Vibration-induced Behavioral Responses and Response Threshold in Female C57BL/6 Mice

Angela M Garner,¹ John N Norton,¹ Will L Kinard,² Grace E Kissling,³ and Randall P Reynolds^{1,*}

Despite documented adverse effects, limits for rodent exposure to vibration in the laboratory animal facility have not been established. This study used female C57BL/6 mice to determine the frequencies of vibration at which mice were most sensitive to behavioral changes, the highest magnitude of vibration that would not cause behavioral changes, the behavioral changes that occur in response to vibration, and the extent to which mice habituate to vibration. Mice were exposed to frequencies of vibration between 20 and 190 Hz at accelerations of 0.05 to 1.0 m/s². Behavioral responses were videorecorded and subsequently scored. Mice showed the most behavioral responses at 1.0 m/s². At intermediate accelerations of 0.5 and 0.75 m/s², behavioral responses were most prevalent at frequencies of 70 to 100 Hz. In contrast, at an acceleration of 0.05 m/s², mice did not show any discernible behavioral response. Behavioral responses induced by the initiation of vibration were transient, generally lasting only 2 to 10 s. Behaviors in awake mice included abrupt freezing of motion, hunched posture, and surveying the cage environment. In mice that were asleep, responses consisted of lifting the head suddenly with or without prior shifting of body position. When exposed to multiple periods of vibration over a short time, responses seemed to decrease. In summary, mice were particularly sensitive to vibration between 70 to 100 Hz, did not respond to the slowest acceleration (0.05 m/s²), and exhibited transient responses at the initiation of vibration.

DOI: 10.30802/AALAS-JAALAS-17-00092

Vibration in animal facilities is an important environmental factor that can adversely affect the reliability and reproducibility of research results as well as animal welfare. Several studies have demonstrated that vibration can cause alterations in rodent reproduction as well as mortality and morbidity in other laboratory species.^{6,12,30} The many potential sources of vibration in an animal facility include construction or renovation projects, animal husbandry equipment, routine husbandry procedures, and sources outside of the animal facility such as trains¹ and other municipal traffic. Because of these concerns, the *Guide for the Care and Use of Laboratory Animals*¹⁷ has emphasized minimizing the vibration that is inherent in the animal facility. Environmental animal facility parameters including temperature, light, and humidity are carefully regulated according to information in the *Guide*. However, unlike the limits established for humans when occupational exposure is a concern,^{6,24} no standard limits have been determined for vibration exposure in laboratory animals. Information regarding the maximal level of vibration that is acceptable for laboratory animals is needed to prevent vibration from adversely affecting animal welfare and research integrity.

Vibration is the periodic movement of an object back and forth past a point of equilibrium. In a complete cycle of vibration, an object moves from an extreme position in one direction to the other extreme direction before returning to equilibrium.⁵ The number of cycles of vibration that an object undergoes within a given time period is defined as its frequency. Frequency is most commonly measured in Hertz, where 1 Hz is equal to 1 complete cycle per second. How far an object moves past its

point of equilibrium is defined as the magnitude of the vibration (for example, displacement, velocity, or acceleration).^{5,11} The perception of vibration in a human or animal depends on both the frequency and magnitude of the vibration. In addition, each object has a resonance frequency range, which is the frequency range at which an object tends to vibrate most readily and can amplify the vibration. The resonance frequency is located within this range, and vibration increases most at frequencies close to the center of this range and somewhat less at the ends of the range. These frequency ranges are unique to each animal or object and are dependent on the subject's physical composition in regard to 'stiffness' and mass. Therefore different species will perceive vibration to a lesser or greater degree, depending on the frequency of the vibration.²⁷ Evidence suggests that the resonance frequency range of mice is 30 to 110 Hz,^{21,27} therefore mice are likely to perceive vibration in this range. Regarding support for this assignment, mice demonstrate increases in heart rate and blood pressure when exposed to vibration at 80 and 90 Hz.²¹

Adding complexity to determining the frequencies of vibration that affect animals is that once an object (for example, cage, cage rack, floor) begins to vibrate, there are multiple frequencies, spaced far apart, that tend to amplify the magnitude of vibration. The lowest frequency that amplifies the vibration is the fundamental frequency, and the higher frequencies are the harmonic frequencies. Harmonic frequencies occur at whole-number multiples of the fundamental frequency, such that if x is the fundamental frequency, then harmonic frequencies occur at $2x$, $3x$, and so forth. If a mechanical system has any freedom to vibrate, even at microscopic levels, the harmonic frequencies of the system can contribute to the level of vibration to which an animal would be exposed.⁹

Although previous studies evaluating the effects of vibration on rodents used telemetry measurements,²¹ we here

Received: 04 Aug 2017. Revision requested: 24 Oct 2017. Accepted: 21 Dec 2017.

¹Division of Laboratory Animal Resources, Duke University Medical Center, Durham, North Carolina; ²Brüel and Kjær North America, Duluth, Georgia; and ³Biostatistics and Computational Biology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina.

*Corresponding author. Email: Randall.Reynolds@Duke.edu.

used behavioral observations to evaluate vibration-induced responses. Behavioral observations are a readily available tool used to evaluate not only animal wellbeing but also an animal's perception of their overall environment. Researchers have evaluated behavior in response to environmental parameters such as light cycles and room temperature by observing overall behavior.^{3,14} Numerous experimental rodent models rely on behavioral observations to evaluate phenotypes, experimental treatments, and models of neurocognitive and neuropsychiatric disorders.¹³ One commonly used behavioral response in rodent research is the startle reflex, which has been well characterized in other species, including humans, also.³⁷ The startle reflex is elicited in response to acoustic, vestibular, or tactile stimuli and is mediated through cranial nerves and various portions of the CNS.³⁴ The startle reflex is commonly used to test perception to acoustic stimuli and has been used for hearing sensitivity testing.^{35,37} In addition, tactile stimuli such as facial air puffs have been used to elicit the startle reflex, which involves a characteristic hunched posture in which the head and neck are retracted even if for an extremely short period of time.^{10,37} Other behaviors indicative of fear can follow the startle response in rodents and include the 'freeze response,' in which the animal abruptly stops all voluntary motion and visually scans the environment for possible danger.²⁰

We evaluated C57BL/6 mice in the current study because of their common research use as a wildtype or background strain. Our goals were to determine the frequencies of vibration at which mice were most sensitive to behavioral changes, the threshold (that is, acceleration) at which mice stopped displaying behavioral responses to vibration within their resonance frequency range, the behavioral changes induced by vibration, and the extent to which mice habituate to vibration. We hypothesized that mice would show the most pronounced behavioral changes at vibration frequencies within their resonance frequency range and at frequencies that altered cardiovascular parameters in previous studies.²¹ Other important insights gained were the need to establish an appropriate period for acclimation to the testing room prior to vibration experiments and the contribution of harmonics in increasing vibration exposure.

Materials and Methods

Animal housing and care. All experiments involved female C57BL/6 mice (age at acquisition, 6 wk; weight, 20 to 30 g; Charles River, Raleigh, NC). Female mice were used to provide information on vibration levels that might alter reproductive parameters in pregnancy and pup rearing. All mice were housed in polycarbonate microisolation cages (Allentown Caging, Allentown, NJ) under a 12:12-h light:dark cycle, temperatures of 70 to 74 °F (21.1 to 23.3 °C), and humidity of approximately 50%. Mice were given free access to water and rodent laboratory chow (no. 5053, Lab Diets, St Louis, MO) in the animal housing room. The room where experiments were conducted was kept under the same environmental conditions as the animal housing room. All procedures were reviewed and approved by Duke University's ACUC and performed in an AAALAC-accredited facility. All mice were free from pathogens specified on the Charles River rodent health report for C57BL/6 mice.⁷ before testing mice were acclimated to the facility for 1 wk after arrival, based on studies indicating that mice need 3 to 5 d to return to normal feed and water consumption after major transport.^{8,31}

Vibration testing. In preparation for testing, mice were placed in a clean polycarbonate cage with 2 cups of fresh bedding (Enrich-O'Cobs, The Andersons, Maumee, OH) and 1/8 cup

of dirty bedding from the home cage, along with a mixture of powdered rodent chow (Lab Diet, 5053) and hydrogel (Clear H₂O, Westbrook, ME) in a plastic container (approximately 50 mm × 20 mm). The cage was covered and transported by hand to the experimental room and placed on a custom aluminum platform affixed to the vibration shaker (V408 Shaker, Brüel and Kjær North America, Duluth, GA). Once mice were in the testing room, the standard cage top was replaced with an acrylic glass top with evenly spaced air holes. The cage was tightly adhered to the platform by using fabric hook-and-loop fasteners straps. A camera was placed on a separate platform in front of the experimental cage. The mice were allowed to acclimate to the experimental room for approximately 20 h prior to experimental procedures. The cage was not manipulated prior to testing, and extreme care was taken to avoid disturbing the mice prior to vibration initiation. During the experiments, the researcher operating the equipment sat below the direct line of sight of the mice to minimize the amount of movement that mice could see outside of the cage. All testing was performed at approximately the same time daily to exclude any potential influence of time of day on behavior. Vibration was measured in the vertical direction with the accelerometer attached to the platform immediately adjacent to and in the middle of the cage side. Continuous sinusoidal vibration (that is, vibration applied at a single frequency) was used to allow determination of specific frequencies at which mice might be particularly sensitive to vibration in regard to exhibiting behavioral changes. Random vibration, which contains multiple frequencies at varying accelerations or magnitudes, is more common in a natural setting but does not allow for the isolation or amplification of specific vibration parameters. Vibration measurements did not differ between locations on the cage, thus confirming that vibration exposure was independent of the location of the mouse within the cage.

Acclimation period before behavioral testing. Because behavioral testing was conducted in a different room from the housing room, data were collected to determine the time the mice required to acclimate to the test cage within the experimental room. Using ethograms from previous studies, we hypothesized that as the mice became more acclimated to the new environment, their exploratory behaviors (for example, rearing, locomotion) would become less frequent and the inactive and maintenance behaviors (for example, sleeping, grooming) would become more frequent until the 2 groups of behaviors stabilized during the light hours of the light:dark cycle.^{14,31} Mice ($n = 5$) were individually videorecorded for 10 min immediately after being placed on the vibration platform (0 h) and at 0.5, 1, 2, 2.5, 3, 6, and 24 h afterward. Videos were scored by a blinded observer who was trained to identify common rodent behaviors prior to the study. Recordings were scored by using an ethogram adapted from a previous study¹⁴ (Figure 1). Each recording was divided into 10-s intervals. The observer was instructed to record the category of behavior occurring during the first 3 s of each interval and was not provided with any information regarding the time point relative to when the mice were placed in the cage. The ethogram used divided behaviors into active, maintenance, and inactive behaviors. When an active, inactive, or maintenance behavior was noted during the interval, a score of 1 was assigned to the appropriate behavioral category. When multiple behavior categories were noted in an interval, a score of 1 was given to all appropriate categories for that interval. The total number of times a behavior in each behavioral category was observed during each recording was summed for analysis. Maintenance and inactive behaviors were

Active behaviors	Maintenance or inactive behaviors
Locomotion	Grooming
Rearing	Feeding or drinking
Sniffing	Still but alert
Digging	Sleeping

Figure 1. Observed behaviors used to determine acclimation time and vibration-induced changes.¹⁴

combined because they both represented behaviors that were distinct from active behaviors.

Behavioral scoring associated with vibration exposure testing.

Mice were videorecorded before and after vibration was initiated by using the shaker apparatus, and the recordings were scored for the behaviors listed in Figure 2. The scoring system used in Figure 2 was developed based on preliminary analysis and repeated observations of mice during vibration exposure, compared with behaviors in the same mice before vibration was initiated. Mice that were awake during testing were scored as having a response to vibration when they exhibited at least one of the fear-related behaviors (more than one behavior was often noted) in the first column of Figure 2. When they were asleep, a mouse categorized as responding when it lifted its head abruptly.

Videorecordings were scored by 2 independent, blinded observers who were trained to recognize vibration-induced behaviors. The observers were provided with video clips containing the first minute of the control period or the first minute of the vibration-exposure period, including the initiation of vibration. The observer was instructed to give a score of 1 when an observed behavior was exhibited within the first 20-s interval after the beginning of the clip and to record the time relative to the beginning of the clip at which the behavior was initially observed and when it ended. In light of analysis of preliminary recordings, a time interval of 20 s was chosen for sampling because most mice showed reactions within 10 s, and all mice exhibited reactions within 20 s after vibration initiation. A score of 0 was assigned to a clip when no behaviors from Figure 2 were noted within the time interval. A key was maintained to identify each clip and included mouse number, whether the clip was a control or vibration period, and which frequency and acceleration were tested. The various clips were presented to the observers in a random fashion, so that the observer would not be aware of whether the clip was of a control period or a vibration exposure period. Although vibration could be observed in the cage from some video footage at 0.5 m/s² and greater, vibration at lower levels was not discernable from the video clips. When disagreement regarding a clip arose between observers, a score of 0 was given. Disagreement occurred in approximately 10% of the clips and occurred only in clips when vibration was 0.15 m/s² and greater.

Behavioral response to sound produced by the experimental apparatus. To ensure that behavioral changes were due to vibration only and not to sound generated by the vibration equipment, 5 mice were exposed to only the sound produced after vibration was initiated. Mice were placed individually in a clean experimental cage and transported to the experimental room as previously described. The cage was placed on a mock vibration platform located on a separate table adjacent to the shaker. The mock platform was the approximate height and size as the shaker. Another experimental cage was affixed to the actual vibration platform, as described. The mice were exposed to the sound produced from vibration at an acceleration of 1.0 m/s² (the maximal sound produced in any of our experiments) and at frequencies of 70, 80, 90, and 100 Hz. These frequencies

of vibration were chosen because the highest number of mice exhibited a behavioral response at these frequencies in preliminary experiments. Mice were videorecorded for 6 min before the sound was initiated and for 6 min during exposure to the sound produced by the vibration equipment. Between tested frequencies, mice were given a 10-min period during which the shaker sound was turned off. All videorecordings were scored by a blinded observer for the frequency of active, inactive, and maintenance behaviors before and during sound exposure (Figure 1) and for behavioral changes observed during vibration exposure (Figure 2).

Behavioral response to vibration. Vibration frequencies and accelerations were chosen by using both the inherent resonance frequencies of mice as well as vibration levels that have induced cardiovascular changes in mice.²¹ Five mice were exposed randomly to frequencies of 20 to 190 Hz in 10-Hz intervals at each acceleration level tested. The mice were videorecorded for 6 min before and during vibration exposure; 10 min without vibration exposure occurred between frequencies. Mice experienced a washout period of at least 5 d before being tested at other accelerations. Each mouse was exposed to vibration at accelerations in the order of 1.0, 0.75, 0.5, 0.25, 0.15, and 0.05 m/s². In addition, mice were tested at 0.1 m/s² and 90 Hz because one mouse among the 5 repeatedly reacted to this frequency and acceleration; no other frequencies were tested at this acceleration. The video clips were scored as described above for vibration exposure testing.

Given the high level of fear-related behavioral responses at 1.0 m/s² between 70 and 100 Hz, the full recordings (6 min) at these levels also were scored by using Figure 1 to determine whether there were any differences in the number of active or maintenance-inactive behaviors that mice exhibited between the control and vibration periods. The video clips were scored as described earlier for acclimation period testing.

Confirmation of minimal vibration threshold. Ten mice that had not previously been exposed to experimental vibration were used to confirm the maximal acceleration level that failed to produce behavioral changes. Of the 10 mice, 5 were exposed to the acceleration level that did not result in behavioral changes in the previously described study. The remaining 5 mice were exposed to vibration at an acceleration of 0.25 m/s², as a positive control, to ensure that the mice responded to vibration in similar numbers as the previously tested group of mice that were exposed to repeated trials, as described in the earlier section describing behavioral response to vibration testing. The frequency of 90 Hz was chosen for both acceleration levels in light of previous data showing that this frequency was in the middle of the frequency range at which mice were most sensitive to vibration and that this was a sensitive frequency in regard to vibration-induced cardiovascular changes.²¹ Mice were videorecorded for 4 min before vibration exposure, to provide a control, and for 4 min during vibration exposure. Independent, blinded observers scored each recording for behavioral changes (Figure 2), as described for vibration exposure testing.

Repeated exposure to vibration. To determine whether mice adapted or habituated to repeated episodes of vibration, 10 mice were randomly assigned to 2 groups of 5 mice, with one group exposed to repeated vibration at 90 Hz and 1.0 m/s², whereas the other was exposed at 90 Hz and 0.5 m/s². Mice were videorecorded for 30 s without vibration and then while exposed to vibration at 90 Hz and 1.0 m/s² or 0.5 m/s² for 1.5 min. Mice experienced a 1.5-min period without vibration before vibration exposure began again. Each mouse was individually videorecorded for a total of 10 cycles, and vibration-induced

Mouse awake	Mouse asleep
Freezes motion abruptly Assumes hunched posture abruptly Looks up abruptly with ears in forward position Looks down or to the sides of cage abruptly	Lifts head abruptly with ears in forward position, with or without shifting body position before lifting head

Figure 2. Observed behavioral responses to vibration.

behavioral responses (Figure 2) were scored as described for vibration exposure testing.

Statistics analyses. Data were recorded into spreadsheets for analysis (Excel, Microsoft, Redmond, WA). A P value of 0.05 or less was used to define significance. Data were analyzed by one-way repeated-measures ANOVA for differences that resulted from various levels of vibration accelerations and frequencies. Because each animal was measured multiple times, normality of the repeated-measures ANOVA residuals was assessed. The residuals from each of the repeated-measures ANOVA were normally distributed according to Shapiro–Wilks tests. Data from the habituation study were analyzed by using the Student t test. Data generated from the experimental room acclimation study were analyzed by 2-way repeated-measures ANOVA. Differences between levels were confirmed by using the Tukey Honest Significant Difference test after ANOVA.

Results

Acclimation to the experimental room. Immediately after being placed in the test cage, mice showed significantly more active behaviors of rearing, sniffing, and locomotion compared with maintenance or inactive behaviors (Figure 3, 0 h). Active behaviors remained significantly ($P \leq 0.05$) more frequent than maintenance and inactive behaviors (for example, grooming and eating) for as long as 1.5 h after mice were placed in the test cage. By 3 h, the numbers of maintenance and inactive behaviors were no longer significantly different from active behaviors. At 6 and 24 h, the incidences of maintenance and inactive behaviors were also not significantly different from active behaviors. Even though the groups of behaviors were not significantly different beyond 1.5 h, the pattern of decreasing numbers of active behaviors and increasing numbers of maintenance and inactive behaviors led us to select an acclimation period of approximately 20 h for acclimation before experiments.

Effect of sound from vibration equipment on mouse behavior. Unlike mice exposed to vibration, mice exposed only to the sound produced by the vibration apparatus at frequencies of 70, 80, 90, and 100 Hz at 1.0 m/s² did not show significant differences in the mean number of observations of active, maintenance, and inactive behaviors than occurred with exposure to ambient noise only. In addition, the scores assigned by the independent observers fully agreed and indicated that no mice showed any obvious vibration-induced behaviors (Figure 2) when exposed to the sound produced by the shaker table (data not shown).

Behavioral changes caused by vibration. Behavioral responses to vibration (Figure 2) occurred within 20 s of vibration initiation, after which the mice often returned to their previous behavior, including sleeping, even though the vibration continued. At higher accelerations, mice tended to react to vibration almost instantaneously. At lower accelerations, mice seemed to react more slowly to the initiation of vibration. The time between the initiation of the behavior and the resumption of the previous behavior, or the demonstration of a different behavior, was 2 to 10 s.

Mice that were sleeping when vibration was initiated looked up abruptly, occasionally preceded by a shifting in body positioning. In mice awake at vibration initiation, all showed a

Figure 3. Number (mean ± SEM) of active behavioral responses and maintenance or inactive behavioral responses during acclimation. The number of responses differed significantly (*, $P \leq 0.05$) between active compared with maintenance or inactive responses at 0, 0.5, 1, and 1.5 h.

combination of freezing, with or without obvious hunched posture or visual scanning of the cage. Approximately 7.5% of awake mice exhibited a hunched posture in response to vibration whereas about 65% of mice also looked at the cage floor or sides, and 15% looked up abruptly. The mice appeared to be alert to their surroundings as they recognized the physical sensation, but then to quickly lose concern.

Behavioral response to vibration at various accelerations and frequencies. Over the 13 frequencies tested from 20 to 140 Hz, the number of mice that responded differed significantly ($P \leq 0.05$) between either of the highest 2 acceleration levels (1.0 and 0.75 m/s²) relative to either of the lowest 2 acceleration levels (0.25 and 0.15 m/s²; Figure 4). A decreasing dose-response relationship was observed for the total number of mice responding from the highest to lowest acceleration.

At an acceleration of 1.0 m/s², at least 2 of 5 animals reacted at all frequencies tested between 20 and 140 Hz (Figure 5 A). The number of mice reacting at this relatively high acceleration did not show an obvious frequency-specific sensitivity pattern to the frequencies tested. When the accelerations of 0.75 and 0.5 m/s² were tested at the same frequencies, bell-shaped curves emerged (Figure 5 B). At 0.5 m/s² acceleration, the behavioral responses were significantly ($P \leq 0.01$) greater in the frequency range of 70 to 100 Hz than the 4 lower frequencies. At the acceleration of 0.75 m/s², significantly ($P \leq 0.01$) more mice reacted at 70 to 100 Hz than at either the 4 lower or higher frequencies (Figure 5 B), indicating that mice may be particularly sensitive to vibration-induced behavioral changes within 70 to 100 Hz. Responses at 0.25 m/s² and 0.15 m/s² appeared sporadically in mice across several frequencies between 50 to 130 Hz (Figure 5 B), whereas at 0.1 m/s², 1 of 5 mice reacted at 90 Hz during repeated experiments; therefore further testing at this acceleration was not performed (data not shown). However, no mice showed reactions to vibration at 0.05 m/s² at any of the frequencies tested from 30 to 110 Hz, the resonance frequency range for mice (Figure 5 B).

To confirm that 0.05 m/s² was an accurate threshold, mice with no previous exposure to vibration were exposed to 0.25 m/s², as a control, or at 0.05 m/s² at 90 Hz. In addition, the mice were tested without vibration exposure. At 0.25 m/s²,

Figure 4. Total number (mean $\pm$ SEM) of mice responding at each acceleration level over the 13 frequencies tested from 20 to 140 Hz. Different lowercase letters indicate values that are significantly ($P \leq 0.05$) different.

Figure 5. (A) Number of mice responding to vibration at various frequencies and at 1.0 m/s². Mice are similarly sensitive to all vibration frequencies at this high level of acceleration. (B) Mice demonstrated more ($P \leq 0.01$) responses at frequencies of 70 to 100 Hz at accelerations of 0.5 m/s² (relative to the 4 lower frequencies) and 0.75 m/s² (relative to both the 4 lower and 4 higher frequencies), producing a bell-shaped curve. Behavioral responses at accelerations of 0.05, 0.15, and 0.25 m/s² occurred over various frequencies. No mice reacted at the acceleration of 0.05 m/s².

3 of 5 mice responded, whereas no mice responded without vibration exposure or at 0.05 m/s², indicating that 0.05 m/s² is at or below the threshold of vibration that would induce observable behavioral changes in mice housed in cages as described. Figure 6 is a representative spectrogram showing the vibration peak at 0.25 m/s² and 90 Hz, with relative levels of the established threshold of 0.05 m/s² (50 mm/s²), at which mice failed to respond, and the maximal ambient vibration levels.

At frequency ranges where mice exhibited the highest number of responses to vibration (1.0 m/s² at 70 to 100 Hz), changes in the numbers of active behaviors and maintenance or inactive behaviors were more pronounced at frequency levels greater

than 70 Hz (Figure 7; $P \leq 0.01$). Relative to the control period, the numbers of neither active nor maintenance or inactive behaviors differed during vibration exposure, except at 80 Hz (Figure 7). At 80 Hz, the number of active behaviors significantly ($P \leq 0.05$) increased and maintenance and inactive behaviors decreased between the control and vibration periods.

Secondary resonance as a factor in vibration exposure. Behavioral changes were noted at experimentally induced vibration of 160 and 190 Hz at accelerations as low as 0.15 m/s² (Figure 8). At 150 and 160 Hz, simultaneous secondary vibration peaks occurred at higher frequencies of 300, 320, 450, 480, and 600 Hz (data not shown). Because this relatively high-magnitude vibration was produced at multiple frequencies when vibration was applied at 150 and 160 Hz, for example, the effects of each frequency on behavioral responses could not be separated, and an upper threshold limit of vibration could not be established.

Repeated exposure to vibration. Mice exposed to repeated periods of vibration at 90 Hz and an acceleration of 1.0 m/s² continued to react to the vibration for all 10 trials, whereas the reactions of mice exposed at 0.5 m/s² decreased numerically as the number of exposures increased (Figure 9). The mean number of mice responding in the first 2 trials was greater ($P \leq 0.01$) than the mean of the animals responding in the total number of subsequent trials at both the 0.5 m/s² (4.0 and 1.1, respectively) and the 1.00 m/s² (5.0 and 2.7, respectively) acceleration. Although inconclusive, these data suggest that the responses of mice may decrease rapidly during repeated exposure to vibration.

Discussion

Prior to vibration exposure, mice were allowed roughly 20 h to acclimate to the testing cage to prevent significant differences between the incidences of active behaviors and inactive or maintenance behaviors. During vibration exposure at higher acceleration levels such as 1.0 m/s², response rates did not differ, regardless of the frequency tested. However, at intermediate accelerations of 0.75 and 0.5 m/s², mice showed the most responses to frequencies between 70 to 100 Hz. Mice exposed to vibration at 1.0 m/s² between 70 to 100 Hz, did not show any significant differences between active and maintenance or inactive behaviors except at 80 Hz, where active behaviors were increased during the vibration period relative to the control period. The most prevalent behavioral responses included brief, abrupt changes in body positioning and interruptions in previous normal behavior. At an acceleration level of 0.05 m/s² mice failed to show a noticeable response even at frequencies between 70 to 100 Hz. Mice exposed to repeated short periods of vibration at 1.0 m/s² continued to react during all trials tested while mice exposed repeatedly to accelerations at 0.5 m/s² did show some decreases in response rate.

To ensure that any behavioral differences were attributable to vibration and not the new testing environment, our first goal was to determine an adequate acclimation period for our studies. Our study indicated that between 6 to 24 h might be needed for mice to acclimate to a new environment from a behavioral standpoint. These results agree with a previous study that showed that active behaviors, such as rearing and climbing, significantly increased after rodents were transported to a new environment and took as long as 1 d to decrease back to baseline.³¹ After being transported to a new animal facility, mice are often acclimated to the new housing area for at least 3 to 5 d.^{8,31} Previous studies have indicated that during this time, food and water consumption return to baseline after major transport.³¹ After even minor changes in location or common husbandry procedures, mice have transient changes

Figure 6. Spectrogram showing applied vibration at 90 Hz and 0.25 m/s². When exposed, 3 of 5 mice reacted at this level; all mice failed to respond at 90 Hz and 50 mm/s² (blue line). Maximal ambient vibration is represented by the green line.

in corticosterone levels, intestinal microbiota, and overall behavior.^{22,31} Studies that involve behavioral testing on rodents usually provide only 30 to 60 min for the animals to acclimate to the new environment.^{4,36}

We chose to test the same mice at different acceleration levels to decrease variability and to reduce the number of animals used. As expected, the number of behavioral responses to vibration decreased stepwise as the acceleration of vibration decreased. In addition to the slower rate of acceleration, mice had previously been exposed to vibration and were at least 5 d older when the next acceleration level was tested; both of these factors might have contributed to the decreased responses as the acceleration level was lowered. Many acoustic and tactile startle protocols use 100 or more repeated trials in a single day to achieve short-term habituation, and repeated trials over several consecutive days are necessary for long-term habituation.^{26,35} In our study, in which the same mice were tested at multiple acceleration levels, trials were performed on only 1 d for each animal with at least 5 d between exposures, to prevent habituation to vibration. In addition, repeatedly using the same mice resulted in testing the same mice at ages of 7 to 13 wk. C57BL/6 mice in this approximate age range (8 to 12 wk) have been grouped together for comparison to other age groups regarding behaviors including freezing and other fear behaviors.²⁹ In addition, freezing behavior, as noted in all vibration-induced behavior responses, generally does not decline with age in rodents and does not show an age-related change until at least 11 mo in C57BL/6 mice.¹⁹ The same number of mice responded to 0.25 m/s² and 90 Hz (3 of 5) regardless of whether the mice were vibration-naïve or had previously been exposed experimentally to vibration. This finding supports the absence of any effect of habituation or age as variables to decrease responses to different acceleration levels.

At an experimental acceleration of 1.0 m/s², the highest number of mice reacted across all frequencies. The high number of reactions at this acceleration is unsurprising considering 1.0 m/s² is a fairly high magnitude of vibration, comparable to an earthquake strong enough to be readily perceived by humans and able to move heavy furniture.³³ Although the greater number of mice reacting at this level of vibration was expected, the nature of the behavioral changes was somewhat surprising in

Figure 7. (A) Number (mean $\pm$ SEM) of active behaviors per frequency for control (immediately before vibration) and vibration periods at 1 m/s². Number of behaviors varied significantly ($P < 0.01$) across frequencies during the vibration period. At 80 Hz, the number of active behaviors differed significantly (*, $P \leq 0.05$) between the control and vibration periods. (B) Number (mean $\pm$ SEM) of maintenance or inactive behaviors per frequency for control (immediately before vibration) and vibration periods at 1.0 m/s². Number of behaviors varied significantly ($P < 0.01$) across frequencies during the vibration period. At 80 Hz, the number of maintenance or inactive behaviors differed significantly (*, $P \leq 0.05$) between the control and vibration periods.

that mice appeared to only show a brief response during the first 20 s after vibration initiation and then returned to their previous (control) behavior. This characteristic of behavioral change was consistent among all frequencies and accelerations at which mice responded to the introduction of vibration. Therefore, from the

Figure 8. Behavioral responses at frequencies between 150 and 190 Hz, with likely contribution of vibration at higher frequencies.

Figure 9. Number (mean $\pm$ SEM) of mice responding to repeated vibration exposure at 90 Hz and at accelerations of 1.0 and 0.5 m/s². At both acceleration levels, the mean number of mice responding during the first 2 trials was greater ($\dagger$, $P \leq 0.01$) than that responding in subsequent trials.

behavioral aspect, mice have a very transient reaction to only the initiation of vibration. The transient response may be due to the fact that mice are exposed to vibration on a relatively regular basis, and once the sensation is recognized, it is dismissed as nonthreatening.

Mice that were sleeping during vibration initiation raised their head with ears forward, sometimes preceded by shifting in position. In contrast, awake mice always abruptly halted (freezing) any voluntary movement, with or without turning their heads up, down, or toward the sides of the cage, as though closely observing their environment. The freeze response, including visually scanning the environment, are components of fear behavior in mice.^{2,34} This behavior includes cessation of voluntary movement for variable periods of time, thereby potentially escaping detection by a predator, as well as continuing to visually scan the environment for threats while remaining poised to flee as needed.²⁰ When first exposed, the mice in our study indicated that they perceived vibration as a possible threat.

Along with the freeze response, the mice sometimes responded to vibration by assuming a hunched posture, as would occur with a startle reflex. We occasionally noted a discernible startle reflex prior to the freeze response. Startle reflexes in rodents have previously been elicited in response to either auditory or tactile stimuli (for example, electrical stimulation of the skin or sudden air-puffs).^{15,25,26,28,35,37} In response to these stimuli, rodents exhibit a reflexive action that involves acute muscle contraction; contracting the head and neck musculature results in a hunched posture.^{10,37} In the current study, vibration served as a form of tactile stimulation, but the characteristic hunched posture that mice exhibit in the classic startle reflex was not always apparent and tended to be more frequent at the higher acceleration levels (that is, 0.75 and 1.0 m/s²). Only about 7.5% of awake mice that exhibited a positive response to vibration

displayed the characteristic hunched posture noted in the startle reflex. This percentage may have been decreased given that measurements were done by visual observation rather than with the use of more sensitive equipment. However, the findings in this study represent what an observer in the laboratory animal facility would perceive.

We noted variability between mice at some frequencies across acceleration rates in regard to the number of behavioral responses. For example, at an acceleration of 0.25 m/s², a total of 3 mice reacted to vibration at 50 Hz, but at 0.5 m/s², only 1 mouse reacted at the same frequency. At higher accelerations (that is, 0.75 m/s² and 1.0 m/s²), reactions such as hunched posture were very prominent and clearly visible. However, as acceleration decreased, reactions became less evident, although mice still exhibited the consistent behavioral signs, such as freezing their motion and looking up, at the cage floor, or sides of the cage. The variability at lower accelerations may have been due to the subtle nature of the responses, the mice not consistently perceiving the vibration as a threat, or the type of behavior they were exhibiting when the vibration was initiated.

Compared with responses at other frequencies, mice exhibited more fear-related behavioral reactions between 70 and 100 Hz, indicating that mice are more sensitive to vibration within this frequency range. This range falls within the previously proposed resonance frequency range of 30 to 110.^{21,27} In contrast to the mice when they were not exposed to vibration, there was a significant difference in active compared with maintenance or inactive behaviors across the frequencies tested when mice were exposed to 1.0 m/s². Active behaviors were increased and maintenance and inactive behaviors were decreased at the frequency levels above 70 Hz. In addition, at 80 Hz, mice exhibited significantly more active and fewer maintenance and inactive behaviors during vibration than during the control period. This finding supports the results of a previous study at which vibration at frequencies of 80 and 90 Hz—but not at higher or lower frequencies—led to elevations in heart rate and blood pressure.²¹ The behavioral observations in our study confirm that the frequencies of 80 and 90 Hz are within the sensitive frequency range for mice exposed to vibration. However, behavioral changes occur at a much wider frequency range and at lower accelerations than those that cause increases in heart rate and blood pressure, indicating that behavioral changes are likely a more sensitive indicator of vibration exposure.

At frequencies of 150 and 160 Hz, secondary peaks in the magnitude of vibration occurred at 300 and 450 Hz and at 320 and 480 Hz, respectively, due to harmonics. These secondary resonance peaks likely accounted for the tendency for more behavioral responses at the higher frequencies tested. Harmonic peaks in vibration magnitude are often observed in mechanical systems when there are irregularities, looseness, or imbalance between machine components, even at microscopic levels.⁹ One or more of these conditions likely would be present in any animal caging system, given that cages are not secured so tightly that all movement is prohibited. In the testing system we used, harmonics may have resulted from microscopic movement between the cage and the platform even though the cage was tightly secured. The harmonic frequencies likely contributed to additional vibration of the cage and caused the numerically increased behavioral responses at 150 and 160 Hz. The fact that secondary peaks of vibration can occur may result in the inability to determine standards for an upper-frequency range at which mice are no longer susceptible to vibration in laboratory animal housing conditions. In addition, this finding indicates that more than just the frequencies of the

primary source of vibration must be considered in regard to animal exposure.

In a previous study, vibration caused by transporting animals on various types of carts, with or without buffering materials between the cart and cage, produced mean vibration levels ranging from 1.24 to 8.60 m/s² in the vertical direction.¹⁶ Carrying cages by hand yielded a mean vibration acceleration of 1.98 m/s². Another previous study showed that 0.75 m/s² can increase heart rate and blood pressure in mice,²¹ and the current study demonstrates that behavioral changes can occur with accelerations as low as 0.1 m/s². Therefore, mice are likely to experience levels of vibration that can alter behavior and increase heart rate and blood pressure during transport. In addition, recent studies have demonstrated that trains traveling outside of a vivarium resulted in the exposure of mice in an animal room to vibration levels of around 0.025 g (approximately 0.25 m/s²). Exposure to vibration at these levels increased fecal corticosterone levels among female mice.¹ In the current study, vibration at 0.25 m/s² produced behavioral reactions at several frequencies. Therefore, in addition to vibration produced within the animal facility, vibration sources outside of the facility can potentially alter behavior.

The acceleration of 0.05 m/s² did not elicit behavioral responses in mice. Although the median human limit of vibration perception is 0.01 m/s²,²² the mice in our study were in bedded cages, as in a typical laboratory animal facility. The bedding might have dampened vibration, such that the acceleration actually perceived by mice might have been lower than 0.05 m/s².

Although inconclusive, data from mice that were exposed to repeated, short periods of vibration suggest that behavioral responses may be less prevalent when repeated vibration exposure occurs with short pauses between the episodes. Habituation occurred in studies using sound or tactile stimuli to elicit the startle reflex.^{25,26,28} Although our data are inconclusive in regard to habituation, they prompt the question of possible acclimation to vibration with repeated or continuous exposure, especially with repeated vibration exposure over consecutive days. However, the potential for acclimation to vibration may depend on many factors, including the strain of mice and the duration and interval of vibration, as has been shown to occur with other stimuli. Studies have shown differences among common strains such as DBA and BALB/c mice in regard to the degree of habituation to tactile and acoustic stimuli.²⁵ For investigators using different strains of rodents than the C57BL/6 mice we tested, the potential for differences in the ability of mice to acclimate to vibration remains.

Although we now have more information regarding the behavioral changes that occur due to vibration, the frequencies of vibration to which mice are particularly sensitive, and an observational vibration threshold, several questions remain. For example, we used female mice in this study, but results differ for male mice or pregnant or nursing dams. Whether different strains of mice react differently remains unknown. In addition, vibration exposure in the current study was acute, but if vibration exposure were to occur as several exposure sessions during several hours or for a single prolonged duration, further behavioral effects may be noted. Little is known regarding the effects of vibration on physiologic or biochemical parameters. However, fecal corticosterone is increased in mice exposed to vibration due to trains traveling near animal facilities.¹ Rats repeatedly exposed to low-frequency vibration at 20 Hz exhibit altered LDL- and HDL-cholesterol levels.¹⁸ Furthermore, several studies demonstrate the physiologic effects of noise on rodents, including increased norepinephrine and cholesterol levels in rats

and decreased splenic lymphocyte counts in mice.³² Whether similar changes occur in mice exposed to vibration is unknown. These topics warrant investigation in future studies to fully discern the potential effects of vibration on research outcomes as well as the wellbeing of laboratory mice.

In conclusion, our mice showed behavioral reactions consistent with a fear response in response to short-term, acute vibration, suggesting that they perceive vibration as a potential threat. The total number of responses elicited from all frequencies tested was higher at the higher acceleration levels and appeared to be dose-dependent in regard to the magnitude of vibration administered. Responses to vibration were transient, and mice often returned to their previous behavior after the initiation of vibration, even though vibration exposure continued. Behavioral responses decreased with decreasing acceleration and were unapparent at the vibration acceleration rate of 0.05 m/s². This level of acceleration may serve as observational threshold limit to vibration for mice in bedded cages. In addition, 70 to 100 Hz appears to be the most sensitive range for mice to perceive vibration and alter their behavior. However, vibration at any frequency should be minimized due to the sensitivity of mice at the higher frequencies and secondary harmonic peaks of vibration at frequencies other than those generated by the primary source. Our findings demonstrate that not only the frequency of vibration induced by the primary source but also the vibration induced at harmonic frequencies should be considered. Behavioral changes due to vibration may not be apparent if mice habituate to the vibration. Although subtle, the responses to vibration could pose a source of research variability, especially in behavioral studies. Due to the known and unknown effects of vibration on animals, care should be taken to minimize all sources of vibration in animal facilities.

Acknowledgments

We thank Andy Butcher and the engineers at Brüel and Kjær North America for their technical support, Dr Ramona Rodriguez for her expertise in mouse behavior, Drs Daniel Schmitt and Christine Wall for the use of their vivarium space, Dr Jai Tubbs for her assistance with manuscript preparation, students Christine Zavala and Andy Smith, and the staff of the Duke University Medical Center, Division of Laboratory Animal Resources. This study was supported by a grant from the American College of Laboratory Animal Medicine Foundation.

References

1. Atanasov NA, Sargent JL, Parmigiani JP, Palme R, Diggs HE. 2015. Characterization of train-induced vibration and its effect on fecal corticosterone metabolites in mice. *J Am Assoc Lab Anim Sci* 54:737–744.
2. Balogh SA, Radcliffe RA, Logue SF, Wehner JM. 2002. Contextual and cued fear conditioning in C57BL/6J and DBA/2J mice: context discrimination and the effects of retention interval. *Behav Neurosci* 116:947–957. <https://doi.org/10.1037/0735-7044.116.6.947>.
3. Bedrosian TA, Vaughn CA, Weil ZM, Nelson RJ. 2013. Behaviour of laboratory mice is altered by light pollution within the housing environment. *Anim Welf* 22:483–487. <https://doi.org/10.7120/09627286.22.4.483>.
4. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. 2012. The mouse forced swim test. *J Vis Exp* 59:1–5. doi: 10.3791/3638.
5. Canadian Centre for Occupational Health and Safety. [Internet]. 2017. Vibration—introduction. [Cited 13 November 2017]. Available at: https://www.ccohs.ca/oshanswers/phys_agents/vibration/vibration_intro.html.
6. Carman RA, Quimby FW, Glickman GM. 2007. The effect of vibration on pregnant laboratory mice. International congress and exposition on noise control engineering (Inter-Noise). Istanbul,

- Turkey, 28–31 August 2007. Institute of Noise Control Engineering, 1–11.
7. **Charles River**. [Internet]. 2017. North American Health Reports. [Cited 06 June 2017]. Available at: <http://www.criver.com/Health-Data/na/H07R020M.pdf>.
 8. **Conour LA, Murray KA, Brown MJ**. 2006. Preparation of animals for research—issues to consider for rodents and rabbits. *ILAR J* 47:283–293. <https://doi.org/10.1093/ilar.47.4.283>.
 9. **Crocker MJ, editor**. 1998. Handbook of acoustics. New York (NY): John Wiley and Sons.
 10. **Curzon P, Zhang M, Radek RJ, Fox GB**. 2009. The behavioral assessment of sensorimotor processes in the mouse: acoustic startle, sensory gating, locomotor activity, rotarod, and beam walking. In: Buccafusco JJ, editor. *Methods of behavior analysis in neuroscience*, 2nd ed. Boca Raton (FL): CRC Press/Taylor and Francis.
 11. **Encyclopedia Britannica**. [Internet]. 2017. Frequency. [Cited 06 June 2017]. Available at: <https://www.britannica.com/science/frequency-physics>.
 12. **Felt SA, Cowan AM, Luong R, Green SL**. 2012. Mortality and morbidity in African clawed frogs (*Xenopus laevis*) associated with construction noise and vibrations. *J Am Assoc Lab Anim Sci* 51:253–256.
 13. **Fernando AB, Robbins TW**. 2011. Animal models of neuropsychiatric disorders. *Annu Rev Clin Psychol* 7:39–61. <https://doi.org/10.1146/annurev-clinpsy-032210-104454>.
 14. **Gaskill BN, Gordon CJ, Pajor EA, Lucas JR, Davis JK, Garner JP**. 2012. Heat or insulation: behavioral titration of mouse preference for warmth or access to a nest. *PLoS One* 7:1–11. <https://doi.org/10.1371/journal.pone.0032799>.
 15. **Halberstadt AL, Geyer MA**. 2009. Habituation and sensitization of acoustic startle: opposite influences of dopamine D1 and D2 family receptors. *Neurobiol Learn Mem* 92:243–248. <https://doi.org/10.1016/j.nlm.2008.05.015>.
 16. **Hurst K, Litwak KN**. 2012. Accelerative forces associated with routine inhouse transportation of rodent cages. *J Am Assoc Lab Anim Sci* 51:544–547.
 17. **Institute for Laboratory Animal Research**. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
 18. **Kasprzak C, Damijan Z, Panuszka R**. 2004. Influence of low-frequency vibration on changes of biochemical parameters of living rats. *J Acoust Soc Am* 115:2443–2443. [10.1121/1.4782140](https://doi.org/10.1121/1.4782140).
 19. **Kennard JA, Woodruff-Pak DS**. 2011. Age sensitivity of behavioral tests and brain substrates of normal aging in mice. *Front Aging Neurosci* 3:1–22 [PubMed]. [10.3389/fnagi.2011.00009](https://doi.org/10.3389/fnagi.2011.00009).
 20. **Kozłowska K, Walker P, McLean L, Carrive P**. 2015. Fear and the defense cascade: clinical implications and management. *Harv Rev Psychiatry* 23:263–287 [PubMed]. [10.1097/HRP.0000000000000065](https://doi.org/10.1097/HRP.0000000000000065).
 21. **Li Y, Rabey KN, Schmitt D, Norton JN, Reynolds RP**. 2015. Characteristics of vibration that alter cardiovascular parameters in mice. *J Am Assoc Lab Anim Sci* 54:372–377 [PubMed].
 22. **Ma BW, Bokulich NA, Castillo PA, Kananurak A, Underwood MA, Mills DA, Bevins CL**. 2012. Routine habitat change: a source of unrecognized transient alteration of intestinal microbiota in laboratory mice. *PLoS One* 7:1–11 [PubMed]. [10.1371/journal.pone.0047416](https://doi.org/10.1371/journal.pone.0047416).
 23. **Parsons KC, Griffin MJ**. 1988. Whole-body vibration perception thresholds. *J Sound Vib* 121:237–258. [https://doi.org/10.1016/S0022-460X\(88\)80027-0](https://doi.org/10.1016/S0022-460X(88)80027-0).
 24. **Paschold HWM, Mayton AG**. 2011. Whole-body vibration: building awareness in SH&E. *Prof Saf* 56:30–35.
 25. **Pilz PK, Carl TD, Plappert CF**. 2004. Habituation of the acoustic and the tactile startle responses in mice: 2 independent sensory processes. *Behav Neurosci* 118:975–983. <https://doi.org/10.1037/0735-7044.118.5.975>.
 26. **Plappert CF, Pilz PK**. 2005. Long-term habituation of the startle response in mice evoked by acoustic and tactile stimuli. *Behav Brain Res* 162:307–310. <https://doi.org/10.1016/j.bbr.2005.03.022>.
 27. **Rabey KN, Li Y, Norton JN, Reynolds RP, Schmitt D**. 2014. Vibrating frequency thresholds in mice and rats: implications for the effects of vibrations on animal health. *Ann Biomed Eng* 43:1957–1964. <https://doi.org/10.1007/s10439-014-1226-y>.
 28. **Sasaki A, Wetsel WC, Rodriguiz RM, Meck WH**. 2001. Timing of the acoustic startle response in mice: habituation and dishabituation as a function of the interstimulus interval. *International Journal of Comparative Psychology* 14:258–268.
 29. **Shoji H, Takao K, Hattori S, Miyakawa T**. 2016. Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. *Mol Brain* 9:1–18. <https://doi.org/10.1186/s13041-016-0191-9>.
 30. **Snyder JM, Treuting PM, Brabb T, Miller KE, Covey E, Lencioni KL**. 2015. Hepatic lipidosis in a research colony of big brown bats (*Eptesicus fuscus*). *Comp Med* 65:133–139.
 31. **Tuli JS, Smith JA, Morton DB**. 1995. Stress measurements in mice after transportation. *Lab Anim* 29:132–138. <https://doi.org/10.1258/002367795780740249>.
 32. **Turner JG, Parrish JL, Hughes LF, Toth LA, Caspary DM**. 2005. Hearing in laboratory animals: strain differences and nonauditory effects of noise. *Comp Med* 55:12–23.
 33. **U.S. Geological Survey**. [Internet]. 2016. The severity of an earthquake. [Cited 06 June 2017]. Available at: <https://pubs.usgs.gov/gip/earthq4/severitygip.html>.
 34. **Valentinuzzi VS, Kolker DE, Vitaterna MH, Shimomura K, Whiteley A, Low-Zeddies S, Turek FW, Ferrari EAM, Paylor R, Takahashi JS**. 1998. Automated measurement of mouse freezing behavior and its use for quantitative trait locus analysis of contextual fear conditioning in (BALB/cJ × C57BL/6J)F2 mice. *Learn Mem* 5:391–403.
 35. **Valsamis B, Schmid S**. 2011. Habituation and prepulse inhibition of acoustic startle in rodents. *J Vis Exp* 55:1–10.
 36. **Yang M, Crawley JN**. 2009. Simple behavioral assessment of mouse olfaction. *Curr Protoc Neurosci* 48:8.24.1–8.24.12. <https://doi.org/10.1002/0471142301.ns0824s48>.
 37. **Yeomans JS, Li L, Scott BW, Frankland PW**. 2002. Tactile, acoustic, and vestibular systems sum to elicit the startle reflex. *Neurosci Biobehav Rev* 26:1–11. [https://doi.org/10.1016/S0149-7634\(01\)00057-4](https://doi.org/10.1016/S0149-7634(01)00057-4).