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Noise and Vibration Generation and Response of Mice (*Mus musculus*) to Routine Intrafacility Transportation Methods

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Intrafacility transport of mice is an essential function for both laboratory and husbandry personnel. However, transport may induce a stress response that can alter research findings and negatively impact animal welfare. To determine minimally adverse intrafacility transport methods, in-cage noise and vibration exposure during transport on a variety of transport vehicles (hand carrying, stainless steel rack, flatbed cart, metal teacart, plastic teacart, and a cart with pneumatic wheels) were measured. Under-cage and in-cage padding was tested for its ability to decrease noise and vibration on each vehicle. Behavioral (open field test and elevated plus maze) and corticosterone responses of mice were then measured following transport on the most adverse (metal teacart) and least adverse (pneumatic cart) methods of multicage transport. Behavioral measures showed no difference between transported mice and untransported mice in both single- and group-housed settings. Plasma corticosterone was significantly elevated in mice transported on the metal teacart immediately following transport and continued to have elevated trends in circadian peaks during the 48 h of sampling. The cart with pneumatic wheels was most effective at reducing noise and vibration, reflected in posttransport corticosterone readings that remained equivalent to those in untransported mice. This study demonstrates that mitigation of noise and vibration during cart transport may decrease the impact of transport on certain stress parameters in mice.

Abbreviations and Acronyms: dBZ, Z-weighted dB; EPM, elevated plus maze; OFT, open-field test; RMS, root mean square

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Introduction

Exposures to elevated noise and vibration levels have been associated with a range of often negative behavioral and physiologic changes in mice and rats. These animals are especially susceptible to noise exposure due to their vastly extended range of hearing, expanding far beyond the human limits of 20 kHz up to 80 to 100 kHz.[27] Consequently, current recommendations suggest that average vivaria noise levels be maintained at <70 unweighted or Z-weighted decibels (dBZ), and that peaks as low as 80 dBZ are sufficient to induce a startle response in most laboratory rodents.[35] In addition, cochlear damage and subsequent hearing loss have been documented with prolonged noise exposures greater than 85 dBZ in both humans and laboratory animals.[36] Although less is known about the effects of vibration, levels of 0.25 m/s^2 or lower are considered acceptable for rodent facilities, although lower levels can be perceived by rodents to a less defined effect.[35] Noise and vibration levels above recommendations have the potential to impact behavior, reproduction, and a vast number of physiologic parameters affecting nearly every field of research.[1,3,4,8,11,12,22,24–28,33–36] Therefore, it is essential to identify and mitigate sources of excessive noise and vibration to optimize animal welfare and research data.

Many routine activities in rodent housing facilities can result in elevations in noise and vibration.[8,27,35] One of these activities is the transportation of animals within vivaria, typically performed on various types of carts to allow for efficient transport of multiple cages. Intrafacility cart transport results in elevations in noise and vibration within rodent cages that exceed current recommendations, although the effects of this exposure have not been fully examined.[15,27,31] The current body of literature has limited association of specific noise and vibration exposures during transport with stress responses. However, temporary physiologic disruption has been demonstrated in rats after transport.[2,7,8,14,21,36] One study similarly reported elevations in corticosterone in mice in response to transport in hand-carried cages to a laboratory space.[34] However, the responses of mice to different methods of cart transport have not been explored. Association of transport noise and vibration generation with the responses of mice to cart transport and attenuation strategies would better determine the impact of these variables on transport stress, best strategies to mitigate their impact, and acclimation time needed for transported mice to return to baseline.

In this study, we hypothesized that sound and vibration elevations during routine intrafacility transport causes physiologic and behavioral changes in mice and that attenuation of these noxious elements will reduce the impact of transport on these changes. To test this, we aimed to 1) measure noise and vibration levels caused by several methods of intrafacility transport at our institution, 2) examine methods to mitigate the elevated levels, and 3) determine the temporal effects on behavioral studies and corticosterone levels in mice. The goal was to determine the least aversive transport method to lower stress, optimize acclimation periods, and improve overall animal welfare.

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Materials and Methods

Sound and vibration recording meters. ***Measurement of noise***. A sound meter (NSRT_mk3; Convergence Instruments, Sherbrooke, QC, Canada) was used to measure the noise generated within cages (Figure 1). The type 1 sound meter measured 19 × 42 × 160 mm and weighed 100 g. This device collected Z-weighted equivalent continuous sound pressure levels (Leq) once per second with a bandwidth of 20 Hz to 20 kHz, saturation level of 120 dBZ, and noise floor of 52 dBZ. Maximum noise levels were reported as the maximum Leq reached during a trial, and average noise levels were reported as average Leq across the duration of the trial. A manufacturer's certificate of calibration was received along with the sound meter at the time of purchase.

Measurement of vibration. A vibration meter (VSE mk2-8g; Convergence Instruments, Sherbrooke, QC, Canada) measuring 7.62 × 3.94 × 2.06 cm and weighing 65 g was used to measure vibration along 3 axes (x = side to side, y = forward and backward, z = up and down) once per second for the duration of the trial (Figure 1). Vibration was reported as root mean square (RMS) acceleration measured in meters per second squared (m/s^2) within a dynamic range of ±78.4 m/s^2. Maximum vibration levels were reported as the maximum RMS acceleration reached during a trial, and average vibration levels were reported as average RMS acceleration across the duration of the trial. The vibration meter was calibrated prior to each daily use along all axes using earth's gravity as a reference.

Cage setup. The sound and vibration meters were positioned centrally side by side on bedding within a standard cage to best estimate the noise and vibration levels experienced by the animals. Each cage was covered with a polycarbonate filter top lid and had a metal food hopper hanging on the rear wall. An upside-down sipper-style water bottle was placed in the appropriate slot to reflect the standard intrafacility transport setup used at our institution.

Experiment to define the levels of noise and vibration generated by different transport techniques. ***Study design***. Instrumented cages without mice were placed on transport devices (1 flatbed, 1 stainless steel rack, 2 plastic carts, and 1 metal cart) or handheld and moved through a predefined route within the facility. Measurements were recorded automatically once per second. Controls were obtained in IVC housing conditions explained below. Maximum level achieved and mean level for the entire transport were compared between the transport methods. To examine simple methods to reduce noise and vibration, the experiments were repeated on each transport device using external attenuation (1 or 2 towels under the cage) or internal attenuation (brown crinkle paper within the cage).

Transportation methods. For control conditions, data were collected by placing the instrumented cage centrally into an individually ventilated system with top-mounted ventilation units (EcoFlo; Allentown, Allentown, NJ). Three recordings of 3-min duration were taken with 2-min intervals between recordings. IVC measurements were used as the primary control comparison as this is the standard rodent housing method throughout most of our institution.

For initial comparisons of transport methods, data were measured continuously during transport. For hand carrying, the cage was held horizontally in a 2-hand hold with the cage long axis perpendicular to the direction of travel. For cart transport, the cage was placed centrally with the long axis perpendicular to the direction of travel on the top shelf of the cart. For the stainless-steel rack, the cage was placed on the central shelf. Cart details can be found in Table 1. All rack casters and those closest to the cart and flatbed handles swiveled while the remaining

Figure 1. Setup of sound and vibration meter cage. Left: cage interior: sound meter placed on left on cage floor, vibration meter placed on right of cage floor. Right: cage exterior.

Table 1. Characteristics of carts used

Cart	Length (m)	Width (m)	Height (m)	Weight (kg)	Wheel diameter (mm)	Wheel width (mm)	Rubber wheel type	Shelf number	Manufacturer
Flatbed	1.2	0.6	0.1	25.8	152	52	Hardened	1	Ancare
Stainless steel rack	1.6	0.6	1.8	70.8	127	32	Hardened	7	Ancare
Plastic teacart	0.8	0.4	0.7	11.8	127	25	Hardened	2	Uline
Pneumatic (plastic) teacart	0.9	0.6	0.7	30.0	203	64	Pneumatic	2	Uline
Metal teacart	0.8	0.5	0.7	19.1	127	25	Hardened	2	Uline

Manufacturer locations: Ancare, Bellmore, NY; Uline, Pleasant Prairie, WI.

wheels remained fixed. All cart shelves were supported by L-shaped struts 7.6 cm wide and 0.6 cm thick on both plastic carts and 3.8 cm wide and 0.07 cm wide on the metal cart. The stainless-steel rack shelves were adjustable clip-in shelf mounts on 2.5-cm hollow square struts.

Transportation route. The route was a standardized, 96-m loop on even epoxy resin flooring. At the halfway point, an elevator was entered and ridden up one floor, exited, and then an adjacent elevator was entered and ridden back to the original floor where the loop was completed. The route was completed in an average of 3.9 min including wait times for the elevators. One individual with an average walking speed of 4 km/h performed all of the transports. The route was repeated a total of 6 times, split between 2 different days, for each type of transport.

Attenuation methods. The transportation process was repeated with each cart using 3 different attenuation methods: a double-folded towel (approximately 20 mm thick) placed under the cage, 2 double-folded towels (40 mm thick) placed under the cage, or 6 g of brown crinkle paper within the cage but under the sensors.

Experiments to determine the effects of transport on indicators of stress. ***Study design***. The transport methods generating the lowest level of noise and vibration (pneumatic cart) and the highest levels (metal cart) were used in subsequent animal testing. Transport stress was assessed with behavior testing or blood biomarkers in separate experiments. In the behavioral study groups, mice (n = 10/group) were implanted with a temperature transponder one week prior to experimentation and group housed 2 to 3 mice per cage. Each mouse underwent transport followed by an open-field test (OFT) directly after transport and an elevated plus maze (EPM) test 24 h later. Body temperature readings were obtained immediately before and after transport. Control mice were not transported prior to testing aside from transfer from housing to the behavior testing room prior to acclimation to the testing room. After the testing, the group-housed mice remained in standard housing for one month. The mice were then singly housed for one week followed by repetition of the transport and behavioral studies. Figure 2 summarizes the timeline of the behavior measurements.

For evaluation of blood biomarkers, mice were catheterized for automated serial blood collection and singly housed after surgery. After 3 d of recovery, a baseline blood sample was collected. Mice were disconnected from the automated collection device and transported on their assigned transport device (metal cart or plastic pneumatic cart). After transport, automatic blood collection was resumed immediately and at predetermined intervals for 48 h. Control mice were disconnected from the automatic collection device after baseline sampling, held in the same room in a clean shoebox cage for 15 min, and reconnected for sampling. Blood samples were processed upon completion of sampling and analyzed for corticosterone levels. Figure 3 summarizes the timeline of the biomarker measurements. Experimental groups for animal studies are summarized in Table 2.

Animals. Male (10 to 12 wk of age) C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were used in all animal experiments. Male mice were selected to limit variability in biomarker and behavioral responses as a result of estrus cyclicity.[9,11,13,20] Mice were housed in a 18- × 29- × 12.5-cm polycarbonate mouse cage (Allentown, Allentown, NJ) on approximately 300 mL of a 50/50 blend of ¼- and ⅛-in. irradiated corncob bedding (Anderson's Bed-o'Cobs; Frontier Distributing, Maumee, OH) with 6 g of brown crinkle paper encased in a white tea bag material (EnviroPak; Shepherd Specialty Papers, Watertown, TN). Individually housed mice were also provided with a clear red plastic mouse igloo (Bio-Serv, Flemington, NJ) or a 2- × 2-in. compressed cotton square (Ancare, Bellmore, NY) if cannulated for blood collection. Prior to experimental use, mice were acclimated for at least 7 d in ventilated microisolation cages in a temperature-controlled room (22.2 ± 1.1 °C and relative humidity of 60% ± 10%). Mice were

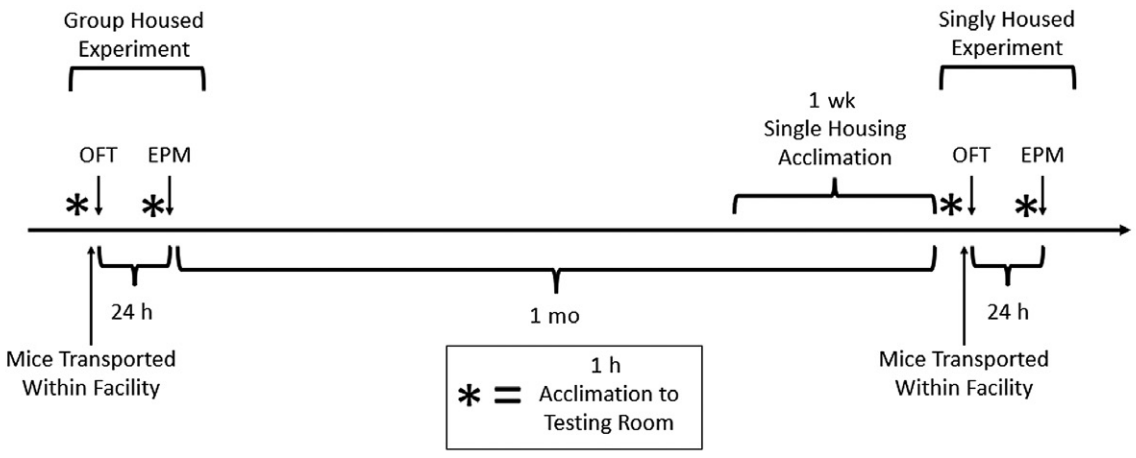

Figure 2. Timeline of behavior study.

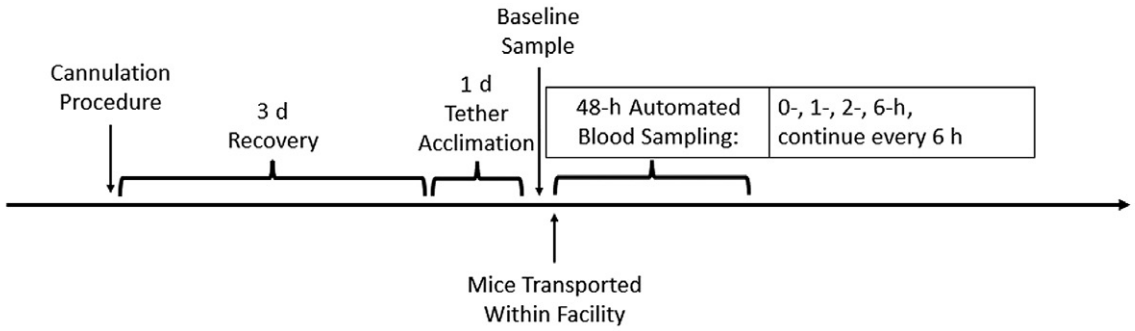

Figure 3. Timeline of stress biomarker study.

group housed by experimental condition assignment with up to 3 mice per cage. Mice that underwent surgical cannulation were individually housed following surgery to protect their exterior catheter site. Housing was in a SPF barrier facility with a 12:12-h dark:light cycle. Mice were monitored for pathogens via serology and PCR analysis of soiled-bedding sentinel mice and by an exhaust air duct PCR assay and were negative for the following pathogens: mouse hepatitis virus, minute virus of mice, mouse parvovirus, epizootic diarrhea of infant mice virus, ectromelia virus, Sendai virus, pneumonia virus of mice, Theiler murine encephalomyelitis virus, reovirus, lymphocytic choriomeningitis virus, mouse adenovirus, polyomavirus, *Mycoplasma pulmonis*, and pinworms. The Jackson Laboratory routinely tests free of mouse norovirus and *Helicobacter* spp., although mouse norovirus, *Helicobacter* spp., and other bacterial pathogens are not tested for routinely at our institution. All mice were apparently healthy and free of any known pathogens, excluded or otherwise. Mice had ad libitum access to food (Laboratory Rodent Diet 5001, PMI; LabDiet, St. Louis, MO) and water. All procedures involving animals were approved by the University of Michigan Animal Care and Use Committee prior to implementation. All animals were housed in an AAALAC-accredited facility, and all procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*.[16]

Transportation methods. The metal teacart and plastic pneumatic cart were tested since they produced the largest differences in sound and vibration during the initial transport studies. Cages containing mice were transported one at a time alongside an unoccupied cage that was instrumented to record noise and vibration (Figure 1). The 2 cages were placed side by side centrally, approximately 5 cm apart, with the long axis perpendicular to the direction of travel on the top shelf of the cart. The transported cages were covered with a lightweight polypropylene gown (Medline Industries, Northfield, IL) per institutional policy.

Transportation route. The transport route was extended to 612 m to simulate transportation of mice between 2 housing areas within the facility. The route was a moderately trafficked loop on even epoxy resin flooring with utilization of the adjacent elevators described for the initial testing. The route was completed by the same individual in an average of 15 min. Cage components and setup remained consistent for all conditions and transport sessions.

Table 2. Animal experimental groups and animal numbers

Study	Control (untransported)	Metal teacart	Pneumatic (plastic) teacart
Behavioral assays: group housed	10	10	10
Behavioral assays: single housed[a]	10	10	10
Serial blood collection: single housed	9	9	9

[a]Animals previously used in group housing.

Behavioral assays. Mice were transferred in their home cage on a metal cart across a hallway from the housing room to the testing room (< 10 m transport distance) and acclimated for at least 1 h prior to transport. Immediately following testing, an OFT began with each mouse placed in the center of a square open-field chamber measuring 43 × 43 × 30 cm^3 made of dark gray plastic. Video recording began 3 s after the mouse was detected in the arena and continued for 30 min. Four mice (of 3 groups in 4 separated chambers) were tested at a time. The mice were removed and returned to their home cage and housing room upon completion of the test. Twenty-four hours following the OFT, mice were again acclimated to the testing room for 1 h prior to undergoing EPM testing. The EPM apparatus was constructed of white painted wood board consisting of 2 open arms (34 × 7.5 × 0.5 cm^3) and 2 closed (with walls) arms (34 × 7.5 × 15 cm^3), crossing perpendicularly in the middle with a small center area. The entire platform was raised approximately one meter above the floor. The test began by placing the mouse in the center zone facing the same open arm. Video recording began 2 s after the mouse was detected in the arena and continued for 5 min. When the test was finished, the mice were returned to their home cage and housing. EthoVision XT (Noldus, Leesburg, VA) software was used to record, track, and analyze the videos from both tests, calculating total distance traveled, average velocity, time spent in each zone, overall movement, and rearing. Testing chambers and objects were wiped down after each trial with Sani-Cloth wipes (PDI Healthcare, Woodcliff Lake, NJ) and cleaned with water to eliminate olfactory cues from previous mice. Lighting intensity was set at 300 lx for OFT and 20 lx for EPM using a ceiling bounce light generated by LED floor lamps. Room temperature, humidity, and background noise were kept consistent throughout the experiments.

Temperature measurement. Mice assigned to serial behavioral testing were anesthetized with isoflurane (VetOne, Boise, ID) for aseptic surgical implantation with an intraperitoneal temperature transponder (12 mm long and 2 mm in diameter) (IPTT-300; Bio Medic Data Systems, Seaford, DE). They were allowed to recover for at least a week prior to the study. A handheld reader (DAS-8007; Bio Medic Data Systems, Seaford, DE) was used to record body temperature directly before and after transport.

Automated blood sampling. Surgical cannulation and automated sampling (Culex; BASi Research Products, West Lafayette, IN) allowed for serial blood collection in conscious, free-moving, and undisturbed mice.[30] Mice assigned to

serial blood collection underwent surgical catheterization of the carotid artery. Anesthesia was induced with 5% isoflurane (VetOne, Boise, ID) and maintained on 1% to 3% isoflurane to effect on a nose cone. Lubricating eye ointment (Puralube; Dechra Pharmaceuticals, Northwich, UK) was applied. The ventral and dorsal neck were prepared for aseptic surgery and a small incision was made superior to the clavicle, exposing the carotid artery. A catheter made of MicroRenathane tubing (0.025-in. outer diameter × 0.012-in. inner diameter, Braintree Scientific) was inserted into the artery and ligated in place with 7-0 silk suture (Fine Science Tools, Foster City, CA). The catheter was tunneled subcutaneously and exteriorized at the back of the neck via a stainless steel tubing connector (made of 25-gauge needle and silicon). The skin was closed with sutures. The exteriorized catheter was filled with heparinized saline and tightly plugged with stainless steel surgical wire. Carprofen (5 mg/kg, SC) (Rimadyl; Zoetis, Parsippany, NJ) was given preemptively and once daily for 2 d after surgery. Mice were individually housed and allowed to recover for 72 h following surgery in the housing room. Following recovery, mice were transferred with their home cage bedding to the caging system and the exteriorized catheter was attached to a tether. The platform of the cage rotated automatically in an opposite direction to mouse movement allowing free movement of the mouse without tension or tangling of the sampling line. The system was programmed to flush a small volume (5 to 10 µL) of heparinized saline (10 U/mL) once every 15 min to maintain catheter patency. Mice were acclimated for 24 h and then 50 µL of blood was collected for baseline controls. Mice assigned to transport conditions were then untethered and transferred to a clean transport cage and underwent their transport route. Control mice were untethered and transferred to a clean cage that remained stationary for 15 min. After transport or allotted time in a clean cage, mice were returned to their infusion cage and automated serial blood draws (50 µL) were conducted immediately, then at 1, 2, 6, 12, 18-, 24, 30, 36, 42, and 48 h. An equal volume of saline was administered for fluid replacement at each time point. All blood samples were diluted in equal parts 50% heparinized (Sagent Pharmaceuticals, Schaumburg, IL) saline (Baxter, Deerfield, IL) (10 U/mL) and held in a refrigerated carousel until completion of the last sampling time point. The samples were centrifuged (14,000 × *g*, 30 s) and plasma stored at −80 °C until analyzed.

Stress biomarker assays. Plasma corticosterone concentrations were measured with a commercially available ELISA kit (K014-H5W; Arbor Assays, Ann Arbor, MI) according to the manufacturer's instructions. Corticosterone samples were diluted to a final concentration of 1:100 in the provided assay buffer. Optical density was determined by a microplate spectrophotometer (EPOCH2; BioTek, Broadview, IL) at 450 nm, and output was processed by corresponding imager software (Gen5, BioTek, Broadview, IL). All samples and standards were run in duplicate and averaged for final concentration. Any readings below the limit of detection were assigned half the lower limit of detection.

Euthanasia. At the conclusion of experiments, mice were euthanized via 40% CO_2 inhalation with cervical dislocation performed after breathing had ceased.

Statistical analyses. Group size was determined via a prior power analysis using G*Power version 3.1.9.7 (Heinrich Heine University Düsseldorf, Düsseldorf, Germany) with an α of 0.05 and power of 0.8. All analytics were performed on GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA). Data normality was verified using Kolmogorov–Smirnov or Shapiro–Wilk tests depending on sample size and expressed as mean and SE. *P* values were determined using one-way and 2-way ANOVA with $P < 0.05$ considered significant. Corrections were applied for multiple comparisons of noise and vibration levels and repeated measures for behavior and biomarker data. Post hoc Tukey–Kramer and Dunnett tests were applied for analysis of multiple groups. All reported *P* values are based on post hoc testing pursued after indication of a significant main effect.

Results

Cart transport produces severe elevations in noise and vibration. Noise levels measured in cages without mice reached a maximum of 71.5 (±0.1) dBZ under individually ventilated housing conditions (Figure 4B), and the IVC vibration levels remained under 0.1 m/s^2 RMS along all 3 axes (Figure 4D). Overall, the vibration readings along the *z*-axis were much higher and more variable than on the *x*- and *y*-axes, with average readings of $4.3 \pm 3.3\ m/s^2$ RMS compared with $0.9 \pm 0.5\ m/s^2$ RMS and $0.7 \pm 0.4\ m/s^2$ RMS and with average maximums reaching $12.4 \pm 6.2\ m/s^2$ RMS compared with $3.2 \pm 1.6\ m/s^2$ RMS and $3.0 \pm 1.5\ m/s^2$ RMS, respectively. Therefore, the *z*-axis is represented in our comparisons due to the degree of severity and variability among transport conditions.

Hand carrying cages produced significantly ($P < 0.05$) higher average and maximal noise levels than did IVC controls (Figure 4A, B) while there was no statistical difference in vibration levels between hand-carried cages and IVCs (Figure 4C, D). Average and maximal noise and vibration levels were significantly ($P < 0.05$) lower for hand-carried cages compared with all other transport methods (Figure 4).

Sound meter data indicated no significant differences among the average and maximal noise levels (Figure 4A, B) produced by the transport methods with hard rubber wheels (flatbed, stainless steel rack, plastic teacart, and metal teacart). Noise levels on these carts averaged 85.9 ± 1.9 dBZ with maximums reaching 114.7 ± 1.3 dBZ, significant ($P < 0.05$) elevations from IVC housing and hand carrying and far exceeding the threshold for activation of the startle reflex and, depending on the duration of exposure, may present risk for impacts on hearing.[35,36]

The *z*-axis vibration levels showed similar trends to noise measurements with no significant differences among the average and maximal levels produced by the transport methods with hard rubber wheels (Figure 4C, D). Vibration levels produced by these carts averaged 5.0 m/s^2 (±1.3) RMS with maximums up to 23.0 m/s^2 RMS, also significantly ($P < 0.05$) elevated from IVC controls and hand carrying and exceeding the recommended chronic maximum of 0.25 m/s^2.[35] Maximum acceleration levels reached by transport methods with hard rubber wheels also exceeded Earth's gravitational force (9.81 m/s^2), indicating the potential for animals to become airborne during transport (Figure 4D).

Pneumatic wheels reduce in-cage noise and vibration. The plastic cart with pneumatic wheels produced significantly ($P < 0.05$) lower average and maximal noise and vibration levels in cages without mice compared with the transports with hard rubber wheels (Figure 4A–D). Noise produced by the pneumatic cart remained significantly ($P < 0.05$) higher than the IVC and hand carrying while there were no differences in average vibration levels among IVC, hand carrying, and pneumatic cart conditions (Figure 4A–D).

Under-cage padding reduces in-cage noise and vibration. Under-cage padding with 2 towels significantly ($P < 0.05$) reduced average noise in cages without mice on one of 5 types of

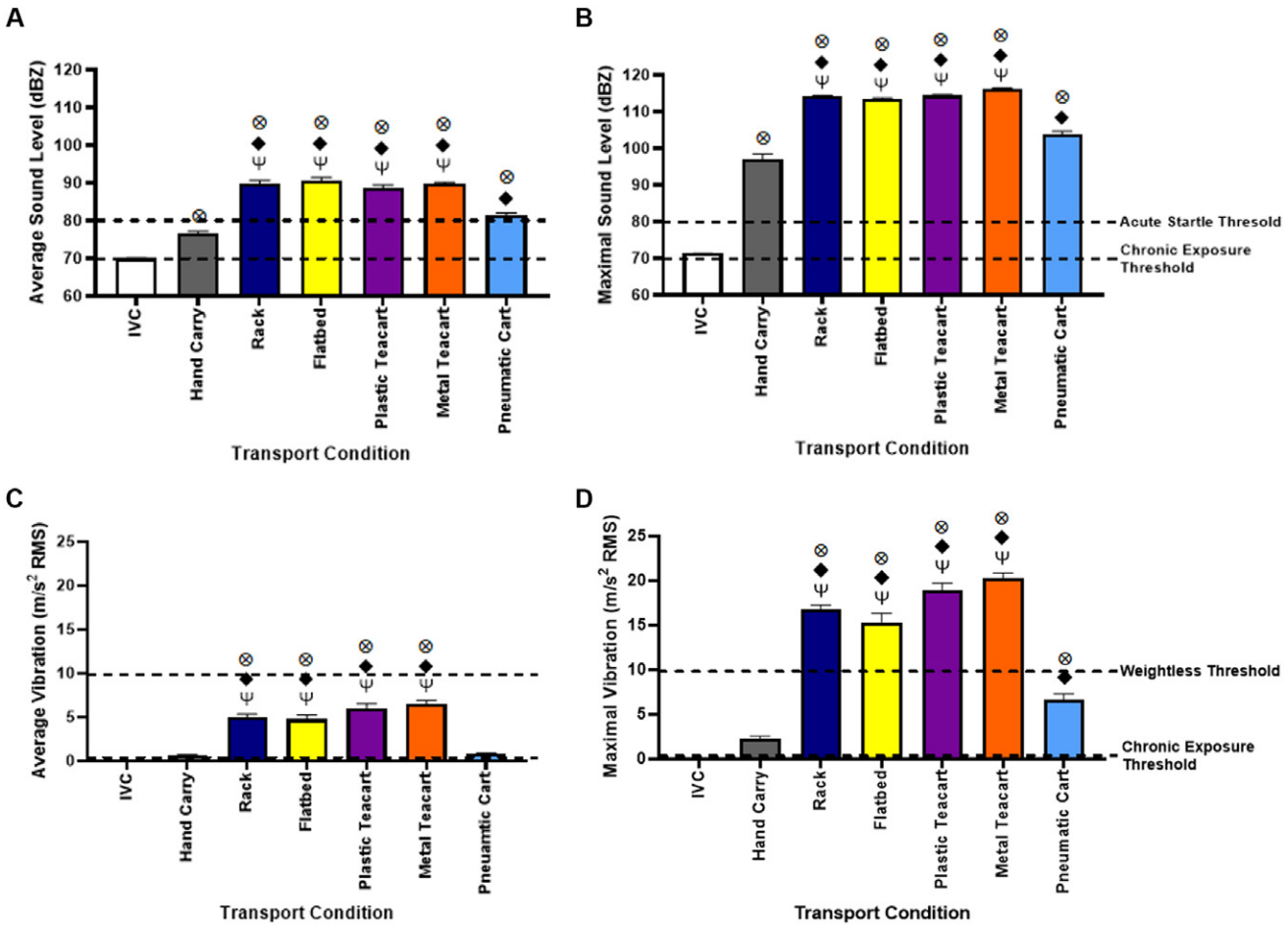

Figure 4. Comparison of average noise (A), maximal noise (B), average vibration (C), and maximal vibration (D) levels among transportation methods. $n = 6$ trials/group; data are presented as mean ± SE. ⊗, significant increase from IVC (control); ◆, significant increase from hand carry; Ψ, significant increase from pneumatic cart; $P < 0.05$. Noise chronic exposure threshold = 70 dBZ, acute startle threshold = 80 dBZ.[35,36] Vibration chronic exposure threshold = 0.245 m/s^2, weightless threshold = 9.81 m/s^2.[35]

carts, and padding with one or 2 towels significantly ($P < 0.05$) reduced maximal noise in all of the transport devices with rubber wheels compared with controls (Figure 5A, B). Average vibration levels were significantly ($P < 0.05$) reduced by 2 towels in 3 of 5 types of carts and maximal vibration levels in 2 of 5 types of carts (Figure 5C, D). Under-cage padding with one towel was not as effective and significantly ($P < 0.05$) reduced maximal vibration levels in one of 5 types of carts (Figure 5D). Increasing the amount of nesting material in the cage did not affect noise or vibration levels.

Overall, it appeared that the metal cart produced the most aversive noise and vibration that could be dampened somewhat with under-cage padding. The pneumatic cart was least aversive, and under-cage padding provided no additional improvement. These carts were used in subsequent experiments with mice.

Transported mice experienced significantly different noise and vibration exposure. During transportation of the mice used in behavioral and biochemical assays, average and maximal noise and vibration levels detected in an adjacent cage were significantly ($P < 0.05$) higher in cages transported on the metal cart compared with the plastic cart with pneumatic wheels (Figure 6), consistent with our initial data (Figures 4 and 5). Average noise and vibration values for all conditions were higher compared with values recorded during trials without mice. This may have been due to the extended route, increasing the ratio of time the carts were in motion rather than at rest waiting for and riding elevators.

Common anxiety-sensitive behavioral assays are minimally influenced by brief intrafacility transport. In group and singly housed mice, an OFT conducted directly after transport showed no significant differences in mouse movement, distance and velocity traveled, or time within the central, intermediate, and outer zones between the mice transported on the 2 different carts and compared with nontransported, control mice (Table 3, Figure 7A, C). Similarly, no significant differences in time or entries on the open arms or distance traveled on the EPM were noted in either group or singly housed mice (Table 3, Figure 7B, D). However, transported mice tended to spend more time in the open arms compared with mice at a level that approached significance in group-housed mice ($P = 0.09$) and to a lesser degree in singly housed mice ($P = 0.16$) (Table 3, Figure 7B, D). Singly housed mice in general spent significantly ($P < 0.05$) less time on the open arms and traveled farther on the EPM than did group-housed mice (Figure 7B, D).

Intrafacility transport does not impact core body temperature. There were no significant ($P < 0.05$) differences in changes in core body temperatures measured with an implanted thermistor before and after transport among transported and control mice in either group- or single-housed settings. In group-housed mice, average changes in body temperature were 0.18 ± 0.80 °C, 1.16 ± 2.04 °C, and 0.59 ± 1.06 °C for untransported, metal teacart

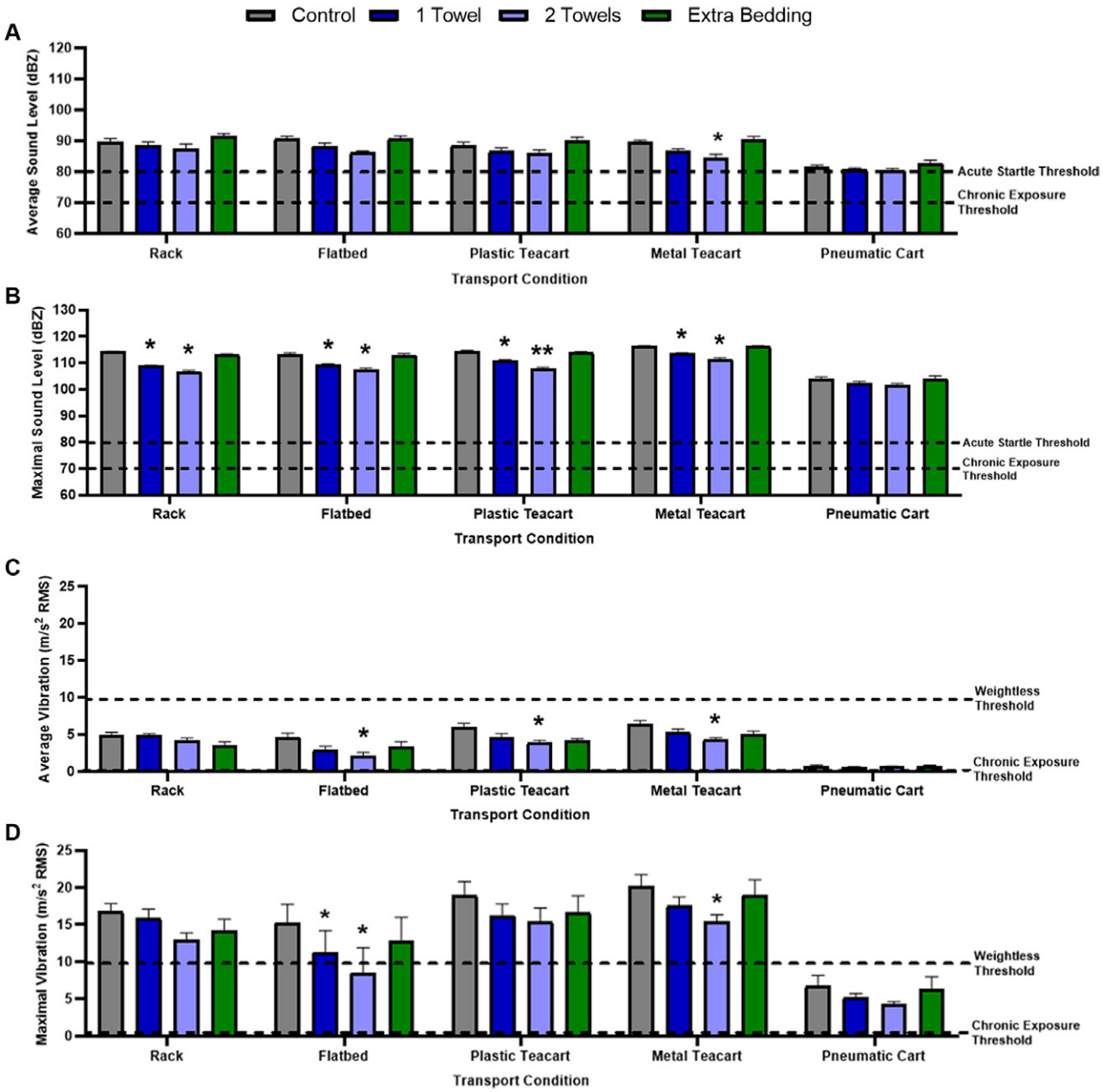

Figure 5. Comparison of average noise (A), maximal noise (B), average vibration (C), and maximal vibration (D) levels among attenuation types on each cart. n = 6 trials/group; data are presented as mean ± SE. *, Significant decrease from control; **, significant decrease from all other groups ($P < 0.05$). Noise chronic exposure threshold = 70 dBZ, acute startle threshold = 80 dBZ.[35,36] Vibration chronic exposure threshold = 0.245 m/s^2, weightless threshold = 9.81 m/s^2.[35]

transported, and pneumatic cart transported mice, respectively. Single-housed mice had similar values with average changes in body temperature of 0.42 ± 0.79 °C, 0.22 ± 0.73 °C, and 0.71 ± 1.05 °C, respectively.

Elevated noise and vibration exposure during transport impacts serum corticosterone. Nearly all mice experienced significant ($P < 0.05$) elevations in corticosterone at the initial time point immediately following return to the sampling cage compared with baseline. Corticosterone levels were found to be significantly ($P < 0.05$) elevated in mice immediately following transport on the metal cart compared with untransported mice (Figure 8). Corticosterone levels at this time point also trended higher ($P = 0.06$) in mice transported on the metal cart compared with the pneumatic cart. Corticosterone levels returned to within range of control mice within 1 h after transport, although mice transported on the metal cart continued to have higher trends in circadian peaks within the 48-h sampling period.

Discussion

This study vastly expands data on noise and vibration generation during intrafacility transport. Noise generation of all transport methods tested far exceeded recommended chronic maximum levels of exposure (70 dBZ), acute startle thresholds (80 dBZ), and thresholds of hearing loss (85 dBZ) with prolonged exposures even in the presence of attenuation.[35,36] Previous work supports these findings with peak noise readings from a single rat cabinet transport reaching 110 dBZ and under-cage

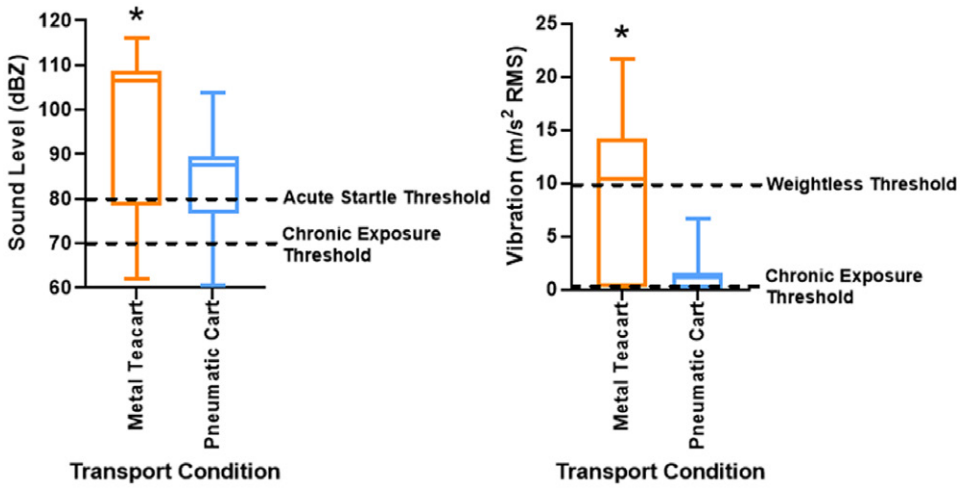

Figure 6. Comparison of sound (left panel) and *z*-axis vibration (right panel) levels between metal and pneumatic carts during mouse transport based on combined data from all trials. $n = 24$ trials; data are expressed as range (whiskers), quartiles (box), and means (line within box). *, $P < 0.05$. Noise chronic exposure threshold = 70 dBZ, acute startle threshold = 80 dBZ.[35,36] Vibration chronic exposure threshold = 0.245 m/s^2, weightless threshold = 9.81 m/s^2.[35]

attenuation only reducing these levels by about 10 dBZ.[31] More promising, the novel addition of pneumatic wheels to a plastic transport cart resulted in average vibration levels that were not significantly different from exposures experienced in IVC housing or during hand carrying. As with noise readings, vibration levels for all other tested transports far exceeded the maximal recommended exposure of 0.25 m/s^2,[35] even in the presence of attenuation. Vibration data collected in this study are corroborated by previous research in which reporting vibration levels were most severe along the *z*-axis and metal carts resulted in the highest vibration within the mouse cage.[15] Our data also support previous findings that thick under-cage padding with towels significantly reduces vibration levels and may be an accessible method of vibration attenuation during transport, although neither study showed under-cage attenuation reduced vibration exposure to acceptable levels below 0.25 m/s^2.[15,35] Only the pneumatic cart tested in our study was able to reduce average vibration closer to acceptable levels (0.8 ± 0.2 m/s^2 RMS). The noise and vibration levels during transport measured in our study demonstrate that mice are often exposed to adverse environments during intrafacility transport, and attenuation efforts should be considered for the benefit of both the animals and staff.

A core aim of this study was to correlate noise and vibration exposure to the behavior and physiologic responses of mice to intrafacility transport. Differences in noise and vibration exposure in mouse cages transported on the metal or pneumatic cart were shown to be significant ($P < 0.05$). However, variance in mouse behavioral and physiologic responses were dependent on the parameter measured. Of the behavioral and biochemical assays conducted, only corticosterone assays showed significantly

Table 3. Statistical analysis of anxiety-like behavior after transport

Parameter	Control (mean ± SEM)	Metal teacart (mean ± SEM)	Pneumatic cart (mean ± SEM)	Statistics	
				F	*P*
Group-housed mice					
Open field test					
Time spent in center (%)	23.04 ± 3.12	19.88 ± 2.33	20.82 ± 3.42	0.29	0.75
Distance traveled (m)	75.73 ± 3.50	78.41 ± 4.39	75.46 ± 3.52	0.18	0.83
Time spent moving (%)	56.48 ± 2.18	55.63 ± 2.22	55.40 ± 1.57	0.08	0.92
Elevated plus maze					
Time spent in open arms (%)	12.43 ± 2.76	18.16 ± 2.47	21.01 ± 2.85	2.61	0.09
Entries into open arms (no.)	10.90 ± 2.10	12.30 ± 1.86	14.60 ± 1.45	1.05	0.36
Distance traveled (m)	13.87 ± 1.05	14.05 ± 0.47	14.37 ± 0.43	0.13	0.88
Singly housed mice					
Open field test					
Time spent in center (%)	17.16 ± 1.02	20.23 ± 2.71	16.05 ± 2.32	1.03	0.37
Distance traveled (m)	78.76 ± 5.65	68.91 ± 5.20	64.07 ± 2.14	2.65	0.09
Time spent moving (%)	59.03 ± 1.89	53.65 ± 3.00	52.03 ± 1.41	2.78	0.08
Elevated plus maze					
Time spent in open arms (%)	4.40 ± 1.38	4.98 ± 1.06	8.03 ± 1.66	1.98	0.16
Entries into open arms (no.)	3.20 ± 0.84	4.00 ± 0.92	5.30 ± 1.00	1.32	0.23
Distance traveled (m)	10.91 ± 1.29	9.03 ± 0.54	10.83 ± 0.88	1.24	0.31

n = 10/group; data are presented as mean ± SEM.

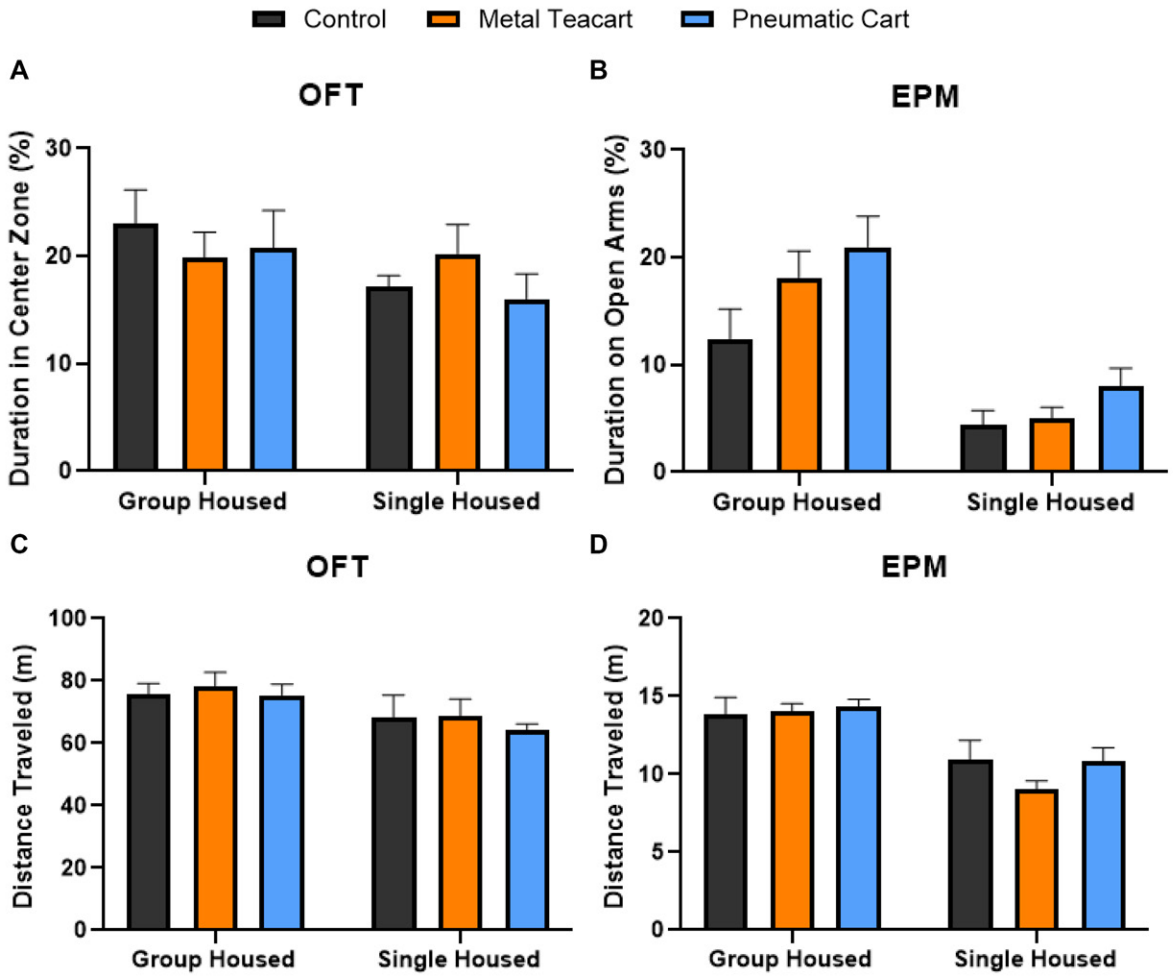

Figure 7. Anxiety-like behavior and exploratory locomotion in mice after transport. (A) Time spent in center in OFT. (B) Time spent on open arms in EMP. (C) Distance traveled in OFT. (D) Distance traveled in EPM. $n = 10$/group; data are presented as mean ± SE.

($P < 0.05$) higher elevations immediately following transport in mice transported on the metal cart compared with mice transported on the pneumatic cart and untransported mice.

Corticosterone levels are commonly used as a measurement of stress in mice due to its increased secretion as a result of the pituitary adrenal responsiveness to any environmental stress.[13,23] To eliminate the impact of direct human handling of animals or on-site physical presence of laboratory staff during blood sampling on hormonal secretion, we took advantage of automated blood sampling technology to collect blood from conscious, free-moving, and undisturbed mice. Therefore, plasma corticosterone measured in this study is effective at detecting the impacts of both acute and chronic stressors under otherwise stress-free conditions.[29] Under standard housing conditions, corticosterone exhibits a circadian behavior in mice with typical daytime peaks around 1600,[5] observed in this study in all groups without apparent disruption by daytime transport. Mice exposed to elevated levels of noise and vibration on the metal cart during transport exhibited elevated corticosterone acutely but returned to control ranges within an hour following transport. However, circadian peaks continued to trend higher than for controls or mice transported on the pneumatic cart during the 48-h sampling period, indicating potential chronic effects of transport. Corticosterone levels of mice transported on the pneumatic cart remained within control ranges throughout the sampling period. These observations indicate acclimation of at least 1 h after intrafacility transport is likely adequate for this parameter and that transport methods that attenuate noise and vibration may decrease acclimation needed following intrafacility transport. Given the high pulsatility of corticosterone secretion,[5] more frequent sampling within the 60-min period following transport would provide further insight into the changes in its secretion pattern and a closer estimation on the acclimation period for this mouse strain.

OFT and EPM have been repeatedly validated as measures of stress and anxiety-like behavior in mice. Specifically, mice tend to avoid unknown open areas in direct light, especially when preconditioned with stressful stimuli.[10,19,32] Thus, mice with higher stress levels tend to exhibit higher thigmotaxis, or the tendency to remain close to walls, decreasing their time in the center zone of the OFT and open arms in the EPM.[18,19,32] In addition, C57BL/6 background mice exposed to acute stress consistently show increased time in motion, distance traveled, and rearing in OFT and increased latency and entries into the open arms of the EPM even 24 h after the stressful event.[17,37] Social deprivation (single housing) can increase the anxiety-like behaviors observed during OFT and EPM testing in the form of decreased time in the center or open arm zones and decreased distance traveled in the EPM,[6] reflected in this study's EPM results. Transported mice on either cart did not show significant differences in these parameters compared with untransported mice, although transported mice trended toward spending more time in the open arms of the EPM. These results indicate that the adverse conditions

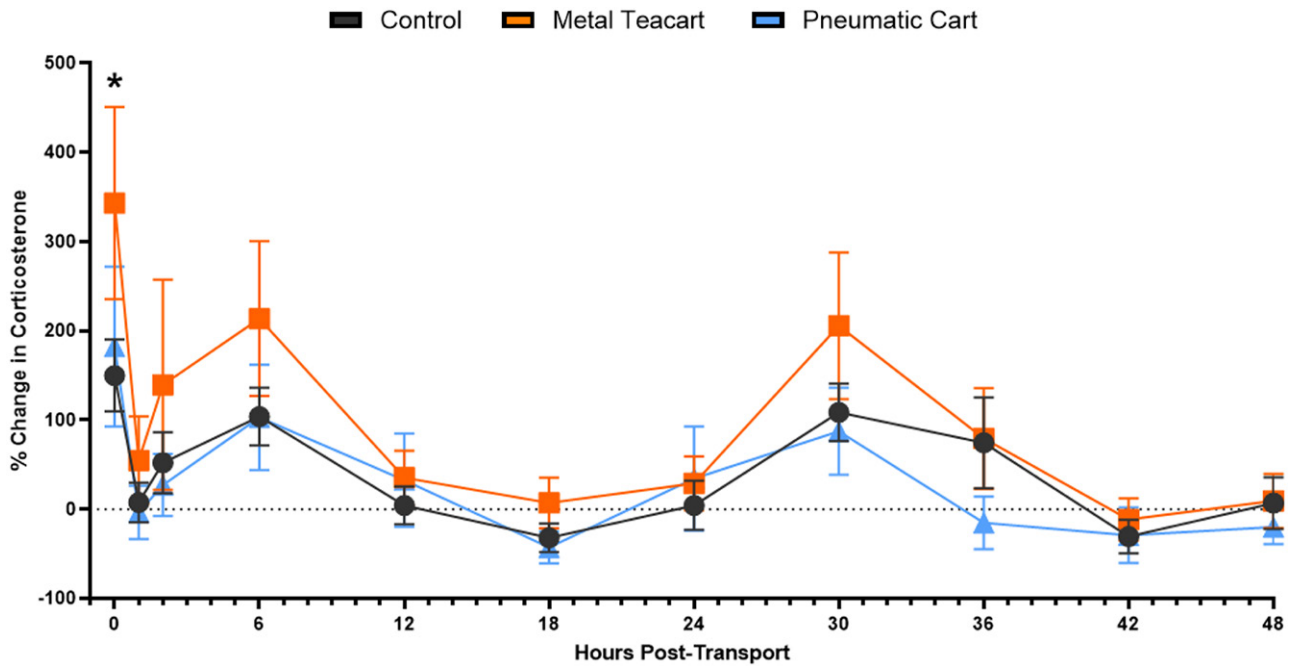

Figure 8. Comparison of plasma corticosterone levels among untransported control mice, mice transported on a metal teacart, and mice transported on a plastic pneumatic cart. $n = 10$/group; data are presented as group mean ± SE. *, $P < 0.05$ between control and metal cart, $P = 0.06$ between pneumatic and metal cart.

experienced during transport did not surpass the threshold to induce behavior changes in this cohort of mice and may even have responses contrary to the increased thigmotaxis expected after transport. Mouse strain may be a contributing factor, as unexpected lack of differences in these assays in response to adverse stimuli have been previously reported in C57BL/6 mice, and individual variability in responses can be present even within this inbred strain.[17,18] Behavioral responses of mice of different genetic background, sex, and age would be of interest in future studies.

While this study provides important validating and novel information on noise and vibration generation during transport and the responses of transported mice, further study is needed to broaden the understanding of the impact of intrafacility transport on mice. This study was limited to comparison of a single cart of each type operated by one individual in one vivarium at one institution, limiting analysis of the vast scope of variables that can modulate noise, vibration, and stress responses during transport. This study was also limited to including a single sex, strain, and age group of mice, all of which factor into behavioral and physiologic responses to stressful stimuli. Previous research has demonstrated the differential effects of mouse sex on corticosterone secretion with vibration exposure, accentuating the need for expanded study of this topic.[4] Study of additional behavioral and physiologic parameters as well as the impact of social housing and enrichment would provide additional insight into the effectiveness of transport attenuation and acclimation recommendations.

This study supports previous evidence that noise and vibration levels often become elevated during intrafacility cart transport, but readily available materials can reduce these elevations by providing padding under transported cages or replacing hard rubber wheels with pressurized air-filled wheels. Reductions in noise and vibration provided by pneumatic wheels were reflected in reduced plasma corticosterone levels in mice following transport, providing further support of study and implementation of transport noise and vibration attenuation. Understanding the impacts of intrafacility transport on animals and effectiveness of mitigation methods could optimize animal welfare and production of quality research data while performing these routine tasks.

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Conflict of Interest

The authors have no competing interest to declare.

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