinous epoxy flooring, concrete block walls with an epoxy finish and plaster ceilings with an epoxy finish. These materials could potentially affect the sonic qualities of the room and should be considered when comparing our findings with other facilities. The room housed ten male rhesus macaques in ten separate but interlocking six-square-foot modular primate cage units of dimensions 32.5" × 30" × 29" (Primate Products, model 1C-A3V6). The cages were all oriented side by side in the same direction, facing the front of the room. Two pairs of macaques were socially housed with access to two upper and two lower modules (two cage units). The remaining six macaques were individually housed with access to an upper and lower module (one cage unit). The primate housing units enabled visual, auditory and olfactory contact between macaques within the housing room. Additionally, supplemental visual, auditory and/or olfactory environmental enrichments were provided daily. Examples of enrichments included television, mirrors and busy boxes. The macaques used in this study were acquired from the California National Primate Research Center and the Oregon National Primate Research Center. For recordings, the device was placed inside an unoccupied primate cage unit in either an upper or lower module, with an individually housed macaque separated into the other module by a dividing grate and a wood-chip-filled metal tray. In this way, the device was able to measure the naturalistic environment inside of a cage while still remaining separate from the macaques. A potential limitation of this setup is that, during recordings, the increased weight added by the device may have resulted in a lower intensity of vibration within the cage.

All environmental variables were recorded for three 1-week-long periods, with the exception of week 1, which had only 6 days' worth of data owing to a cage change conflict. The device was placed inside of a cage located in a different part of the room for each of the 3 weeks. The three recording locations consisted of the device placed inside the top compartment of a cage in the middle of the room (TM), a bottom compartment of a cage in the middle of the room (BM) and a top compartment of a cage on the edge of the room (TE). These three locations in the room were selected to determine if noise levels differed by the vertical aspect of location (that is, top versus bottom cage) and/or the horizontal aspect of location (that is, middle versus edge cage). As data were recorded every second for 1 week in each of the three locations, the final dataset consisted of approximately 1.7 million datapoints for each environmental variable.

Using the Sensory Sentinel device and application, spectra showing sound level versus frequency (Hz) were also captured for common noise events within the facility. Spectra were obtained using the peak hold function, which retains the highest dB SPL value at each frequency during the period of recording. Spectra were captured for periods where noise levels were high, such as during the sounds of monkeys banging on a cage, monkeys interacting with enrichment devices, and various monkey vocalizations, including chirps, grunts and coos. Sequentially on the same day, a single spectrum was captured for each of these noise events using the peak hold function for a period of a few seconds.

Statistical analyses. Following data collection, descriptive statistics including mean, median, IQR, skewness and kurtosis were calculated for each individual day

of recording using Excel. Two sets of these descriptive statistics were calculated for each day, one using data from the full 24 h, and the other using data from only the 12-h waking hours period during which the lights were on in the facility (typically 5:00–17:00). This waking hour period was selected for further analysis because it typically corresponded to the noisiest hours in the facility. Additionally, weekly amount of time when the noise levels were higher than 70, 80, 85 and 100 dB SPL were calculated for each recording location to estimate a noise dose. To visualize noise levels, overall sound levels were plotted as a function of time for each day using data from every second of the 24-h day (a total of 86,400 datapoints per day, Fig. 2). The same data were also used to generate distributions of noise levels for each day using 1 dB SPL bins (Fig. 3). A log *y* axis was used in Fig. 3 to highlight the presence of the louder noise levels, which were otherwise unapparent using a standard *y* axis.

Calculations of descriptive statistics and visualization using histograms indicated that the daily noise distributions were non-Gaussian. As a result, Kruskal–Wallis *H* tests were performed using MATLAB (R2020b; Mathworks) to determine if there were significant differences in sound levels between days of recording within each location, and between weeks of recording for different locations. Mann–Whitney *U* Tests were then used as a post-hoc test to determine which specific groups differed from each other. Finally, linear mixed-effects models ('fitlme' in MATLAB) were used to determine which factors (for example, the vertical location, horizontal location and the type of day) contributed significantly to sound levels in the facility. Owing to the large sample size gathered in this study (approximately 1.8 million total datapoints), many of the conducted statistical tests yielded unusually large test statistics.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The datasets generated during the current study and that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

A.R.M., J.A.B. and R.R. conceptualized and designed the study. A.R.M. and J.A.B. collected the data. A.R.M., J.A.B. and C.A.M. performed the analysis, with input from R.R. A.R.M. and J.A.B. wrote and edited the paper. C.A.M. and R.R. edited the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41684-022-01017-9>.

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Software and code

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Data collection The Sensory Sentinel V2.0 software was used in conjunction with the Sensory Sentinel environmental monitoring device (both developed by Turner Scientific) to collect all data.

Data analysis MATLAB (R2020b; Mathworks Inc.) was used to conduct Kruskal-Wallis H tests (`[p,tbl,stats] = kruskalwallis(__)`), Mann-Whitney U Tests (`[p,h,stats] = ranksum(x,y)`), and linear mixed effects models (`lme = fitlme(tbl,formula)`). Microsoft Excel 2016 was used for calculations of mean (= AVERAGE), median (= MEDIAN), IQR (= QUARTILE), standard deviation (= STDEV.S), skewness (= SKEW), and kurtosis (= KURT).

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Study description	The present study was conducted to assess environmental conditions in the Wilson Hall Animal Facility (WHAF), which houses nonhuman primates (NHPs) used for auditory research at Vanderbilt University Medical Center. Noise, vibration, temperature, humidity and luminance were recorded using an environmental monitoring device placed inside of an empty cage in a macaque housing room. Recordings lasted one week each, at three different locations within the room. Vibration, temperature, humidity and luminance all varied within recommended levels for NHPs, with one exception of low luminance levels in the bottom cage location. Noise levels at each cage location were characterized by a low baseline of 58 – 62 dB SPL, with transient peaks up to 109 dB SPL.
Research sample	The sample for this study was collected from a room that houses ten rhesus macaque monkeys in modular primate cages. Similar studies have been conducted in rodent housing facilities, but there is a general lack of information regarding environmental conditions, particularly noise levels, in NHP facilities. This study was conducted to characterize environmental conditions specifically in the WHAF housing rooms, although the results are likely applicable to other NHP facilities as well.
Sampling strategy	A Sensory Sentinel environmental monitoring device capable of measuring noise, vibration, temperature, humidity and luminance was used to record a value for each of these variables every second for 24 hours per day. The microphone and accelerometer both utilized a sampling rate of 192 kHz to generate an averaged sound level that was recorded each second, whereas the temperature, humidity, and luminance sensors simply sampled once per second. All environmental variables were recorded for three one-week-long periods, with the exception of week 1, which had only 6 days' worth of data due to a cage change conflict. The device was placed inside of a cage located in a different part of the room for each of the three weeks. No statistical methods were used to predetermine sample size. Rather, a total recording period of 3 weeks was chosen to allow us to assess potential variations in environmental conditions across three different locations for all 7 days of the week. This resulted in a total of approximately 1.8 million data points for each environmental variable, which was more than enough for our analysis.
Data collection	For recordings, the device was placed inside an unoccupied primate cage unit in either an upper or lower module, with a macaque separated into the other module by a dividing grate and a wood-chip filled metal tray. In this way, the device was able to measure the naturalistic environment inside of a cage while still remaining separate from the macaques. Recording was initiated, and then the device was left in the same position to record for 1-week periods. Using TeamViewer, we routinely monitored the status of the device without having to enter the housing room.
Timing and spatial scale	From 03/18/21 to 03-24-21, the device recorded in a top cage in the middle of the room (TM). From 03/24/21 to 03/31/21, the device recorded in a bottom cage in the middle of the room (BM). From 03/31/21 to 04/07/21, the device recorded in a top cage in the edge of the room. The first week of recording (03/18/21 to 03/24/21) only lasted 6 days due to a cage change conflict. The other two locations were each recorded in for the full 7 days of the week.
Data exclusions	Data were not excluded from the analyses.
Reproducibility	We consulted with Turner Scientific regarding the methods of our study and confirmed that they are in accordance with similar studies and site visits. The methods included in the manuscript are sufficient for replication by other groups.
Randomization	Randomization was not relevant to our study as our goal was not to manipulate any variables but simply to characterize baseline conditions.
Blinding	Blinding was not relevant to this study as the data collection and analyses did not involve any subjective judgments.
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