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Sound levels in modern rodent housing rooms are an uncontrolled environmental variable with fluctuations mainly due to human activities

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Abstract

Noise in animal housing facilities is an environmental variable that can affect hearing, behavior and physiology in mice. The authors measured sound levels in two rodent housing rooms (room 1 and room 2) during several periods of 24 h. Room 1, which was subject to heavy personnel traffic, contained ventilated racks and static cages that housed large numbers of mice. Room 2 was accessed by only a few staff members and contained only static cages that housed fewer mice. In both rooms, background sound levels were about 80 dB, and transient noises caused sound levels to temporarily rise 30–40 dB above the baseline level; such peaks occurred frequently during work hours (8:30 AM to 4:30 PM) and infrequently during non-work hours. Noise peaks during work hours in room 1 occurred about two times as often as in room 2 ($P = 0.01$). Use of changing stations located in the rooms caused background noise to increase by about 10 dB. Loud noise and noise variability were attributed mainly to personnel activity. Attempts to reduce noise should concentrate on controlling sounds produced by in-room activities and experimenter traffic; this may reduce the variability of research outcomes and improve animal welfare.

Noise in rodent housing facilities is rarely controlled as an environmental variable and is often monitored only in areas where human hearing may be at risk, such as cage washing facilities. This lack of environmental control contrasts with the stringent monitoring that is standard for many other aspects of rodent housing, such as veterinary care, infection status, sanitation, heating, ventilation and air conditioning. Noise can directly affect auditory studies by damaging subjects' hearing, and it can indirectly affect many other aspects of research by causing animals physiological stress[1–5]. Involuntary exposure to noise is recognized as a source of distress in humans, and by extension, there may be appreciable animal welfare concerns if the noise in the animal facility is loud enough to cause stress, cause hearing damage or disrupt sleep.

To our knowledge, the most recent comprehensive surveys of variability and sources of noise in rodent housing rooms were conducted between 10 and 20 years ago[1–3]. These surveys showed that noise levels varied significantly during the work day but not during non-work hours[1]. Sources of noise included mechanical systems, electronics, utilities such as running water and telephones, sanitation equipment and animal care activities[2,3]. Many of these sources produced sounds in the range of mouse hearing[1–3].

In the years since the publication of those surveys, noise-generating mechanical infection-control systems such as individually ventilated cages and cage changing stations have become

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more prevalent in rodent facilities. A study has shown that overall sound levels in rooms containing ventilated caging systems are higher than sound levels in rooms containing no equipment[6]. Although the ventilated cages themselves generate only a modest increase in noise, use of these caging systems might also have indirect profound effects on sound levels: compared with static caging systems, ventilated systems enable rodents to be stocked at higher densities, which can result in an increase in personnel traffic and associated noise. Other types of equipment that may generate noise include mechanical sanitation devices such as steam cleaners and other high-tech housing solutions. To our knowledge, the effects of these new systems on the overall soundscape in modern rodent facilities have not been previously reported.

We monitored sound levels over several periods of 24 h in two mouse rooms that were similar in size and layout but differed in the types of caging systems they contained, the numbers of cages they contained and the numbers of investigators who accessed them. Infection-control systems (barrier caging and use of cage changing stations) were implemented in both rooms. We collected data for the following purposes: (i) to measure baseline and transient sound levels during work and non-work hours; (ii) to identify sources of loud sound (>80 dB); and (iii) to compare the soundscapes in the two animal rooms.

We found that sound levels varied more during work hours than during non-work hours, and that patterns of noise differed between the two rooms. Most noise was produced by human activities. On the basis of our results, we recommend methods to reduce loud sounds and sound level variability in modern rodent housing rooms.

METHODS

Room characteristics

All measurements were carried out at the Johns Hopkins Medical Institutions, a facility accredited by the American Association for the Accreditation of Laboratory Animal Care, International.

We monitored two mouse rooms (room 1 and room 2) that were located on the same corridor. Both rooms were maintained on a light:dark cycle of 14 h:10 h. Surfaces in the two rooms (epoxy-coated cinderblock walls and stainless steel equipment) could be easily sanitized but were sound-reflective. Each room contained a cage changing station (Aniguard II, Baker, Sandford, ME).

Room 1 (area of 17.3 m^2) contained four racks. The racks held a total of 280 shoebox cages that housed mice for 51 investigators. Three of the racks contained individually ventilated cages (Allentown Caging Equipment, Inc., Allentown, PA). Motors were located on top of or next to their respective ventilated racks. The fourth rack contained static filter-top cages. Room 1 was located across the corridor from an anteroom adjacent to the main entrance. The door between the anteroom and the corridor was typically propped open to ease traffic flow in and out of the facility. Room 1 was also close to a room that was used as a personnel office and supplies storage area.

Room 2 (area of 12.6 m^2) contained four racks. These racks held 100 static filter-top cages that housed mice for three investigators. Room 2 was located at the end of the facility corridor in a low-traffic area.

Sound measurement equipment

We measured overall sound levels in the range of 20 Hz to 23.6 kHz using a sound level meter with data-logging capabilities (Larson Davis LxT2, Provo, UT). The sound level meter was fitted with a free-field microphone (0.5 in diameter). Sound levels were reported using Z-weighting (international standard IEC 61672), which provides a flat frequency response across the detection range of the sound level meter (20 Hz to 23.6 kHz). In contrast, A-weighting (international standard ISO 226) would generally be used for collecting data relevant for human hearing. A-weighting gives increased weight to frequencies important for human hearing by attenuating response to frequencies above and below 1000 Hz. Z-weighting is more appropriate for obtaining data relevant for mouse hearing, because laboratory mice with normal hearing capabilities sense higher frequencies (1 kHz to 100 kHz) than do humans (20 Hz to 20 kHz; ref. [7]), and mouse hearing is most sensitive in the 10–20 kHz region, where human hearing drops off[8–10]. Accordingly, sound pressure levels will typically be higher for recordings made using Z-weighting than with A-weighting.

Confirmation that sounds were in the range of mouse hearing was accomplished using both the sound level meter's third octave band measuring capability and a custom measurement system. The custom system measured frequencies up to 100 kHz using a free-field microphone (0.25 in diameter; Bruel & Kjær Type 4939, Nærum, Denmark) powered by a microphone power supply (Bruel & Kjær Type 2804, Nærum, Denmark). Frequencies were digitized and analyzed by programmable hardware modules (Tucker Davis Technologies (TDT) System 3 hardware, Gainsville, FL) and a computer equipped with custom-designed software to provide a visual display of the frequency spectrum of the sounds.

Microphone placement

We positioned the microphone of the sound level meter inside an empty polycarbonate mouse cage with a wire bar lid and isolator top. The cage was placed on the middle shelf of a rack located approximately 4 ft from the change station in the room that was being monitored. To prevent disturbance to the controls of the sound level meter, we kept the meter in a small locked box, which we placed on the cage rack during data recording. We did not put bedding or food in the cage that contained the microphone to prevent debris from compromising the integrity of the microphone membrane; preliminary measurements showed that these items had very little effect on sound measurements (<1 dB). We positioned the microphone by extending it 3–4 inches into the cage through a hole (0.5 in diameter) that had been drilled in the front of the cage. We rested the microphone on a small piece of foam to prevent it from making contact with the cage. The microphone filled the entire hole. When we used the smaller (0.25-in) microphone with the custom system, we used acoustic foam to fill the extra space in the hole.

Sound measurement protocol

In each room, sound levels in the cage containing the microphone were measured every 2 s for three separate 24-h periods. Data were recorded on different days for each room because only one sound level meter assembly was available. We defined 'daytime' sound levels as those measured during normal weekday work hours for animal husbandry personnel (8:30 AM to 4:30 PM). We defined 'nighttime' sound levels as those measured from 4:30 PM to 8:30 AM. No recordings were made on institutional holidays or weekends. To avoid potentially confounding effects of observer presence during these long recording times, the experimenter was not present in the room except to turn the equipment on and off.

During 1–2-h sessions, an observer noted sound levels that were produced by specific activities and equipment. Any activity that produced sound that was noticeably above background levels (above 75–80 dB) during the observation period was logged. The observer was blind to the results of the daytime and nighttime measurements.

Statistical analyses

We used descriptive statistics to summarize data collected for each room. For each data recording period (daytime or nighttime), we calculated the difference between maximum and minimum sound levels and the percentage of noise exceeding 80 dB. For each room, we calculated the daytime and nighttime means of each of these values. We chose the cutoff level of 80 dB because sounds above this level were clearly distinguishable above background sounds on the sound level meter. We used *t*-tests (with significance set at $P < 0.05$) to compare data between the two rooms and to compare between daytime and nighttime measurements within each room.

RESULTS

Comparison between room 1 and room 2 Daytime measurements (8:30 AM to 4:30 PM)

Sound levels in rooms 1 and 2 during normal weekday animal care personnel work hours (measured on 3 separate days for each room) are shown in Figure 1. The mean difference between maximum and minimum sound levels was 34.47 dB in room 1 and 33.77 dB in room 2. The mean percentage of sound levels greater than 80 dB was 44.80% in room 1 and 21.26% in room 2. A student's *t*-test showed that the differences between maximum and minimum sound levels were similar in the two rooms ($t = 0.24$, $P = 0.82$), but the percentage of noise above 80 dB in room 1 was more than two times that in room 2 ($t = 4.39$, $P = 0.01$).

Nighttime measurements (4:30 PM to 8:30 AM)

Nighttime sound levels for the two rooms are shown in Figure 2. The mean difference between maximum and minimum sound levels was 29.60 dB in room 1 and 24.92 dB in room 2 and did not differ significantly between rooms ($t = -0.19$, $P = 0.86$). The mean percentage of noise above 80 dB did not differ between rooms (0.91% in room 1 and 0.14% in room 2; $t = -1.99$, $P = 0.12$).

Comparison between daytime and nighttime measurements—A visual inspection of the sound level data clearly suggests that overall noise variability was lower during nighttime recording periods than during the daytime (Figs. 1 and 2). The range of sound levels did not differ significantly between daytime and nighttime measurements within either room (room 1: $t = 1.84$; room 2: $t = 1.45$, $P = 0.22$). In room 1, however, the percentage of noise above 80 dB differed significantly between daytime and nighttime measurements ($t = 3.85$, $P = 0.018$), and this difference approached significance in room 2 ($t = 2.65$, $P = 0.06$). The largest decrease in percentage of peaks above 80 dB occurred in room 1, where the percentage decreased from 44.80 to 10.69.

Sources of high sound levels—The following sources of daytime sound levels higher than 80 dB were recorded: using change stations or closing room doors (84–105 dB), changing cages and returning them to the racks (80–97 dB), experimental procedures (75–100 dB), talking or shouting (83–100 dB), cart wheels squeaking (82 dB), vocalizations from dogs housed one floor below (80–82 dB; room 2 only) and miscellaneous noise outside the room when the door was left open (83–92 dB). Opening and closing doors was the most frequent source of brief noise peaks. The sound levels from these sources varied from one instance to another, and they sometimes fell below 80 dB. All of these sources produced sound within the range of mouse hearing.

Other sources of sound were continuously present and combined to produce background noise. These included ventilated rack motors (room 1 only), building ventilation and mouse activities such as obtaining food pellets, digging in bedding and vocalizing (audible to humans in room 2 only). Several times throughout each day in room 1 and for one period on the third recording day in room 2, baseline sound levels increased by about 10 dB owing to operation of the cage changing station next to the housing rack. This is a substantial increase: the dB scale is logarithmic, and therefore a change of 10 dB in sound level reflects a 10-fold change in sound power.

Specific sources of nighttime sounds were not documented, but we presume that nighttime sounds at levels higher than 80 dB originated mainly from animal activity and mechanical systems.

DISCUSSION

We carried out this study to evaluate the soundscape in our rodent housing facility and to document sources of background and transient sounds, loud sounds and noise variability in two rooms with different barrier caging systems. Our results suggest that despite the introduction of new mechanical equipment, the soundscape in our facility is not substantially different from those described in reports published 10–20 years ago[1–3]. Furthermore, our results show that soundscapes can vary significantly among different animal rooms. Like Milligan and colleagues[1–3], we noted that noise varied over time during the work day, and noise fluctuations were reduced during non-work hours. Although it is difficult to make a direct comparison due to the different frequency weighting used in this study, overall sound pressure levels appear to be similar.

The soundscape in an animal facility is composed of background (baseline) noise arising from electrical and mechanical systems and of transient noises attributable to a variety of sources. In this study, sources that contributed to background noise but did not exceed 80 dB included changing stations, ventilated racks, lighting systems and building ventilation systems. Baseline noise is of concern if the sound level is sufficiently high to damage hearing, cause stress or impact research outcomes in other ways. Such noise would probably have the greatest effect on auditory research, because even moderate-level noise might affect auditory processing. For example, rats raised in moderate-level noise develop abnormal neural responses in the auditory system, including abnormal neural plasticity and abnormal processing of the frequency, time and intensity information that is important for perception of biologically relevant sounds[11,12]. Thus, exposure to continuous moderate noise levels may result in effects that are not immediately detected by simple hearing screenings such as the Preyer reflex test or auditory brainstem response measurements. In addition, exposure to noise during gestation has been shown to affect maternal and fetal health in mice, perhaps through causing stress[13–15].

The present study shows that mice are exposed to frequent loud sounds, particularly during work hours. As in previous studies, most of the intermittent loud noises (>80 dB) we observed were attributable to human activities. Hearing in chinchillas can be damaged by irregular noise with intermittent sound peaks that vary in amplitude and distribution[16], a pattern of sound similar to that observed in the present study. Longer exposure to intense sounds can lead to permanent hearing loss in susceptible mouse strains such as C57BL/6 and BALB/c (refs. [17–26]). Susceptibility to noise-induced hearing loss varies across strains and ages; not all strains have been tested for susceptibility[17–27]. Intense sounds can also induce audiogenic seizures in some strains of mice, notably DBA/J and C57BL/6 (refs. [28,29]). In humans and other species, noise can also result in sleep disturbances that can have multiple physiological and behavioral sequelae[30], and noise can induce a host of physiological responses independent of sleep effects[4].

In this study, the soundscape differed significantly between two rodent housing rooms that were located on the same corridor. This suggests that when animals in the same or related studies may be affected by noise, they should not be housed in different rooms unless the soundscapes are monitored and controlled. We do not, however, advocate creating a completely impoverished sound environment for several reasons. First, it is not possible to create a completely silent environment, as the animals themselves create noise through vocalizing, feeding and other behaviors. Second, a normal level of human interaction and animal health checks should be maintained to ensure the animals' welfare. Third, an impoverished sound environment could have unintended effects on neural processing in mice. Rather than eliminate extraneous sounds from an animal's living environment, laboratory staff members should control and monitor, to the extent possible, sounds originating from extraneous sources. Ideally, a housing room should be quiet enough for mice to hear one another vocalizing and should have minimal potentially harmful loud noise.

Caveats to measuring sound levels in the range of rodent hearing

To our knowledge, commercial sound level meters for measuring sound levels that span the entire range of mouse hearing and have 24-h data-logging capabilities are not currently available. Custom-designed systems are very expensive and require specific technical knowledge to operate. We chose to use a commercially available sound level meter with a frequency bandwidth that overlaps the range of mouse hearing so that similar measurements might be taken in other institutions that do not have access to sophisticated equipment. Although this limited the frequency bandwidth we could monitor, the range we did monitor encompassed the range of frequencies at which hearing is most sensitive in mice[8–10]. Furthermore, our measurements and those published previously suggest that most sources of noise to which a laboratory animal may be exposed produce sound within the frequency range audible to mice[1–3].

A second caveat is that the presence of a human observer or recording equipment may have affected the behavior of investigators and animal care staff. Noise levels may actually be higher when no observer or monitoring equipment is present. Care should be taken to measure sounds as unobtrusively as possible to obtain the most accurate assessment of the soundscape.

Guidelines for safe exposure to noise

Current literature does not provide sufficient information to establish guidelines for safe exposure of mice to noise; it focuses mainly on mouse response to short-term, intense sounds[17–27]. Safe levels of exposure to noise probably vary across mouse strains and studies. It seems prudent, however, to minimize loud and intermittent noises, which can reduce hearing ability, affect physiological responses and generally act as an uncontrolled study variable. Additional guidelines for safe exposure will probably become available in the future: the Acoustical Society of America has recently launched an effort to establish noise exposure standards for animals (Accredited Standards Committee S3, Subcommittee 1, Animal Bioacoustics).

Noise control

Controlling vivarium noise may reduce the direct and indirect consequences of noise exposure. Our data suggest that controlling noise attributable to human activities will have the greatest effect on reducing overall animal room noise and noise variability. Efforts should be directed at reducing personnel traffic in animal rooms, locating noisy equipment and activities as far away from housing racks as possible and keeping animal room doors closed. To reduce activity inside animal rooms, biosafety cabinets and change stations should be located in separate procedure rooms when possible. If a change station must be located in an animal room, the distance between the change station and the rack should be maximized: in an anechoic environment (one that is treated to eliminate sound reflections and reverberations), sound level decreases by 6 dB for each doubling of distance from the source[31]. This rule is probably not completely accurate for animal vivaria because they contain highly sound-reflective surfaces,

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but it does provide a guideline. To further reduce personnel noise, quiet working practices should be emphasized. One study showed that differing work styles can result in a difference of more than 15 dB in peak noise during cage changing[32]. Particularly noisy activities such as vacuuming should not be conducted in animal rooms[33]. Self-closing doors can reduce the incidence of doors left open by accident, and weighted door closure mechanisms can be installed to reduce door-slamming.

Regardless of noise control procedures, there will inevitably be more personnel activity during the day. If research results might be affected by sleep disruption owing to noise, reversing the light cycle (so that mice are awake during maximum noise disturbance, that is, during work hours) might be advisable, particularly in rooms with much personnel traffic. If the effects of noise disruptions during the dark (active) phase are a concern (for breeding success, for example), reversing the light cycle may not be appropriate.

For auditory research, control of background noise in addition to transient noise may be necessary. Although some noise attributable to various systems in the building is unavoidable, noise caused by ventilated racks can be reduced by placing ventilation motors outside the room or fitting the motors with covers. Most auditory research is carried out inside sound attenuation booths to control the effects of extraneous noise; however, sound exposure outside of experimental time may also affect the animals.

Finally, a system for sound surveillance should be considered so that loud noises and noise variability can be detected and minimized. With an ideal monitoring system, sound levels would be measured continuously, stored digitally and reviewed regularly. Such a system can be expensive, however, and may take up already limited space. As an alternative, periodic scheduled monitoring may also be effective in alerting animal care and veterinary staff when noise levels are not well controlled.

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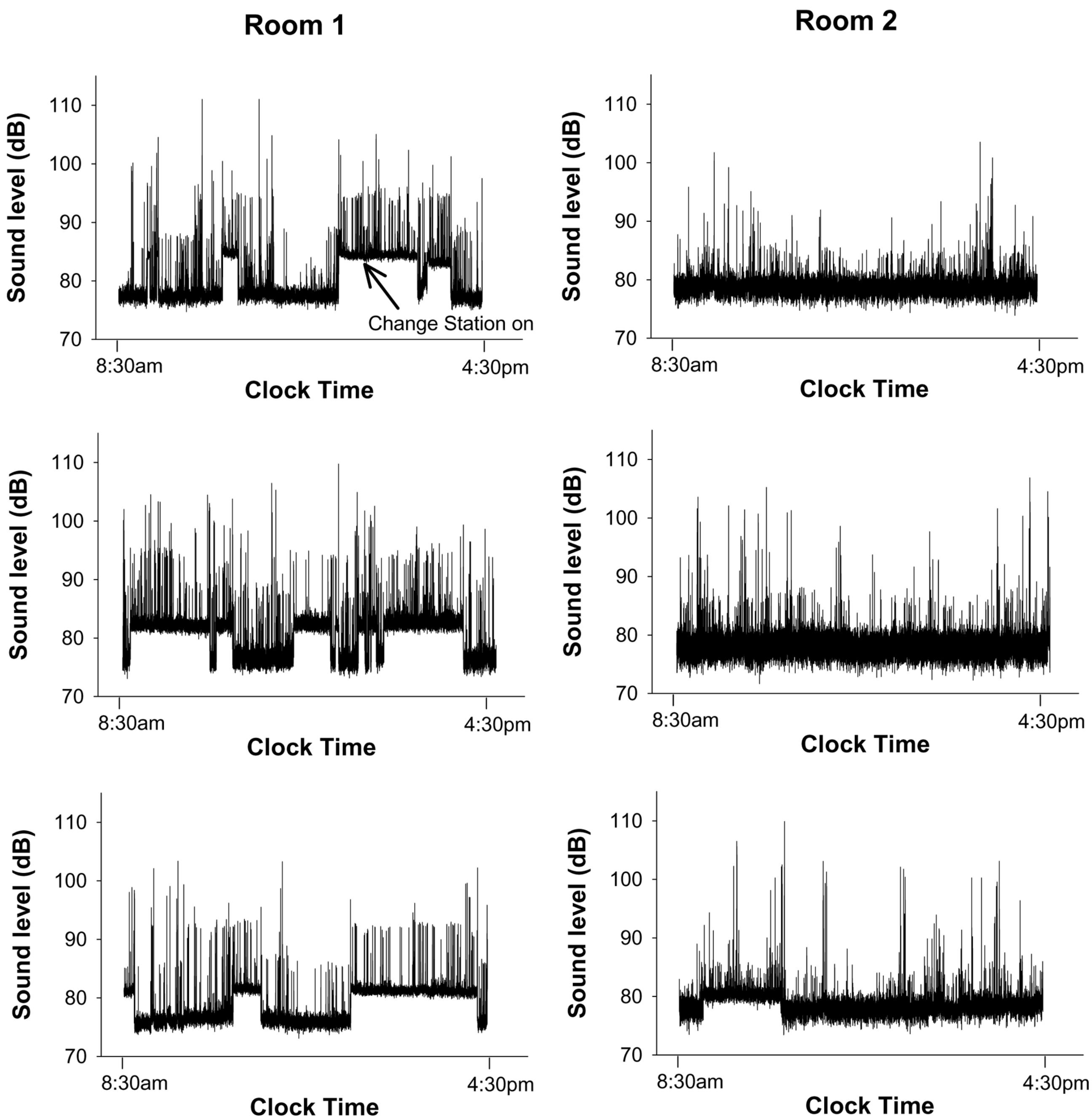

FIGURE 1.
Daytime sound levels (recorded from 8:30 AM to 4:30 PM) for room 1 (high-traffic, high stocking density, used by numerous investigators) and room 2 (low-traffic, low stocking density, used by few investigators). Frequent ‘spikes’ in sound level occur throughout the day.

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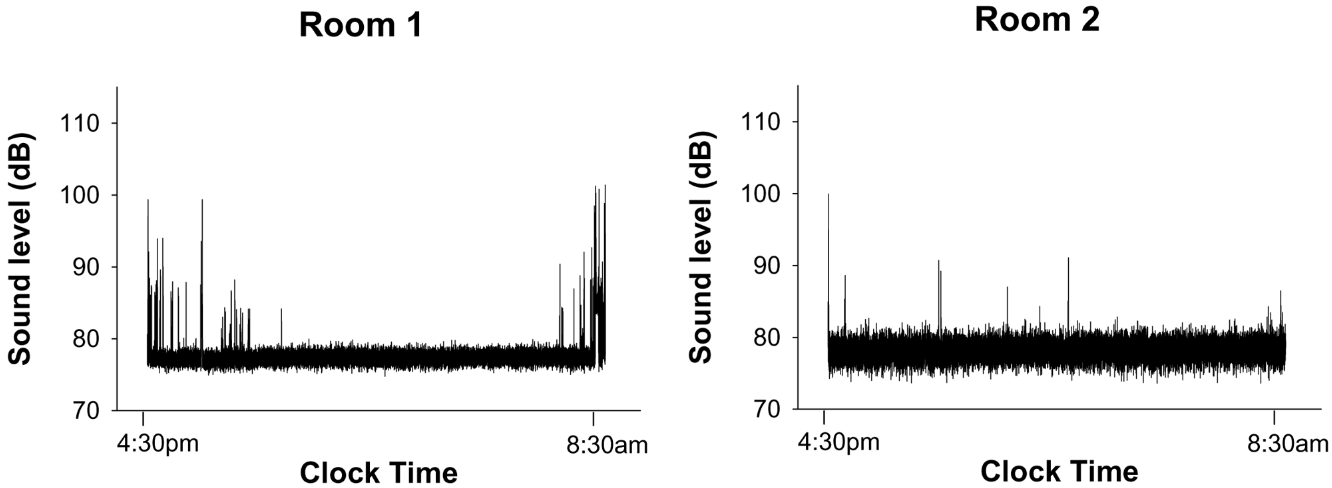

FIGURE 2.

Nighttime sound levels (recorded from 4:30 PM to 8:30 PM) for room 1 (high-traffic, high stocking density, used by numerous investigators) and room 2 (low-traffic, low stocking density, used by few investigators). Compared with daytime measurements (Fig. 1), only a few spikes in sound level occur throughout the data collection period.