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Noise as an Extrinsic Variable in the Animal Research Facility

Jeremy G Turner, PhD[1*] and John R Manker, BA[1]

Animal research facilities are noisy environments. The high air change rates required in animal housing spaces tend to create higher noise levels from the heating and cooling systems. Housing rooms are typically constructed of hard wall material that is easily cleaned but simultaneously highly reverberant, meaning that the sound cannot be controlled/attenuated so the sounds that are generated bounce around the room uncontrolled. (Soft, sound-absorbing surfaces generally cannot be used in animal facilities because they collect microbes; various wall surface features and sound control panel options are available, although rarely used.) In addition, many of our husbandry tasks such as cage changing, animal health checks, cleaning, and transporting animals produce high levels of noise. Finally, much of the equipment we have increasingly employed in animal housing spaces, such as ventilated caging motors, biosafety, or procedure cabinets, can generate high levels of background noise, including ultrasound. These and many additional factors conspire to create an acoustic environment that is neither naturalistic nor conducive to a stress-free environment. The acoustic variability both within and between institutions can serve as an enormous confounder for research studies and a threat to our ability to reproduce studies over time and between research laboratories. By gaining a better appreciation for the acoustic variables, paired with transparency in reporting the levels, we might be able to gain a better understanding of their impacts and thereby gain some level of control over such acoustic variables in the animal housing space. The result of this could improve both animal welfare and study reproducibility, helping to address our 3Rs goals of Replacement, Reduction, and Refinement in the animal biomedical research enterprise.

Abbreviations and Acronyms: L_{eq}, equivalent continuous sound level; SONAR, sound navigation and radar; SPL, sound pressure level; USN, ultrasonic noise; WHO, World Health Organization

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Introduction

Animal research housing spaces tend to be harsh acoustic environments with hard reverberating walls and surfaces that are more easily cleaned. These spaces also require high air exchange rates and are often fitted with rack ventilation systems, which can also make significant noise. Because such systems are running continuously, this can generate a high level of background noise in facilities that may vary by season and will vary between institutions. A range of additional equipment in the rooms (such as biosafety/cage-changing hoods), as well as the husbandry practices themselves, can generate more acute high levels of noise for animals. These factors combine to produce animal housing spaces that generally have high levels of noise and a high degree of variability between rooms and across different institutions as a function of many variables such as caging style, the presence of different types of equipment, construction, etc.[2,32,43] The impacts of noise on animal biology and behavior are far-reaching, with consequences for both animal welfare and study reproducibility in virtually every aspect of biomedical research.[43] The net result is a layer of additional research variability, which makes it difficult to study model systems in a controlled manner, resulting in poor replication of studies across time and institutions, wasted resources and animals, and relatively poor translation of experimental treatments for disease from research animals to humans.

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[1]*Turner Scientific Monitoring, Jacksonville, Illinois*
Corresponding author. Email: jturner@turnerscientific.com

Estimates suggest that a staggering 70% to 90% of findings in biomedical research are not reproducible.[49] Even using a much more conservative approach and assuming that only half of animal studies are not reproducible, this yields a financial impact of at least \$28B US dollars wasted annually (as of 2015) on animal research findings that cannot be replicated.[11] While these financial losses are significant, the real losses are to animal life and delayed treatments and cures. This problem has produced a crisis in public confidence in biomedical research and an act of the U.S. Congress (House Resolution 34 – 21st Century Cures Act) prompted the NIH to impanel an Advisory Committee to the Director (ACD) to study the problem and suggest avenues for improving reproducibility and rigor of the research it supports. The 2021 report entitled "ACD Working Group on Enhancing Rigor, Transparency, and Translatability in Animal Research"[23] identified some key areas of improvement. Among the main recommendations of the report (Recommendation 4.3) was to better ensure that researchers know which extrinsic environmental factors (such as noise, vibration, light, and other extrinsic factors) in an animal facility can affect research outcomes and therefore critical to document. Like vibration and light levels, most facilities do not monitor their animal facility noise levels, as is done daily for temperature and humidity.

This article will provide some key context and background information on noise and the typical 'soundscape' in animal facilities that will enable facility administrators, veterinarians, and researchers to make decisions about why and how to control this key extraneous environmental variable.

Soundscapes and the Umwelt

All animals (even insects and aquatic species) exist in a sound environment (soundscape) filled with a combination of sounds from various sources; some self-generated (vocalizations, noise from movement, etc.), some produced by their conspecifics for social interactions, others from predators or prey, some from the natural environment (trees blowing in the wind, water rustling through a stream), and others that are anthropogenic, human generated (traffic from planes, trains, automobiles, ships, factories, and various sounds created by lab care staff or equipment used during normal operations in a research facility). The soundscape is used by animals for a wide range of functions, but especially for many of the prey species (for example, rodents and fish) used in biomedical research, the soundscape primarily serves as an efficient tool for detecting the presence of threats. In an increasingly 'noisy' world and research animal facility, soundscapes have become more chronically intrusive, intense, and variable, producing challenges for laboratory research animals. A research mouse or zebrafish's soundscape typically consists of a constant mechanical noise in their housing environment from mechanical ventilation and water pump/filtration systems. Additional dynamic stimuli from workers and other activities can create an especially complicated and noisy soundscape.

Many in the animal care and use space assume that with repeated exposure to extrinsic environmental factors like noise, the animals would become accustomed to the stimuli and demonstrate reduced harmful impacts. This is a rational assumption given that central to the survival of all species is the ability to discriminate novel, potentially harmful stimuli from familiar and presumably safe stimuli.[40] While relatively little is known about how laboratory research animals in their home cages adapt to dynamic environmental conditions in their housing spaces, considerable laboratory research has been done on their responses to novel and familiar stimuli in the controlled laboratory setting. This process is so fundamental to survival that it underlies the simplest form of animal learning, habituation, which is present from amoebas and invertebrates to humans[42] and serves as a prerequisite for other forms of associative learning.[31] Habituation is a reduced response of the organism in response to repeated exposure to a previously novel but otherwise generally harmless stimulus, and as a form of learning, it is distinct from sensory adaptation or sensory/motor fatigue.[31] However, with habituation certain principles have to be considered such as the predictability of the stimulus, time between stimuli, intensity of the stimuli, etc., which can determine whether habituation occurs. Indeed, with repeated exposure to stimuli deemed noxious or harmful the opposite can occur, sensitization, which is a heightened response to the stimulus. In addition, just because an organism might behaviorally habituate or adapt to a stimulus in a somewhat predictable environment, that does not mean the stimulus is necessarily without harm. Indeed, extensive research on humans living near noisy roads, railroads, and airports shows that while people tend to become accustomed to the noise in their environments, their health markers nonetheless show signs of the biologic consequences, evidenced by a wide range of increased noise-related biologic health risks such as increased blood pressure and increased incidence of cardiovascular damage.[40,47] Therefore, while we may think that humans and perhaps even research animals seem to behaviorally adapt to our presence and activities, their biologic impacts (for example, change in stress hormones, impacts on sleep, etc.) are more likely to be additive, rather than adaptive, in nature. Indeed, a recent study showed that animals housed in higher cage positions where noise, vibration, and light levels were all measurably higher, the animals showed higher levels of corticosteroids after 3 mo of study but not after 6 mo, presumably a sign of adaptation in corticosteroid response sometime after 3 mo of exposure.[7]

It should also be noted that habituation or adaptation to environmental noise will not likely occur the same over the wide range of species used in biomedical research. For example, zebrafish and mice will not have the same ability to contextualize environmental noise variables as would larger brained species like mini-pigs and NHPs. Mice, for example, demonstrate relatively poor habituation and high variability between strains, across test sessions in a single day, let alone across multiple days of similar exposure.[5]

A key additional variable in the understanding of the soundscape is related to our relatively poor understanding of the umwelt of the animals themselves. Umwelt is a German word for environment, and it is often used more broadly in ethology to describe the unique sensory experiences of an animal.[18] We humans naturally tend to take a very anthropomorphic view of what our animals experience. However, most generally recognize that a dog's color visual experience is different from humans because of their different constellation of cones and that their olfactory sense is much broader and more critical for their behavioral function than it is in humans. This disparity between humans and our lab animals can also extend to other less well-considered senses like hearing. Human and lab animal senses have evolved to aid survival in different competitive environmental niches. The same soundscape can produce a fundamentally different, even unrecognizable, umwelt for a mouse, rat, pig, fish, monkey, and human. For example, all laboratory mammals, fish, and avian species hear a very different soundscape than our human ears. Mice do not hear low-frequency sounds below 1,000 to 2,000 Hz (Hz) well,[14] a frequency range that humans use extensively in our communication. (Mice can, however, 'feel' sound below 1,000 Hz via their vibration sense, if the amplitude is high enough to shake or resonate with the caging or other elements around the animal.) Instead, mice and most commonly used research mammals hear and use sound frequencies in a much higher range, well beyond the upper range of human hearing at 20,000 Hz. Such ultrasonics (sounds above 20,000 Hz), emanating from motorized equipment (computers, certain lights or occupancy sensors, etc.), are routinely present in our animal housing and research lab spaces, creating a hidden, confounding soundscape for many of our research animals and the studies they are involved in.[34,43] Similarly, because we humans cannot hear the noise inside a zebrafish tank, we do not appreciate what their soundscape is like. If we are to make additional progress in understanding the impacts of soundscapes on our research animals, we have to begin by understanding and appreciating not only their soundscape but also their umwelt. Biomedical research has made some progress in understanding the soundscape and umwelt of our research animals, as evidenced, for example, by the many publications and broader awareness of rodent ultrasonic vocalizations. This progress has been enabled by sensors designed to measure such information to help make up for the limitations of our human senses. However, there is clearly more work to do because too few of our animal facilities and researchers make use of such sensors.

Noise and Silence

The concept of noise is used in many fields of study, and it is generally used to describe excessive, additional, or unwanted stimulus energy that contrasts with a target stimulus. The concept of noise is not just used in the study of sound but for all sensory systems (for example, vision, touch, and olfaction). Every sensory system is charged with detecting a signal in a 'noisy' environment, and there is always some level of noise present. A visual image of a face (the signal) is contrasted with the other visual cues present (noise). Detecting a vibratory event requires the sensory system to contrast it with other background vibratory noise. Noise can be generated internally (ongoing neural and/or receptor activity, such as in tinnitus) or external to the animal.

The problem with the definition of noise as excessive, additional, or unwanted sound is that it also depends on the receiver; one person's (or research animal's) noise is another's music, or vice versa. The simplest definition of acoustic noise is the absence of silence. Complete silence is not possible except under highly controlled conditions in a vacuum or in space where there are too few particles in the air to vibrate and make sound waves. Therefore, in a very real sense, there are no noise-free environments, and the goal is not the removal of all noise because that would serve as an impoverishing environment with significant consequences. Some low-level noise can also serve to prime the auditory system and can aid in signal processing. This phenomenon, known as stochastic resonance, has been observed in multiple systems and is associated with improvements in hearing.[37] The practical discussion instead turns to what are the levels of noise in the space of interest and do they cause harm or impede function in some way. In the present context, noise will be treated synonymously with sound and defined simply as the level of sound present (usually in decibels).

Sound Measurement

While content from the following sources will be reviewed here, for a more thorough review of sound and its measurement, see other references.[17,33] Noise or sound results when a source vibrates, thereby causing molecules in the medium to compress and bunch together into areas of high pressure (condensations) and spread apart in adjacent areas of low pressure (rarefactions), creating waves that propagate through the medium. Noise is otherwise identical to vibration, and the only difference between them is whether the medium is a thinner medium like air, something with more densely packed molecules like water, or something denser still like rock or steel, which can have unequal features over space and alter how vibrations travel. Sound and vibration are the same physical phenomena and are only differentiated by the medium in which they travel. Vibrating sounds in air travel about 767 m/h (343 m/s), about 4× faster in water and 15× faster in iron. Since noise is vibration in air or water, they share many similarities with respect to the way they can be measured and understood. For example, in both cases, the magnitude of the stimulus is measured as the amplitude of the wave, and the frequency is measured as the number of condensation-to-rarefaction cycles per second of time (cycles per second = Hertz).

The decibel scale (named after Alexander Graham Bell) is a relative unit of measurement expressed as the ratio of 2 values on a logarithmic scale, which compares the measured magnitude against a standard reference level. The decibel unit is used in a wide range of measurements, whenever a signal is compared with some standard. That standard is sometimes used in electronics in reference to 1 V, for example, in which case it would be read as dBV. The typical standard reference comparison used for measuring sound intensity in air typically uses the approximate human threshold of hearing at 20 µPa of sound pressure. While the reference should always be indicated, oftentimes it is not. Unless otherwise indicated and when used to measure sound in air, the decibel level likely then refers to using the 20-µPa reference, setting 0 dB as the approximate threshold of human hearing. In this situation, the decibel level is indicated as the decibel sound pressure level (dB SPL, or simply dB re 20 µPa). When calibrating a microphone to this standard, a reference calibrator typically plays a calibrated 1-Pa signal, which happens to be 94 dB SPL, and the resulting measurement system compares all signals to that reference standard.

A logarithmic scale is typically used in sound measurement because sound pressure magnitude can vary so widely. The mammalian ear is capable of processing an incredibly wide dynamic range of sound pressures, spanning approximately 1 trillion Pa pressure units.[16] Such large units make the logarithmic scaling easier to process and more consistent with how the non-linear ear functions. Expressing the intensity of a loud gunshot in decibels is much easier to grasp than indicating that the gunshot is about a million or even a billion times more intense than a whisper. Because the decibel measurement treats all sound frequency content evenly, sometimes an additional weighting scale is used to further refine the decibel measurement for a particular purpose. For example, decibel A weighting (dB A) adjusts the decibel level to accommodate for the human speech frequencies and is used most widely for occupational noise exposure testing, whereas decibel hearing loss weighting (dB HL) adjusts the decibel level to normal human thresholds across the hearing range and is used most widely by audiologists to estimate hearing loss. The decibel zero or unweighting (dB Z) applies no weighting. While there have been proposals to apply species-specific weighting to decibel SPL levels,[4] this can be difficult to accomplish with strain/breed-specific differences in hearing. Instead, we suggest using an unweighted decibel (or dB Z) measurement to fit the low and high-frequency filters to capture the sound frequency range audible to that species, which is known for all commonly used laboratory animal research models. Note that the traditional decibel meter used for workplace safety assessments and on cell phones is nearly useless for measuring noise in the hearing range of animal research species because they are typically fitted with an A filter (dB A) for human speech.

Sound pressure will spread from a source in all directions and the levels drop proportional to the distance from the source as the energy in the wave dissipates as it spreads over a broader area. This rate of decrease will be 6 dB for every doubling of the distance from the stimulus source. In an animal facility, a ventilated caging motor in need of service or a procedure hood generating 70 dB SPL at 1 m from the source will measure approximately 64 dB SPL at 2 m and 58 dB at 4 m. (Note that this rule of sound propagation is complicated by the fact that animal facilities are typically square or rectangle box-style rooms with hard surfaces that reflect/reverberate the sounds back, adding to the measured noise level.) In addition, because sounds are measured using a logarithmic scale, they cannot be simply added together linearly. The 2 sources must first be converted to pressure levels in Pascal and then converted back to the logarithmic scale. Two identical ventilated caging motors, each generating 60 dB SPL would add 3 dB to the overall noise level yielding a 63-dB SPL signal in the room from the 2 motors and 4 identical motors each generating 60 dB would yield an overall decibel level of 66 dB SPL.

Because sound pressures will typically vary over time and consist of steady-state signals intermixed with louder occasional signals, the integration time of the measurements should be clarified. Noise levels can be measured using very fast or impulse settings where the signals are captured at a high sampling rate and integrated over a very short time window of many times per second, over a 1-s integration 'slow' time, or for much longer durations. Sometimes noise surveys will express measurements over a much longer period of time in decibel equivalent continuous sound level (L_{eq}). This is sometimes used to assess the overall averaged noise 'dose' exposure level for a period of time, taking into account dynamic levels over that period of time. The decibel L_{eq8} or L_{eq24}, for example, would describe the overall noise dose over an 8-h workday or a 24-h period of time. These longer-term L_{eq} measurements are typically used to assess 8-h workday exposures for occupational health and safety assessments because they provide a single decibel value 'dose' of noise over a period of time, which can be useful for predicting the amount of hearing damage that might result. Typically, the quietest L_{eq} noise levels that can be attained in a quiet environment is in the 30 to 40-dB L_{eq} range, which would be found in a quiet park or a bedroom at night without an air conditioner running. Normal levels in a workplace office will range from 50 to 70 dB L_{eq}, and normal levels in a typical animal research housing room will range from 50 to 75 dB L_{eq} depending upon many factors; heating and cooling system features (compressors, blower motors, duct, and duct hanger styles), air exchange rate settings, whether ventilated caging motors are used, whether procedure hoods are used in the room, and how much work was done in the room on a particular day.

Previously, we have suggested that the average 24-hr noise levels in an animal housing room should remain below 70 dB SPL (unweighted decibel levels, measured within the frequency hearing range of that particular species) because above this level the risk for noise-induced hearing loss/synaptopathy and/or impacts on brain development and activation of stress pathways are increasingly likely.[43] Incidentally, the World Health Organization (WHO) also suggests that humans maintain an average 24-h noise exposure below 70 dB A and 1-h exposure below 85 dB A because above these levels there is an increased risk for hearing loss and a range of adverse health effects, mostly related to impacts on sleep and cardiovascular function.[47] Many facilities have historically used the Occupational Safety and Health Administration-based standard of 85 dB A as a standard for research animal facilities, but this measure is based on the level of noise known to cause hearing loss in mammals (including humans) over an 8-h noise exposure and not a 24-h day as the animals in a housing facility would experience. Such measurements have also typically been conducted using a sound meter designed for Occupational Safety and Health Administration-based occupational health programs for assessing worker exposures, using an A-weighted filter to focus on the relevant human speech sound frequencies, which are largely irrelevant to many laboratory animals.

Measurements in research animal housing areas should be conducted unweighted, without the A-weighted human filter, and should encompass a wide enough frequency range for the species being tested. For example, most sound meters used for human noise exposure will only focus on sounds up to 8,000 Hz, whereas most laboratory research mammals hear sounds well into the ultrasonic hearing range above 20,000 Hz (the extreme upper limit of human sensitivity). More information about the measurement of noise in the animal housing facility can be found elsewhere, but as a general rule of thumb the smaller the head of the species the higher frequency sounds, they will hear.[43] For example, dogs, cats, swine, and NHPs typically hear sounds up to 40,000 to 50,000 Hz, whereas rats can hear up to 60,000 to 80,000 Hz and mice higher still up to around 100,000 Hz. Many species of bat and aquatic mammals hear much higher frequencies still, as they use them for sound navigation and radar (SONAR) navigation, while many bird and aquatic species specialize in hearing much lower sound frequencies that typically overlap with the human hearing range. Understanding the hearing frequency range of the species of interest and measuring within that range is essential for understanding their exposure levels. Some have suggested fitting different weighting filters for different species (for example, the 'R' scale[4]), but this requires judgments about the loudness functions for each animal species, which can wildly vary by strain in mice and rats. A more simplified approach simply uses no frequency weighting (that is, z or 'Z' weighting) but ensures that the measurement microphone system is set to capture the frequency range of the species of interest. For example, when measuring for mice, we capture all sounds above 1,000 Hz (the mouse ear is not sensitive to sounds below this frequency) and up to about 100,000 Hz, whereas for rats we suggest capturing all sounds above 200 Hz. However, a careful understanding of the hearing range of the particular species and strain of animals being assessed is critical. More information about these ranges can be found in various publications[10,14] and published audiograms and hearing ranges can be found in the literature for all commonly used species and many rarely used. Note, however, that strain or breed differences in hearing within a species are common, especially for inbred mice and some commonly used rats.[45]

As a typical tinnitus (ringing in the ears) sufferer will tell you, it is very difficult to appreciate the value of silence until it is gone. While silence is golden in the proverbial sense, complete silence in the research animal facility and in our lives is neither possible nor desirable. In addition to being virtually impossible (without deafening the animal, which itself can create tinnitus as the brain attempts to amplify the missing sound from the ears), even movement of the animals themselves in their cages will necessarily make some noise. Raising any animal in complete silence creates a form of sensory deprivation, with negative impacts of its own.[46]

The natural habitat for rodents will vary by species, but rats and mice are notoriously opportunistic and adaptable to different environments. Wild mice and rats tend to burrow and build nests underground or in tree trunks or other areas where they are isolated away from the world and its predators. Such burrows tend to have an entrance and exit and a series of chambers for rest and rearing young.[1] Their underground nature makes for a quiet environment where noise and vibration from predators can be easily detected and vocalizations from conspecifics can be easily extracted from the quiet background noise.

The goal of understanding noise levels in our own lives and in the lives of our research animals then should focus not on the impractical and undesirable goal of removing all noise but on understanding and limiting the amount of unnecessary or excessive noise and its secondary impacts. Our field measurements sampled from research animal facilities show that most research animal environments struggle to achieve average background noise levels below about 55 dB SPL in the mouse hearing range and usually closer to 60 to 70 dB SPL for rats or larger species that hear lower sound frequencies from the heating, ventilation, and air conditioning (HVAC) system and other sources. Animal research facilities present very challenging, even harsh acoustic conditions. The solid walls and rectangular rooms we use, with no soft absorbent surfaces, are great for keeping clean and preventing the spread of organisms but make for a very reverberant environment where sounds bounce uncontrollably around. Reverberation is a difficult challenge for animal housing rooms and while there are commercially available exterior-grade sound attenuation panels that can help, such treatments tend to be underutilized by facilities. Their use can make a dramatic difference in an animal room, lowering overall noise levels by approximately 10 dB[51] and making for a much less reverberant room, which makes it much easier for human staff and lab animals to communicate and process relevant cues.

As a field, we have progressively moved away from static caging conditions and toward the use of more ventilated caging options, which can sometimes add considerable noise to the housing room. If our human bedrooms were as loud as many of our animal housing spaces, we would certainly have a more difficult time sleeping and the risk for a range of other health problems would emerge. Over the last couple of decades, facilities have increasingly moved toward the use of cage changing/biosafety hoods inside animal housing rooms. These combined practices of ventilated caging and the use of hoods for cage changing have helped improve the air quality of the room's macro and microenvironment and help to control pathogens from spreading between cages and racks. However, their use has created new noise (and vibration) problems that have to be addressed.[21,24,43] Many, especially older, versions of ventilated caging mounted the motor systems on the rack, sometimes leading to noise and vibration of the rack, particularly as motors age. Motor servicing and careful inspection of the vibration dampeners between motors and racks should be employed as a standard practice in animal housing spaces. Increasingly, facilities have the option to purchase wall or floor-mounted motors, or to place them in interstitial spaces, effectively decoupling the motors from the rack and limiting noise and vibration concerns. The noise and ultrasonic noise (USN) generated from the motor and associated equipment can sometimes be excessive and should be monitored regularly, at least annually, to prevent noise/USN and vibration from becoming animal welfare concerns and from having confounding impacts on research studies.

We have measured from dozens of animal housing spaces, and the quietest mouse rooms are around 45 dB, but more typically in the 55- to 60-dB range, similar to what is reported by others.[6,32] For those species that hear lower frequency content from HVAC systems and other equipment (guinea pigs, rabbits, dogs, pigs, and NHPS), the continuous background noise is typically near or above the 70-dB level. At these levels, the continuous noise is loud enough to impact sleep, mask vocalizations or other communications among animals, and produce a range of other impacts.[47] The U.S. Environmental Protection Agency recommended 4 decades ago that humans maintain a 24-h noise exposure average of less than 70 dB to avoid hearing loss,[9] and the WHO conducted a recent comprehensive review of the human and animal research data and confirmed and adopted the Environmental Protection Agency's 24-h noise exposure threshold of 70 dB.[47]

However, hearing loss is not the only concern. The WHO report also recognized that chronic exposure to levels of noise much lower than this 70-dB threshold, around 45 to 55 dB, can have significant, widespread negative effects on a range of health metrics, largely through activation of stress pathways or impacting sleep patterns and cardiovascular function.[47] The resulting impacts of noise can then include virtually every area of biomedical research. These 'nonauditory' effects of noise, reviewed elsewhere,[30,34,43–45] are often unrecognized by researchers, technicians, and veterinarians and could represent a source of confounding variability in animal studies and unrecognized distress for animals. While such background noise could impact any animal model, the impacts may be especially important for our most commonly used species of nocturnal, quiet tunneling dwelling rodents like mice and rats that have relatively poor vision and rely heavily on their senses of hearing/noise and touch/vibration for survival.

However, more studies need to be conducted to assess the impacts of normal husbandry practices on animals, such as a recent study showing that the act of briefly undocking a cage from a ventilated rack for a daily health inspection did not appear to negatively impact breeding performance or activate stress pathways.[6] While that may sound like good news for noise impacts on our animals, it did just use a single classically resilient strain (C57/Bl6) and undocking would not be expected to cause the mice as much noise and vibration as a cage-changing activity. Interestingly, the study also found that animals housed higher on the rack (where noise levels were significantly higher) demonstrated higher corticosteroid levels. However, vibration and light levels were also higher on the higher cage locations, shining a light on the complexity and sensory variability of our housing conditions on a single rack, let alone between racks, rooms, and institutions. Although there are many practical challenges to doing controlled studies on husbandry practices, the challenges are outweighed by their promise to promote improved animal welfare and study reproducibility across all biomedical and behavioral research.

USN: The 'Silent' Noise in Animal Facilities

An additional important consideration regarding noise in the animal facility is the recognition that much of our modern equipment generates USN, above the human hearing range of 20 kHz. This was first pointed out several decades ago[34] by a study showing that 24/39 sources measured in animal housing spaces and laboratories generated ultrasound that could impact animals. Our group has assessed dozens of animal research facilities (housing and lab research spaces) in the last decade and has found the problem persists. Every facility we have assessed has had USN sources, to varying degrees and in different locations (animal housing or lab research spaces), that can be detected by our research animals. These sources tend to be more prominent in research laboratories where animals are tested, but there are many sources inside the animal housing areas as well. Because we humans cannot hear these signals, and they are rarely measured with proper instruments, they represent a 'silent' confound for much of our animal research. However, for mammalian research models, ultrasonic signals represent key areas of their hearing, often where they vocalize to one another various communication signals. USN is therefore likely a source of animal stress and a serious experimental confound at many sites, which remains undetected/unrecognized, leading to unintended impacts on animal models and assays.

USN should be monitored regularly as new equipment is brought into animal spaces and as equipment ages, as ultrasonics can be an early sign of mechanical lubrication or other failures. The industrial world makes regular use of USN measurements for condition monitoring to track leaks in compressed gas lines and the condition of machinery, such as motors, which can generate USN when beginning to fail or in need of lubrication or other service. Given the many diverse sources of compressed gasses and machinery on hoods and ventilated caging systems, USN condition monitoring represents an opportunity for animal research facilities to leverage the same measurements used to track animal exposures to also identify and track the condition and service needs for their equipment. USN levels should be kept as low as possible but certainly maintained below 45 dB SPL to minimize masking of vocalizations/communications and to limit its potential to disrupt sleep.[47] Certain ultrasound frequencies can have species-specific impacts. For example, sound energy in the 18- to 37-kHz range, centered around 22 kHz, activates the amygdala[25] and represents an anxiety-related aversion call frequency range in rats, whereas higher frequency sounds in the 35- to 80-kHz range, centered around 50 kHz, activate the brain reward pathway in the nucleus accumbens and serve appetitive, mating, and other prosocial interactions.[29,36,50] The 50-kHz vocalizations are also present in rats after treatment with psychoactive drug-induced dopaminergic reward[29,50] and have been described as a marker of positive arousal and emotional state, akin to human 'joy.'[26] Mouse vocalizations are less well understood than rats. This may be due to the widespread high-frequency (ultrasonic) hearing loss present during prime breeding months in some of the most commonly used inbred strains, such as C57BL/6, DBA/2, BALB/c, and 129/J,[45,52] but similar to rats, lower frequency sounds that are audible to humans and ultrasonic vocalizations appear to signal threatening events expressed by young pups in isolation[29] and sometimes from tail snipping or ear notching,[48] while higher frequency vocalizations appear to aid social communication.[12,28] The fact that across rats and mice lower frequency calls tend to signal negative aversive events and higher frequency calls signal more positive events blends nicely with the ethological function that in a burrow or underground tunnel, low-frequency sounds with longer wavelengths would bend around corners more easily and travel longer distances more effectively to alert others. In contrast, higher frequency signals, with their shorter wavelengths, would be more isolated and only useful in a near-field social interaction.

The laboratory environment generally contains many sources of USN; fluorescent lighting ballasts, alternating current/direct current power blocks, uninterruptible battery power supplies, computers, test equipment, and ultrasonic mixers/cleaners.[34,43] The presence of such signals could interfere with the tests being conducted. As an example, consider a benchtop where mice might be weighed and dosed with a medicine, such as a β-blocker, to alter heart rate. If a computer is also on the benchtop, putting out a wide range of ultrasonic signals, animals nearer to the computer might be more aroused than animals farther away on the same benchtop, yielding variability and perhaps very different results that might be attributed to the drug. In addition, many of the classic behavioral tests in the learning and memory research field, such as the Morris water maze or open field tests, could be impacted by these signals the researchers may be unaware of. Because USN quality and levels will vary both within and between laboratories, its effects on animals likely produce variable results that can make reproducibility of research results difficult.

Sources of Noise in the Animal Facility and Research Laboratory

Animal housing rooms tend to be relatively noisy environments due to many factors.[21] One key factor contributing to the overall noise in an animal housing room is typically the HVAC systems used for the room. Animal housing rooms are required to have especially high rates of air circulation. Typically, the room air is replaced with fresh air at a rate of between 10 and 20 air changes per hour. Such high air velocity rates create turbulence in the ductwork/vents, resulting in background HVAC noise levels in animal rooms that can be much higher than is typical of a home or office setting. The resulting background noise levels often approach 70 dB SPL in the human hearing range, sometimes making it a challenge for staff to communicate, and typically fall in the 45- to 60-dB SPL range in the mouse frequency hearing range.[43] Maintaining continuous average noise levels below 70 dB SPL can be a challenge in animal facilities but especially for larger species that hear more of the lower frequency sounds generated by HVAC systems. Facilities can sometimes benefit by lowering the room air changes per hour to the minimum allowable and reducing the energy usage and stress on the HVAC systems while also resulting in lower noise levels in the room. This may be in addition justified in rooms with ventilated caging where higher levels of air control in individual cages can be achieved while keeping the macroenvironmental animal housing room air changes to the minimally acceptable level to provide the needed clean air to the room and limit odors. In a newly commissioned animal housing room, however, if levels of background noise are already approaching 70 dB SPL in the animal's hearing range, before the addition of other equipment in the room such as ventilated caging motors, biosafety hoods, etc., additional engineering noise control in the ventilation system may be needed (for example, silencers/mufflers, and attention to ductwork features and hangers that can impact resulting noise levels).

In addition to the basic challenge of controlling noise levels in animal housing rooms that naturally have poor acoustical absorbing features and high air exchange rates, animal research facilities have become increasingly filled with additional sources of noise and USN over the last few decades, as new technologies have emerged. Typical research facilities now often have advanced building automation systems that provide careful monitoring of ventilation flow rates, duct and/or room temperature and humidity sensors, and automatic watering systems, not to mention the advanced systems employed in animal care and use such as ventilated caging and biosafety hoods to keep dander and odors minimized and to help minimize the spread of pathogens. In addition, computerized cameras and related sensing systems for collecting data from animals in cages or via telemetry have ushered in a new era of animal monitoring. Many of these improvements have simultaneously presented a new challenge for noise and USN in the animal housing and research spaces that we now must become aware of and address. The following paragraphs will highlight the most common sources of noise in the animal housing spaces and research labs, based on the literature and our experiences monitoring dozens of facilities during normal day-to-day activities and during construction.

Ultrasonic motion sensors have been installed in many buildings to aid in energy control by sensing when activity is present and turning on or off the lights when activity is sensed. Based on our assessments of animal facilities, they are found in the majority of research animal facilities that we have assessed (approximately 60%), typically in research laboratories or procedure spaces where students and technicians tend not to turn the lights off when they leave but also sometimes in spaces where animals are temporarily held or transported through. The technology used in these systems is similar to that used in the automobile industry as proximity sensors to aid navigation and aid emergency warning systems when something is too close to the car, parking, etc. The SONAR technology is very old and has many applications from automotive to underwater SONAR to range finders and room occupancy sensors. The systems typically deliver a very intense, approximately 120 dB ultrasonic signal in the 30- to 40-kHz range with a speaker, and a microphone that measures changes in the sounds that bounce back to the sensor. SONAR systems work in a similar manner to bat echolocation by projecting a loud sound and measuring its return to investigate what objects are ahead of it.[7] Because ultrasonic sounds have such short wavelengths, they hit an object and bounce back to the source, rather than bending around it as lower frequency sounds would behave. These systems are efficient but unfortunately generate very intense sounds that are audible to the mammals used in biomedical research.

All commonly used mammalian research models, from mice and rats to other rodents, dogs, cats, swine, and NHPs, can typically hear these signals very well,[14] thereby serving as a potential animal stressor or experimental confound. Figure 1 shows example pictures from animal research facilities of what these systems look like. We have never found them inside an animal housing room because the lights in such rooms are always on a light timer, but they are commonly found in the hallways just outside the animal rooms, with the signal sometimes coming into the room, especially when the door is opened to the room. They are commonly found in hallways, receiving areas where animals may sit for a period of time, and frequently in research laboratory spaces where animals are taken for studies or various assessments. These systems are also often found in classrooms, meeting rooms, and clinic spaces where service animals are increasingly present. Such systems should be replaced with other technology that does not generate such high levels of ultrasound for occupancy sensing, such as the commonly used passive infrared sensors, which sense heat from a person and toggle light switches accordingly. Note that some systems combine infrared sensors and ultrasound. In such systems, the ultrasound use can often be toggled off, but our experience suggests that this can leave the user with a false sense of security if not measured because the ultrasonic signal may still be generated but the system just does not use the returning ultrasound to toggle the lights on/off. The intensity of the ultrasonic signal from such a device is loud enough to cause permanent hearing loss in research animals in a matter of minutes, let alone activation of stress pathways as animals are transported through or held in these environments for testing. The intensity of these systems can be so loud that distance motion sensors in the hallway or adjacent spaces can sometimes penetrate an animal housing room when the doors are opened. However, most of the time these sensors are not problematic inside animal housing rooms, just when animals are transported through or held in spaces with the occupancy sensors.

IVC systems have become more commonly used in animal facilities over the last 2 to 3 decades. Such units typically generate small levels of USN that escape through the motor's ventilation system into the housing room. The frequency spectrum of the USN can vary widely as a function of the manufacturer and age of the equipment, but it is typically not intense enough to reach the nearest adjacent cages. However, in some cases the placement of the motor vent can be directly above or adjacent to a cage of animals, effectively bathing them in a constant tinnitus-like USN tone. In addition, because these systems move air continuously, 24 h/d, the motors and airflow of the systems generate constant noise for the animal occupants of the room, some of which is just audible to the human workers. The noise generated by IVCs can vary dramatically as a function of the manufacturer, style, filter status, age, and service life of the system. Our experience is that many IVCs can add 5 to 10 dB SPL of additional background noise in the hearing range of lab animals and a bit more for humans given their lower frequency hearing range and the fact that HVAC noise typically consists of lower frequency noise below 2,000 Hz. Placing motors in interstitial spaces is ideal but often not an option. If IVCs are placed on the animal's rack or anywhere inside the room with the animals, it is important to regularly service and monitor the noise and USN (and vibration) output of these systems to ensure it is not impacting animals.[43] In addition, such age-related changes to equipment can serve as an important signal of mechanical wear and potential failure of systems, serving an important role in equipment condition monitoring. Ideally, animal facilities should track the noise, USN, and vibration of IVCs regularly (at least annually) and service or replace those units that are showing signs of significant wear or failure that could impact animals or the proper functioning of the equipment.

Biosafety/procedure hoods are also increasingly present inside animal housing rooms. They are used for conducting cage changes in an environment that controls bedding dust and minimizing staff exposure and the spread of pathogens between cages and are oftentimes used as an in-room treatment/assay area. Hoods tend to use very loud blower motors that typically add 5 to 10 dB SPL of noise to the room in the mouse hearing range (more for humans or larger headed species). Like building HVAC, the frequency content tends to be lower frequency with only part of it audible to animals but for a human user at a hood the noise levels typically approach 70 to 80 dB SPL. Many larger animal housing rooms can have multiple hoods running at the same time, adding to the overall room noise level. While the hoods help with bedding dust and pathogen control, and depending on their use can also minimize transport of animals to a lab for treatments/assays, they add a layer of noise to every room using them that should be considered as part of the overall noise exposure to the animals in the room. Procedure hoods, especially those fitted with fluorescent light ballasts, also tend to generate USN that can be audible to the animals inside the hood or in near adjacent cages. When present from a fluorescent light ballast, a clear plastic deflector can usually be effective at shielding the USN from reaching the animals inside the hood. All fluorescent light ballasts (whether in the ceiling, inside a hood, or on a benchtop) will produce USN at low levels, which can be audible to nearby animals and should be shielded from

Figure 1. Examples of ultrasonic motion sensors found in animal research facilities. They can be easily found by looking for at least 2 speaker-like ports, one of which serves as the ultrasonic transducer/speaker and the other of which serves as the microphone sensor. Note that humans do not hear sounds above 20 kHz, so these sounds are inaudible to people, making them especially nefarious. Such ultrasonic motion sensors are typically mounted on the ceiling or wall and routinely generate signals of approximately 120 dB SPL in the 30- to 40-kHz range at the source, with typical levels reaching animals 1 m from the floor on a cart or a benchtop, for example, approximately 100 dB SPL.

animals with a thin clear sheet of plastic or other deflecting material. Procedure hoods also tend to vibrate violently, especially on the work surface where cages are placed. This vibration (in addition to having vibratory impacts on animals in the hood) can generate sound that moves into the animal room as metal rubs against metal or plastic. Animal cages placed on the work surface would ideally be placed on an antivibration material such as a rubber mat or thick filter media. As with IVCs, it should be considered a best practice to test the noise, USN, and vibration produced by blower motors at least annually to track their health/function and service needs.

Computers and test equipment are increasingly found in animal housing rooms and of course in research labs where animals are brought for treatment or testing. Desktop computers with a fan are notorious for generating a range of USN frequencies at levels that would be clearly audible to animals within 1 to 2 m of the source. The USN emanates from the vented fan area of the computer where the USN escapes into the room. Plastic deflectors or judicious placement of the computers when near animals can minimize this problem, effectively diverting the USN away from the animals and allowing it to dissipate over space. Similar USN is typically found emanating from uninterruptible power supplies and a range of behavioral and other test equipment. Ultrasonic instrument cleaners, pulverizers, and drills generate exceptionally high levels of ultrasound. Figure 2 shows an example of an animal weighing area immediately adjacent to a computer vent. The level of USN emanating from such devices is typically less than 60 dB SPL at the source and dissipates quickly with distance, so the USN would not be expected to cause hearing loss but could serve as an arousing stimulus to the animal and potential research confound, particularly in behavioral/neuroscientific research studies where the control of extraneous sensory cues needs to be tightly controlled. We have observed similar equipment, such as cameras, control boxes, robotic arms, etc., near Morris water and other maze styles, open field arenas, treadmills, etc. Such equipment should always be assessed for the possibility of USN, as it could impact the animals and serve as an additional confounding stimulus/directional cue to the animal being tested.

Figure 2. Computer generating USN near animals in a research lab. The oval outline indicates the source of USN from the cart-mounted computer. A clear acrylic or other solid barrier between the computer and weighing station would prevent the transmission of USN to the animal.

Transport carts are often used to move animals from their housing areas to areas for testing or treatment. Transport carts tend to be constructed of light gauge stainless steel and rigid wheels/casters that poorly dampen vibration and generate excessive noise. Measurements of noise and vibration inside cages on typical transport carts, like the one pictured in Figure 3, show that transporting a cage of animals on a cart from their animal housing space, just down the hall to a research laboratory, can generate vibration levels of 1,750 milli-*g* (at 1,000 milli-*g* or 1 *g* the animal becomes airborne) and noise levels of 97 dB SPL. Placing either an antivibration rubber or filter media material on the cart shelves below the cages can cut these vibration levels in half and drop the noise levels inside the cage from 97 to 75 dB SPL. Higher mass carts with higher quality or pneumatic wheels/casters and shelf padding can have major impacts on the noise and vibration experienced by the animals during transport, which can impact their performance in the lab and/or the amount of time needed to acclimate them to the lab after transport before treatment or testing.

Staff activities in the room, in our experience, produce the highest levels of noise (and vibration) that animals experience in their housing rooms. A typical cage-changing event on a rack can lead to dozens of startle-eliciting abrupt noises exceeding 80 dB SPL for all animals on the rack. A wide range of other staff activities in the laboratory can also produce excessive noise, such as food, water, and health checks, moving racks around in the room, mopping, hosing the room, turning on faucets, etc. Many of these activities are unavoidable and necessary for maintaining clean animal housing rooms. However, there are typically lower noise ways to engage in these tasks where the tradeoff

Figure 3. Typical light-gauge stainless steel animal facility transport cart, which can generate excessively high levels of noise and vibration for animals transported in cages.

is that it may take a fraction of a second longer to snap a cage into place, for example, but the resulting time and care might mean that the cages experience an approximately 75-dB abrupt sound instead of a 95-dB startle eliciting sound (and vibration). Staff training is critical in addressing this issue to make staff aware of the umwelt of the animals they are working with and how minor changes in staff behavior can have dramatic impacts on the sensory experiences of the animals they are caring for. Figure 4 shows a typical 24-h day with the corresponding noise and vibration levels experienced by animals as a function of staff working in the room.

Construction and renovation projects are an ever-present consideration in the animal space as demands for expansion and/or renovation increase. These projects and their impacts on levels of noise and vibration can be highly variable depending on the proximity of the work, whether it is internal or external to the animal space, and the means and methods required to perform the work. The most invasive construction operations external to the facility typically include demolition, piling, sheeting, excavation, compaction, hydraulic hammering, and soil nailing. While noise may, at times, be a concern with external projects, concrete, and similar structural walls will prevent much of the noise from penetrating the animal space. This is especially true for higher frequency noises above 1,000 Hz (within the hearing range of mice), as noise at and above these frequencies tends not to penetrate dense surfaces. External projects tend to present more substantial issues relating to vibration.

Internal construction projects, however, tend to introduce concerns for both noise and vibration, as the work may be taking place in closer proximity to the animals with fewer barriers to prevent the transmission of noise. Hammer drills, cutting saws, jackhammers, grinders, and powder-actuated tools have been measured at levels exceeding 90 dB when the work is taking place near the animal space. The number of walls/barriers between the noise source and the animals is the best predictor of impact on animals. In our experience, construction activity in mouse housing areas can be problematic in the nearest rooms, but once the animals have at least 3 walls between them and the source, we often do not find measurable levels of noise in the mouse hearing range. In these situations, temporarily moving mouse housing areas farther from the construction could be a solution. Note, however, that noise can travel down corridors through interstitial spaces and ventilation and such noise can be difficult to control. Working on the ductwork in a facility can propagate a noise signal throughout an entire building very efficiently. Note, however, the same caveat as before, that high-frequency sounds would not travel very well to mice so noise problems inside animal facilities from construction tend to be a greater concern for nonmouse, larger species that hear lower frequency sounds. A range of additional noise mitigations is possible when construction is being done, and the optimal approach depends on so many variables that it would be difficult to encompass here. Nevertheless, one key successful approach is to attempt to isolate and control the noise at the source by constructing temporary sound control walls or assembling noise control shrouds around noisy work, which can have a dramatic impact on the propagation of noise through the building. Table 1 provides a list of suggested considerations

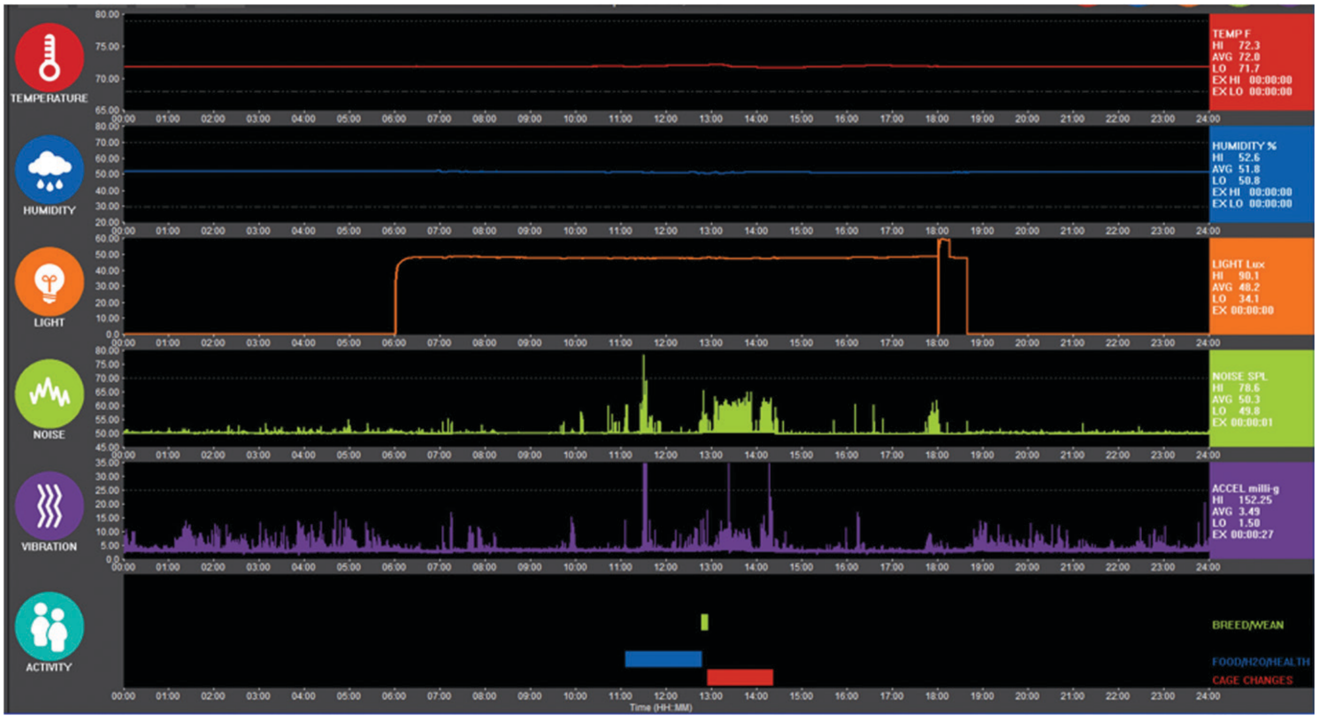

Figure 4. Typical 24-h day for a mouse cage. In this particular example, noise (and other variables) were being monitored in a standard mouse cage on an IVC rack while normal day-to-day activities were being conducted. Note the highest levels of noise (and vibration) were present when staff was doing food/water/health checks (blue bar on bottom timeline) and changing cages (red bar on bottom timeline; the green bar indicates breeding/weaning activities were being conducted). In this particular example, noise only exceeded 70 dB for 1 s in the 24-h period, and vibration during staff activities reached 152 milli-*g*, exceeding the 25 milli-*g* threshold for 27 s. Importantly, in this example, the staff knew that noise and vibration were being monitored, and they had been trained about the importance of doing their work carefully to avoid excessive noise and vibration. When staff become complacent or are not made aware of what is being measured, these levels are substantially higher. In this example, interestingly, someone entered the room and turned the lights on after their typical lights-off cycle had started, producing additional noise. The x-axis represents the 24-h time of day from midnight to midnight on the same day, and the y-axis for each variable represents magnitude of the measurement.

Table 1. Ten key steps to preventing and mitigating construction noise and vibration impacts on research animals

Ten key steps to preventing and mitigating construction noise and vibration impacts
1. Coordinate with all key stakeholders to develop a written communication, measurement, and mitigation plan to help with buy-in, transparency, and relationships. Hold regular weekly updates and look-ahead meetings with a subset of key stakeholders to share previous findings and discuss upcoming expected impacts on animals. Ideally provide a common location where stakeholders (investigators, construction, facility administrators, veterinarians, etc.) can access data and review reports/findings.
2. Measure/monitor N and V levels before (baseline) and during construction so you are working with fats rather than gut in dealing with stakeholders. Conduct a careful set of construction simulation tests before activities start and repeat with each new construction phase/equipment or tool use/activity to determine the expected impact in cages in different areas of the vivarium.
3. Consider cryopreserving sensitive lines, where possible and practical.
4. Consider moving animals to less impacted areas, where possible and called for based on measurements/simulations, noting that sometimes moving animals creates much more N and V than the construction.
5. Consider using reverse light/dark cycles for nocturnal species so their daytime sleep is not disrupted by the construction activities.
6. The best way to prevent N and V issues is to prevent their production. Carefully consider construction equipment “means and methods” as some equipment and approaches are more likely to produce N and V than others. Consider architectural design and planning choices for their N and V features. Weigh relative construction impacts on animals compared with construction timeline; that is, a more acute impacts short-term but accelerated construction timeline compared with a longer term chronic impacts and drawn-out construction timeline.
7. If you cannot prevent production of N and V, try to contain N and V at the source using construction shrouds, acoustical blankets, etc.
8. If you cannot prevent or contain it, attempt to block N and V from reaching animal rooms using temporary walls in corridors, sound control curtains over doors, etc.
9. If you cannot block N and V from reach animal rooms, attempt to block it from reaching animal cages by using antivibration pads under racks or cages, or sound control panels in the room.
10. Final line of defense is to mask the N and V with other stimuli. Ventilated caging can mask some signals and hoods can be effective at masking some construction noises. In some situations, a room-level noise masker like music may be helpful, especially for larger species who better hear lower frequency sounds from construction. When the N and V events are highly predictable/coordinated, such as in timed detonation blasts of bedrock, disperse technicians and others in the housing room during the events to work (change cages, etc.), to both mask the events and to serve as a warning to the animals to expect N and V.

Note that every construction/renovation project is unique and while we have found these steps to be generally useful, each step will contain many site-specific caveats to consider; some may not be possible or necessary and others not listed can be employed. N and V, noise and vibration.

when planning a construction/renovation project in or near an animal research facility.

Aquatic Hearing and Noise

Measuring sound underwater uses a different reference standard whereby 0 dB = 1 µPa, instead of the 20-µPa reference used for air. This yields underwater noise measurements that can be misleading and difficult to interpret for those of us used to measuring airborne noise levels. Generally speaking, the underwater noise levels can be compared with airborne noise by first subtracting the 26-dB difference between the 1 compared with 20-µPa reference signals and in addition subtracting the 36 dB needed to account for the impedance difference between air and water (more pressure is required in air than in water). This yields an approximate 62-dB correction factor when comparing air compared with water noise measurements. Therefore, a circulating water system producing 162 dB (re 1 µPa) in a fish tank would be approximately equivalent to 100 dB (re 20 µPa) in air. The University of Rhode Island curates an excellent resource for understanding more about underwater sound, its measurement, and its impacts on animals.[8]

Species adapted to hearing underwater appear to use additional stimuli and anatomic systems for detecting sounds. In addition to detecting sound pressure, aquatic species are able to detect particle motion, which travels more efficiently in water than in air. When sound waves travel through air or water, they compress particles in the medium and rarefy surrounding media, resulting in fine movement of particles that aquatic species are able to detect. Particle motion detection appears to be especially prominent and useful for detecting low-frequency sounds, which aquatic species use extensively for navigation and communication. Particle motion detection systems in aquatic species behave more like accelerometers detecting vibration than microphones or hydrophones detecting sounds. Particle motion detection in fishes is primarily focused on lower frequencies (generally well below 1,000 Hz). In addition, aquatic species have evolved adaptations that often make the use of a gas/air-containing body near the ear that can further aid their ability to process sounds underwater. Many aquatic species, including adult zebrafish (*Danio rerio*), possess a swim bladder and Weberian ossicles that improve their hearing sensitivities by connecting the inner ear to air-filled cavities, extending upward their detectable frequency range.[20]

Aquatic species have become a much more common tool in biomedical and behavioral research over the last 2 decades. Aquatic species have emerged as an alternative model for the study of many biologic and neurobehavioral conditions. Advancements in imaging, genetics, video tracking, and other methods, paired with a better understanding and appreciation for their highly conserved biologic and neurobehavioral similarities between zebrafish and mammals (for example), have spurred a dynamic time of growth for biomedical research. Zebrafish (*Danio rerio*) account for the majority of this growth and have quickly become a commonly used model in biomedical research,[41] and one article provides an excellent recent review of zebrafish hearing and general aquatic hearing.[27]

To accommodate the increased use, research facilities have increasingly incorporated more and more advanced/automated methods for housing and caring for aquatic species. Zebrafish have much in common with their mammalian cousins in that organ patterning is conserved, making them useful for a range of studies, including those involving the hypothalamic-pituitary-adrenal axis.[13] Zebrafish have a keen sense of their environment, constantly surveilling it for threats and opportunities. As aquatic species, they use sound/vibration traveling through water as a key means for navigating and understanding their surroundings. They are also highly impacted by light patterns as they demonstrate a diurnal pattern like humans with more activity during the light phase and rest during the dark phase but with a wider spectral capacity than humans.[39]

These common factors combine to make the impacts of extraneous environmental variables just as disruptive to them as it is for mammalian models. Their development occurs on a more aggressive timescale than most mammals although, as after just 1 wk of development, zebrafish larvae already display behavioral patterns as complex as adults.[19]

An excellent example is a study of zebrafish sensitivity to noise and vibration exposed zebrafish to sound/vibration in their living environment by activating their well plates with a speaker for a week.[19] The level of noise/vibration was not reported, but it was set to a point where they could not visibly see the water or larvae or well plate vibrating. This study showed that noise/vibration produced a significant disruption/reduction of activity, particularly during the light cycle when they are normally more active. Importantly, the researchers found that the sound/vibration resulted in a dramatic approximately 50% larval survival rate during the 7-d exposure compared with 100% survival in controls. Importantly, they found that sound/vibration appeared to produce the most biologic stress as it was the only extraneous variable they tested that led to mortality; other manipulations including a hypercaloric diet and various manipulations of the light/dark cycle, including a jet lag condition and constant light manipulations did not produce mortality.

Humans can, of course, hear sound pressure underwater but with generally higher thresholds and less directional sensitivity than airborne hearing.[36] However, humans appear to show better responses to underwater sound than can be explained by traditional bone conduction and may leverage some form of particle motion and/or use of the middle ear airspace or perhaps air-filled lungs, as is done by some turtles, frogs, lizards, and salamanders.[15] In many such species, air-filled middle ear and lungs serve as a resonating feature to improve hearing at lower frequencies, below 1,000 Hz. For example, the human air-filled middle ear space behind the tympanic membrane, given its approximately 0.5-mL volume, resonates at a frequency around 660 Hz, allowing the middle ear airspace to serve as an amplifier for low frequency sounds below 1,000 Hz, yielding underwater thresholds around 500 Hz that are much better (lower) than what can be explained by bone conduction alone.[38] Some species appear to use air-filled cavities such as the lungs to actively dampen self-generated signals and improve the signal-to-noise ratio. For example, in tree frogs it has been shown that air-filled lungs and the lung-to-ear air pathway, can actively improve peripheral frequency tuning, aiding noise control and signal-in-noise detection in a complicated multispecies breeding chorus.[22] It is likely that airborne low-frequency sound processing in research mammals and humans can be impacted by such mechanisms, although there is a dearth of data on these topics.[35] Nevertheless, there is an emerging active area of interest around the negative health effects of low-frequency sounds or infrasounds (in some cases below the normal frequency range of airborne hearing for the species) that may be propagated through the body as various body organ systems resonate at lower frequencies (typically below 200 Hz), below the range of hearing of many animals, but which may still have impacts via vibrotactile systems.[3] In this way, hearing and touch/vibration systems again share some things in common. After all, hair cells in the cochlea used for hearing are mechanical sensors like touch receptors but are inside the bony, fluid-filled cochlea. These hair cell sensory receptors bend in response to the ever so slight 'touches' of sound on the eardrum, vibrating the ossicles and causing a fluid wave inside the cochlea.

Conclusions

Noise and USN are ubiquitous in every animal research facility and rarely measured in research reports. This is particularly problematic given that most of the animal models used by researchers hear best in the ultrasonic range at pitches not audible to humans. As a result, noise and USN likely introduce an unintended confounding research variable in many studies involving animals. To mitigate this problem, we suggest animal facilities develop a written plan for noise measurement and perform facility-wide noise measurements at least annually and whenever there are changes to either the facility (for example, equipment) or animal health, using the principles described previously.[43] Once identified, problems should be controlled with appropriate mitigation techniques. Facilities should expect to be able to report on their noise plan and measurements and findings in annual reports and for documentation for regulatory and accrediting bodies such as AAALAC. Finally, we think that for the field to gain an understanding of the impacts of noise on animals and studies and to address the reproducibility crisis, methods sections in published manuscripts should include information on the noise and acoustic features of animal housing and testing areas.

Conflict of Interest

The author(s) have no conflict(s) of interest to declare.

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