Journal of the American Association for Laboratory Animal Science
Copyright 2008
by the American Association for Laboratory Animal Science

Vol 47, No 2
March 2008
Pages 49–56

Development and Application of a Novel Environmental Preference Chamber for Assessing Responses of Laboratory Mice to Atmospheric Ammonia

Angela R Green,[1,*] Christopher M Wathes,[2] Theo GM Demmers,[2] Judy MacArthur Clark,[3] and Hongwei Xin[4]

A novel environmental preference chamber (EPC) was developed and used to assess responses of laboratory mice to atmospheric ammonia. The EPC features 1) a test chamber with 4 individually ventilated, mutually accessible compartments; b) automated tracking of mouse movements by using paired infrared sensors; c) identification of individual mice by using photosensors; d) monitoring and regulation of the NH_3 concentration in each compartment; and e) personal-computer–based data acquisition. In an initial preference study with the EPC, 4 groups of 4 laboratory mice (BALB/c/Bkl; body weight, 13.4 to 18.4 g) were each given a choice among 4 NH_3 concentrations (mean ± SE) of 4 ± 2, 30 ± 2, 56 ± 4, and 110 ± 6 ppm for 2 d after a 2-d familiarization period. Once trained to use the intercompartment tunnels, the mice made extensive use of the EPC, with each group making more than 2000 intercompartment movements during 48 h. Video recording verified the results of the automatic tracking system, which detected and correctly determined mouse location for 79% of the moves. The use of photosensors proved to be ineffective in recognizing individual mice. Although the EPC would benefit from refinement and further development, it simplified analysis of locomotion behavioral data. Results of the preference study indicated that the mice exhibited no clear preference for, or aversion to, any of the experimental concentrations of ammonia and that the mice clearly preferred the upper 2 compartments of the chamber over the lower 2 compartments. Further investigation should be conducted to verify these preliminary results and explore other preferences of laboratory mice for environmental conditions and resources.

Abbreviations: ATS, automatic tracking system; EPC, environmental preference chamber; IR, infrared; IVC, individually ventilated cage

A recent review has highlighted the influence of the environment on the health, behavior, and welfare of the laboratory mouse.[11] Much of this information has yet to be translated into good practice for environmental management of laboratory mice. In particular, the intricate and subtle physiological mechanisms by which mice sense and perceive their environment are generally ignored in specifications for—and provision of—the 'optimal' environment, potentially compromising health and welfare of the animal and the scientific validity of research. Current regulatory standards in both the United States and the United Kingdom are based primarily on human perceptions of environmental conditions and are largely based on human exposure limits.

Provision of a suitable microenvironment in the home cage is a prerequisite for good laboratory science.[12] Most laboratory mice are housed in 1 of 3 types of caging: 1) open top wire-bar lid cages, 2) filter-top cages, and 3) actively (individually) ventilated cages (IVC). The atmospheric environment, specifically ammonia concentration, differs markedly among these 3 cage systems and depends on bedding type, cage cleaning frequency, stocking density and ventilation rate.[16,17] Ammonia concentration in cages is often used as an indicator of the frequency with which cage bedding should be changed. However, cage-cleaning frequency presents a conflict between hygiene and disruption of scent-marking patterns.[15] Cage ventilation requirements are based largely on the need to keep NH_3 to an acceptable concentration. Increasing the ventilation rate can promote drier bedding thereby reducing NH_3 concentration. However, a higher ventilation rate must be achieved without high air velocities, which are aversive to mice, particularly when breeding.[4] Ammonia exposure may compromise olfactory perception by desensitizing olfactory receptors, with adverse consequences for reproduction,[6] health,[7] and potentially behavior. For example, exposure of rats to 25 to 250 ppm NH_3 for 4 to 8 wk or 100 ppm for 1 to 4 wk increased the severity of respiratory mycoplasmosis.[3,19] The morphology of rat tracheal epithelium changed after only 4 d of exposure to 200 ppm NH_3, whereas the delayed type immune response was reduced in guinea pigs exposed to 90 ppm NH_3.[20] Most recently, a study suggested that elevated NH_3 levels in the cage may impair embryo production in superovulated mice.[2]

The human occupational exposure limit of 25 ppm[1] NH_3 for an 8-h working day typically is taken as the tolerable concentration for laboratory rodents. The first signs of response in rodents, such as decreased resistance to pathogenic organisms in rats, develops at about this concentration.[3] Conversely, many scientists consider this threshold too low, because wild rodents live in burrows where they may be exposed to, and tolerate,

Received: 18 Sep 2007. Revision requested: 19 Oct 2007. Accepted: 23 Nov 2007.
[1]Agricultural and Biological Engineering, University of Illinois, Urbana, IL; [2]Royal Veterinary College, University of London, [3]JMC Consultancy, Stonington, CT; [4]Agricultural and Biosystems Engineering, Iowa State University, Ames, IA
**Corresponding author. Email: angelag@uiuc.edu*

considerably higher concentrations; however, there are no data on ammonia concentrations within natural burrows. No studies were found to determine the tolerance of rodents for NH_3. Housing in filter-top cages may provide an aversive environment for laboratory mice unless the bedding is changed frequently (for example, at least every 4 d).[13] The use of IVCs may alleviate this potential hazard by maintaining low NH_3 concentrations over extended periods. The management of bedding, including frequency of changes and substrate type, in IVC systems may depend on the animals' perception of their atmospheric environment, in particular the concentration of NH_3.

The value of preference testing to assess aversion to, or tolerance of, a particular environment has been well documented. Previous studies on swine and poultry showed substantial evidence of a strong preference for fresh air over ammoniated atmospheres that are typical of livestock buildings[8-10], while limitations have also been documented.[5] We adapted the approach used previously to assess the preferences of laboratory mice for atmospheric environments. Our specific objectives were: 1) to develop a novel environmental preference chamber (EPC) for study of behavioral responses of laboratory mice to environmental conditions and 2) to demonstrate the use and performance of the EPC by conducting an introductory study of the preferences of mice for atmospheric NH_3 at concentrations commonly encountered in laboratory animal facilities.

Materials and Methods

Development and specifications of the EPC. The design and functional criteria for the EPC were: 1) mutual access between adjacent compartments while avoiding the need to traverse a third compartment (as necessary in an annular maze); 2) negligible cross air flow or infiltration between compartments with a ventilation rate of 100 air changes hourly; 3) uniform, laminar air flow at a low velocity (that is, less than 0.1 ms^{-1}) through each compartment; 4) transparency for ease of observation and recording of the mice and their activities; 5) similar illumination of all the compartments; 6) automatic tracking and recording of locomotion behaviors of the mice, with the ability to determine locomotion direction; 7) ability to track the individual mouse during its ranging around the EPC; 8) ability to monitor and control NH_3 concentration within the target values; and 9) ability to acquire and save data automatically. On the basis of these criteria, the EPC was developed and its performance evaluated.

The center piece of the test method is the EPC, as shown in Figure 1. It comprises 4 mutually accessible compartments (each measuring 300 × 150 × 150 mm), arranged in 2 tiers of 2 compartments that are connected with ladders and rectangular access tunnels (40 × 20 × 20 mm). Each compartment is connected directly with each of the other 3 compartments. Compartments I and II are on the upper tier, and compartments III and IV are below. This unique design is preferable to the more conventional design of 4 compartments arranged in a Maltese cross on one tier in that there is no central zone or access tubes in which the mice can dwell or tarry, and the 3-dimensional design with ladders encourages climbing activity.[14]

The transparency of the chamber was realized by using clear sheet acrylic as the building material. Special attention was paid to the compartments' illumination to ensure a consistent distribution, and uniform illuminance, within and between the 4 compartments. Specifically, the illumination intensity (all-white light) varied within each compartment from 0 to 5 Lux with room lights off, produced by the light emitting diode of the photosensor in the center of the tunnels, and 25 to 50 Lux with room lights on. Compartments were brighter near the front and end wall and darker near the back wall; intensities were within 5 Lux with lights on and 1 Lux with lights off for a given location. The low light during lights-off was necessary to facilitate video recording with the low-light cameras. The back wall, top, and access tubes of each compartment were covered with paper similar in color to the bedding to create contrast for the cameras and to block some of the light from the room.

Each compartment was ventilated separately at the designed 100 air changes hourly to minimize pollutant accumulation. The low air velocity (less than 0.1 ms^{-1}) and laminar flow inside the compartment were achieved by using an array of circular inlets (41 openings of 7 mm in diameter each) and another array of circular outlets (41 openings of 8 mm in diameter each) in the respective end wall. Cross-flow between the compartments was prevented by ventilating each compartment at the same air exchange rate with its own fan, thus ensuring equal static pressures across the tunnels. This feature eliminated the need for a physical covering over each of the tunnel openings.

Ammonia was introduced into each compartment with a mass flow controller (Bronckhorst High-Tech BV, Ruurlo, Netherlands), in light of its measured concentration in the exhaust air, and was injected into the supply air duct for mixing. A computer-controlled system consisting of a set of stainless steel NH_3 converters (Mattheus Milieu Techniek, Wageningen, Netherlands), a sample point multiplexer, and NO gas analyzer (Thermo Environmental Instruments, Franklin, MA) was used to continuously monitor the NH_3 concentrations in each compartment. In addition, NH_3 concentration was checked daily by using diffusion tubes (Draeger, Pittsburgh, PA) placed in the exhaust pipe of each compartment. Temperature and relative humidity in the room were recorded with a data logger (Tinytag TGU1500, Omni Instruments, Dundee, UK).

Movement of the mice between the compartments was monitored with an automated tracking system (ATS; Figure 2). Specifically, paired infrared (IR) sensors (OP165/OP505, Optek Technology, Carrollton, TX; distributed by Digi-Key, Thief River Falls, MN) were placed in each end of each tunnel. These sensors allowed the travel direction of the mice to be determined (for example, from compartment I to II and vice versa). To determine the identity of individual mice, a photosensor (CdS photoconductive cell, Photonic Detectors, Camarillo, CA; distributed by Digi-Key, Thief River Falls, MN) sensitive to visible light reflected from mice of different fur colors, was mounted in the middle of each tunnel. The fur of each white mouse would be stained a distinctly different color (such as yellow, blue, red, and black), with optimal staining method and color to be determined in preliminary testing. A white-light-emitting diode was used to provide a consistent light source for the photosensor. The tracking sensors were connected to integrated circuits: D-type Octal Latches (74ALS373, Fairchild Semiconductor, South Portland, ME) or an 8-channel analog multiplexer (MPC508, Texas Instruments, Dallas, TX; Figure 3). The integrated circuits were interfaced with a computer-based data acquisition device (PMD 1208-LS, Measurement Computing, Norton, MA). The interface code was written in Visual Basic, VBA, and operated within Excel (Microsoft, Redmond, WA). To provide back-up and verification of the ATS, a time-lapse videocassette recorder was used to record the activities throughout the experiment.

An initial performance evaluation of the EPC was conducted prior to its use with animals. To examine the degree of cross-flow of air between compartments, NH_3 was injected as a tracer into each compartment at the concentrations, and in the arrangements, that would be used in subsequent trials. The

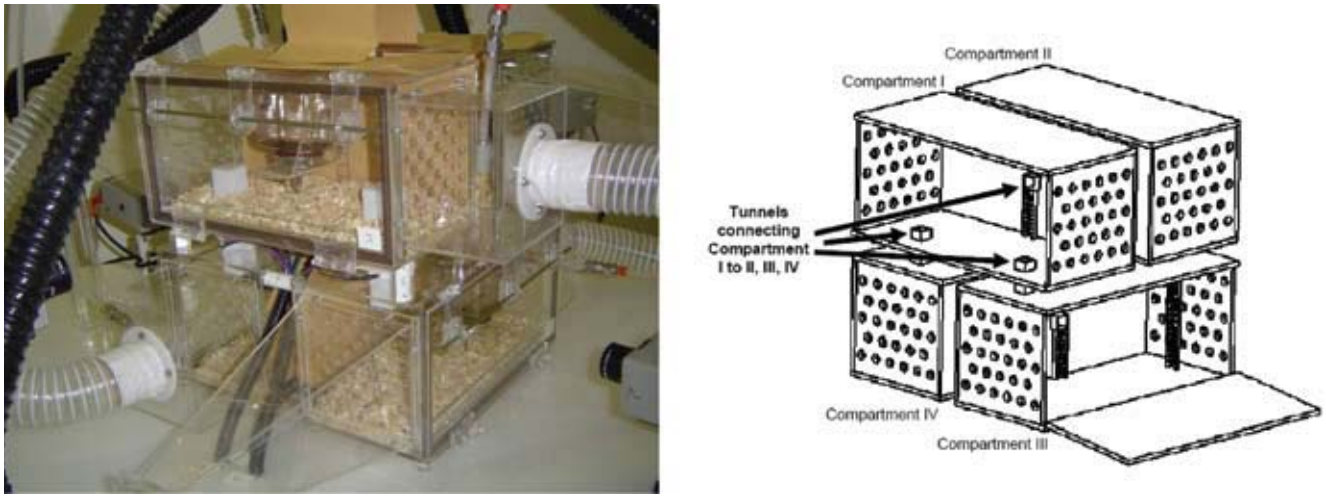

Figure 1. Photographic and schematic representation of the environmental preference test chamber designed for studying responses of laboratory mice to atmospheric conditions.

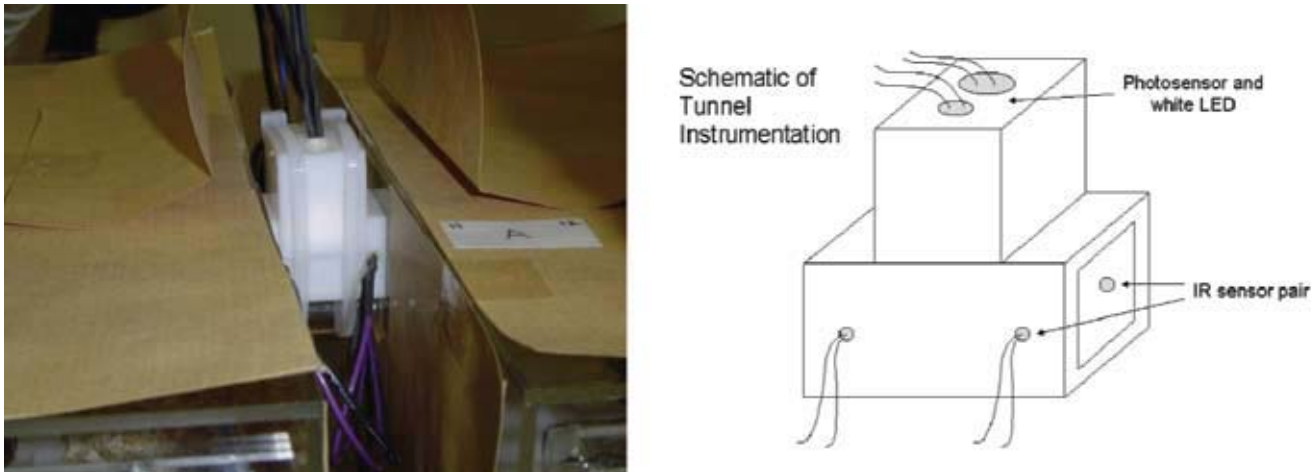

Figure 2. Photographic and schematic representation of intercompartmental tunnel instrumented with paired IR sensors, photosensor, and light-emitting diode for tracking mice locomotion.

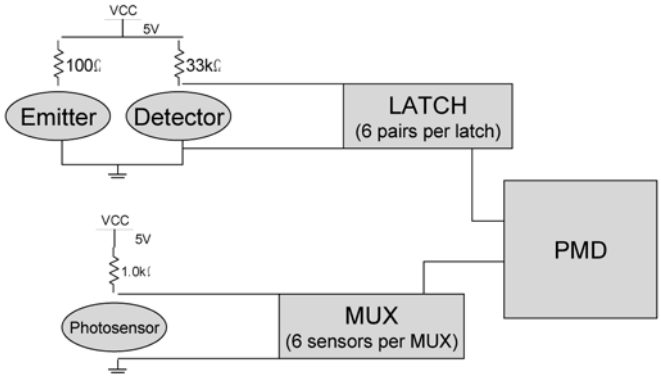

Figure 3. Circuit schematic for data collection boards. MUX, multiplexer; PMD, personal measurement device; VCC, supply voltage

maximal deviation in the fresh air (0 ppm NH_3) compartment was approximately 10 ppm. All other deviations in the fresh air compartment were 3 ppm or less. In light of the magnitude of the NH_3 concentrations to be used in the preference tests that were to follow, these deviations were considered acceptable.

Using the EPC to assess the responses of laboratory mice to atmospheric NH_3. ***Training and Selection of the Experimental Mice.*** A pilot test was carried out to determine the usage of the EPC, particularly the tunnels, by the mice. Exploration of the EPC occurred soonest when mice were placed in separate compartments initially, instead of together in the same compartment. Three groups of female mice were tested for activity and ability to navigate the tunnels: outbred white Bkl:BKW mice (weight, 25 to 29 g; age, 6 to 8 wk; B and K Universal, Hull, UK); outbred white Bkl:BKW mice (weight, 15 to 19 g; age, 3 to 4 wk; B and K Universal); and inbred white BALB/c/Bkl mice (weight, 10 to 15 g; age, 3 to 4 wk; B and K Universal). After initial training, the heaviest mice were too large to comfortably pass through the tunnel, whereas both the lighter groups were able to do so. The smaller outbred mice showed little exploratory behavior and lower motivation to explore the compartments. In contrast, the inbred mice rapidly explored the entire chamber once the initial independent tunnel navigation had occurred. Therefore, we selected BALB/c/Bkl mice for further testing of responses to atmospheric NH_3.

Husbandry and handling of experimental mice. Husbandry of the experimental mice followed normal practices. Holding accommodation was provided by a ventilated cage rack (Bio-ZoneGlobal, Ramsgate, Kent, UK) to ensure that the mice were kept in fresh air prior to the preference test. Temperature and humidity control were provided by the room's system. The mice were provided a 12:12-h light:dark photoperiod. Food and

water were available ad libitum in both the holding rack and preference chamber. The holding cage and test chamber used the same furnishings, that is, bedding material (approximately 1.5 cm thick; 85 g softwood shavings per compartment), which was changed weekly in the holding rack; 30 g pelletted food; a hanging water bottle (supplied with holding rack, purchased for EPCs; model 15 with caps, Thoren Caging Systems, Hazleton, PA), and 1 sheet of 2-ply tissue paper (25 × 25 cm) for enrichment (Figure 4). The entire chamber and its components were cleaned and disinfected by wiping the chamber and briefly soaking the water and food containers with disinfectant (Virkon S, DuPont Animal Health Solutions, Sudbury, Suffolk, UK) between trials.

Experimental protocol. The objective of this initial experiment was to assess the behavioral responses of laboratory mice to atmospheric NH_3 through a preference test using the newly developed EPC. The experimental design was a balanced factorial design based on Latin squares with NH_3 concentration and compartment as the experimental factors with 4 levels each (nominal concentrations of 0, 25, 50, and 100 ppm and compartments I, II, III, and IV, respectively). There are 24 possible arrangements of NH_3 concentration in the 4 compartments of the chamber; however, only 4 (1 complete Latin square, see Table 1) were tested in this initial application of the EPC. The compartments receiving the respective atmospheric NH_3 level were the experimental units. In so doing, 4 groups of mice, each comprising 4 female mice (BALB/c/Bkl, 21 to 27 d old at arrival) were given preference tests that lasted 4 d, including the familiarization period.

Upon arrival, the mice were separated randomly into groups of 4 and placed in prepared cages in the holding rack. Groups of 4 mice represented a typical group size suitable for a 'shoebox cage' and allowed for each compartment of the preference chamber to house 1 mouse at the start of each experiment. The mice were acclimated in the holding rack for at least 7 d prior to placement in the preference chamber. Each mouse was picked up at least once daily during this acclimation period, to accustom it to handling.

After acclimation, the mice were placed in the preference chamber for a 2-d familiarization period under comfortable conditions. Each mouse initially was placed in a separate compartment within the chamber. For the first 24 h, the mice were allowed to explore the chamber without human intervention, and their movements and locations were recorded. For those mice that had not navigated all tunnels in both directions, additional training was necessary and consisted of holding each mouse closely in front of a tunnel until it had entered and exited the tunnel of its own accord. The mice then were allowed a minimum of 30 min of exploration, and the training was repeated until the mice were completely familiar with the chamber. Evidence of familiarity was provided by compliance with the following rules: 1) each mouse had accessed each compartment; 2) each mouse had come into contact with other mice within its group; and 3) each mouse had moved independently through both a horizontal and a vertical tunnel without human intervention. After familiarization, the mice were removed from the chamber and placed in their holding cage in the holding rack for approximately 30 min. The bedding, food, and tissue in the preference chamber were replaced with fresh furnishings, but the chamber was not disinfected. This procedure ensured that equal resources were available in all compartments when the NH_3 treatments were applied. The mice were returned to the chamber, each in a separate compartment, and allowed 1 h of refamiliarization and exploration prior to the application of the NH_3 treatments. For the next 2 d, the treatments were applied, with nominal NH_3 concentrations of 0, 25, 50, and 100 ppm. The compartments were not opened or accessed during the treatment period.

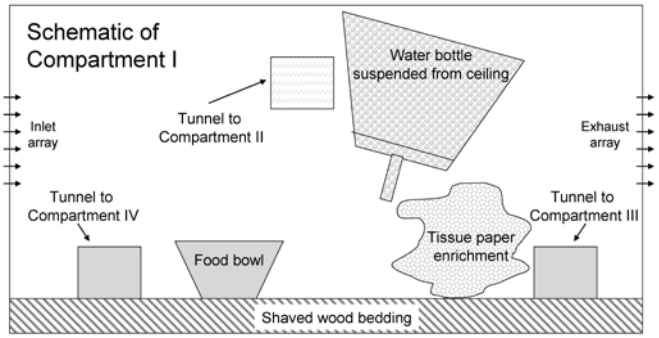

Figure 4. Schematic of compartment I within the chamber.

Table 1. Experimental design, based on a Latin square, for assignment of ammonia concentration to compartments

	Trial			
Compartment	1	2	3	4
I	100	25	0	50
II	0	50	25	100
III	50	0	100	25
IV	25	100	50	0

Compartments I and II were on the upper tier, III and IV on the lower tier.

The live body weight of each mouse was measured before and after the preference test. The experiment was authorized under the Animals (Scientific Procedures) Act (1986)[21] after ethical review with project license number PPL 80/1911.

Collection and processing of behavioral data. During the 2-d test period, the ATS sensors were scanned continuously, and data were recorded at a minimum frequency of 2 Hz. Data were written to the file whenever the beam between any IR sensor pair was broken. The IR sensor pairs and latch returned a voltage signal of either 0 or 1 according to a preset threshold to indicate 'beam intact' or 'beam broken,' respectively. The binary combination for any tunnel's 2 IR sensors was used to indicate 'no mouse in tunnel' (denoted by 0,0) or 'mouse in tunnel' (any other combination). The sequence of binary combinations (ie. the progression of broken beams) indicated the direction of the mouse's movement. The photosensor returned a resistance value that varied according to the reflectance of the object in front of it. The resultant data set was a collection of dates, times, resistance values, and binary digits.

The raw data collected with the ATS were processed into a set of mouse movements and corresponding times, by using the following steps:

1) Identify presence of a mouse in a tunnel (value of 1 in binary IR sensor data)

2) Determine direction of movement through the tunnel in light of the sequence of 0s and 1s of the IR sensor data

3) Summarize list of movement into and out of each compartment

4) Organize data into 4 sets, 1 for each compartment (moves into and out of each compartment)

5) Calculate the amount of time spent in each compartment (time into minus time out of)

6) Calculate the number of mice in each compartment at a given time

7) Calculate the variables for statistical analysis: total occupancy (mouse–hours) in each compartment; percentage of total time per compartment; number of moves into each compartment; average duration of stay in compartment; and mean number of mice in a compartment at a given time

The video recordings were reviewed to verify, and correct as necessary, the measurements of the ATS, specifically the direction and time of a mouse's movement and the number of mice in a compartment at a given time. Corrections were made as needed, specifically for those movements for which ATS incorrectly determined mouse location. (Complete video was available for 3 of the 4 trials. Adjustments made to the other trial were based on available video and logic tests [developed during video verification of the 3] and comparisons with notes taken during the trial) The measurements by the ATS were categorized into 1 of 4 categories: 1) movement detected and mouse location correctly determined after its move; 2) movement detected but mouse location not determined after its move; 3) movement not detected, even though a mouse had entered the tunnel; or 4) movement detected but mouse location incorrectly determined after its move.

Statistical analysis. The processed behavioral data (from step 7 in the algorithm) were subject to statistical analysis by using the SAS PROC MIXED procedure,[18] with NH_3 treatment and location of the compartment as fixed effects of the model and the trial as random effect (analysis A). The analyses were repeated with the level of the compartment (top versus bottom tier) replacing location of the compartment (I, II, III, and IV) in the model statement (analysis B). The 5 response variables were analyzed separately. The treatment effects were considered significant at an α level of less than or equal to 0.05.

Results

Performance of the EPC. Overall, the performance of the EPC was satisfactory when assessed against its design and functional criteria. The accuracy of determining the movement of mice around the EPC was about 79% as determined by the ATS, and the backup video camera system was needed to verify the ATS data.

For the three 2-d trials conducted and verified with complete video, 6480 entrances into the tunnels were detected by the tracking system (Table 2). Of these entrances, 5102 (79%) were correctly identified and deciphered by the IR sensors. A portion of the other moves (1051, 16%) were detected but mouse location was not determined and had to be verified by reviewing the video. These unidentified moves likely were caused by transient disruptions to the main electrical supply to the room in which the EPC was located, which disrupted the ATS, and were not attributable to inadequate design of the EPC. This fault could be avoided by the use of a protected main supply. In other instances where a move was not detected by the ATS, (193, or 3% of the data), the outcome was caused by 1 mouse closely following another through a tunnel, leaving no clear separation. Some moves were detected but the mouse location was incorrectly determined (134, or 2% of the data); these were subsequently clarified by the video observations.

The performance of the photosensors for recognition of individual mice was poor in that they were unable to individually discern the colors on the fur of each mouse. Permanent marker, food coloring, watercolor, pig marker, and sheep marker were all tested. Both color intensity and consistency are required for distinguishable output from the photosensor over several days.

Table 2. Performance of the automated tracking system in detecting moves made by laboratory mice between compartments and determining mouse location in a preference chamber (values from 3 trials)

Classification category	No. of moves	% Total moves
Detected, location correct	5102	78.7
Detected, location undetermined	1051	16.2
Not detected	193	3.0
Detected, location incorrect	134	2.1
Total	6480	100

The pig marker was shown to be the most satisfactory, but it also lost consistency and intensity over time, resulting in difficulty in distinguishing the individual mice.

Behavioral responses of mice to the testing environments. The weights (mean ± SE) of mice before and after the preference test were 18.4 ±0.5 and 18.3 ± 0.5 g (trial 1), 13.4 ± 0.3 and 16.6 ± 0.4 g (trial 2), 15.3 ± 0.2 and 17.9 ± 0.4 g (trial 3), and 17.7 ± 0.6 and 17.9 ± 0.4 g (trial 4), respectively. Using the training procedures described previously, all mice learned to navigate the chamber independently, and thereafter they moved frequently between the compartments. The total number of moves of all mice among the compartments over 48 h (with ammonia present) was: 3309, 2221, 2046, and 2470, for trials 1, 2, 3, and 4, respectively, averaging approximately 13 moves hourly per mouse. The 48-h temporal profiles of mice in each compartment in each of the 4 trials are presented in Figure 5.

The mice were observed to increase activity and exploration when the NH_3 supply commenced and stopped. For up to an hour after the NH_3 treatment commenced, the mice patrolled and investigated all compartments and sniffed frequently at the ventilation inlet. The longest periods of time spent in the same compartments occurred when the mice were sleeping, usually in 1 of the upper 2 compartments, although not all groups preferred the same compartment. The mice ate and drank in all compartments. They also handled nesting material in all compartments, but the most organized nests were made in the preferred sleeping compartments. In 2 of the 4 trials, the mice moved some nesting material through the tunnels into the preferred sleeping compartment, which was the upper tier compartment with the highest NH_3 concentration. In another trial, the mice moved all the nest material from the entire chamber into the same compartment (nominally 100 ppm NH_3). Measured NH_3 concentrations (mean ± SE) were 4 ± 2, 30 ± 2, 56 ± 4, and 110 ± 6 ppm for the 0, 25, 50, and 100 ppm nominal concentrations.

For the first 24 h after the NH_3 treatment had commenced, the mice showed few preferences for different compartments, and only the effects of compartment location (P = 0.05) and trial (P = 0.01) were significant in the model for the number of moves. The number of moves (mean ± SE) into compartment I (376 ± 87) and II (370 ± 70) were greater than those into compartment III (260 ± 40) and IV (222 ± 32), but no different from one another. The number of moves for trial 1 (470 ± 80) was significantly greater than that of trial 2 (257 ± 36), trial 3 (236 ± 32), or trial 4 (265 ± 19).

Clear preferences for the upper (I and II) over the lower (III and IV) compartments were observed during the second 24 h of NH_3 treatment (Table 3). This location effect was significant for all 5 behavioral variables analyzed ($P < 0.03$). The analysis revealed no distinguishable preference for, or aversion to, different NH_3 concentrations. The results of the statistical analysis

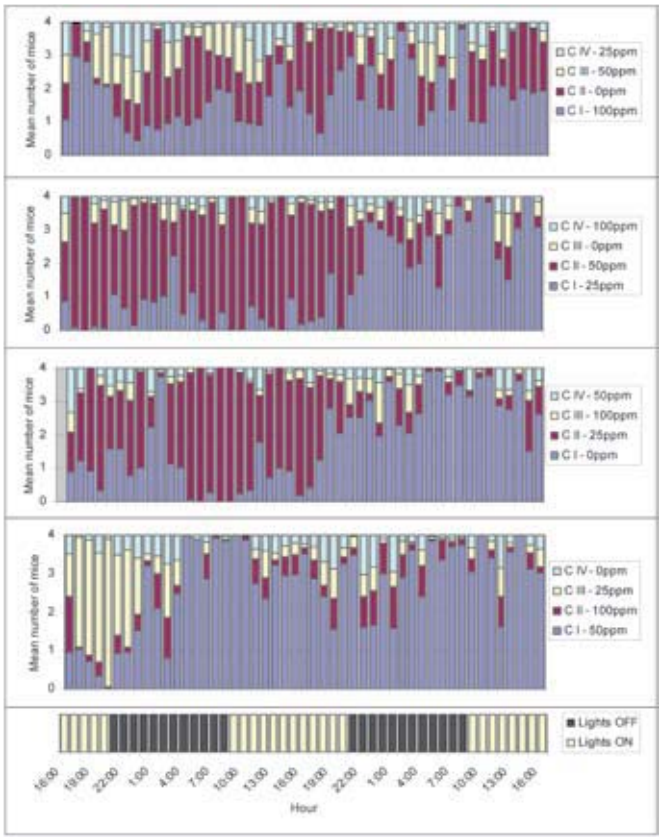

Figure 5. Temporal profiles of mice occupancy in each compartment throughout the 48-h treatment exposure during each trial.

were the same for both analyses (A and B), implying that the experimental design was robust.

Discussion

Design and performance of the EPC. The EPC proved to be a successful design to test the environmental preferences of laboratory rodents with potential applications to many environmental factors, for example, illumination and resource provision. Although further refinements are needed to improve its performance, the design of the chamber is particularly novel in that access to all the available choices is possible from each and every compartment. This design overcomes a considerable limitation of the principal alternatives, for example, a radial maze, where animals have to traverse a neutral central compartment, thereby creating difficulties in interpreting time spent therein, and an annulus, where immediate access to alternative choices is limited to those in the neighboring compartments and where animals have to traverse neighboring compartments to access more distant choices. The inclusion of 4 compartments allows for a favorable range of environmental conditions that can be tested simultaneously, an option that would not be possible in a simple pairwise test of 2 cages side-by-side. An annulus design could be implemented with 3 compartments and might be a suitable alternative, although the access tunnels would be longer and thus provide an area for the mice to dwell between treatment environments. Nevertheless, before a blanket recommendation can be given for our design, it and the alternatives should be compared by using the same environmental resources.

Although the ATS did not entirely eliminate the need to watch the video recordings, the time required for manual labor was reduced substantially. In similar experiments with pigs and chickens in an annular preference chamber, animals were monitored only by video camera,[8-10] with great effort needed to record the animal's movements. We are unaware of a similar ATS for comparison. The accuracy of the ATS may be improved in subsequent experiments, specifically for reducing the number of movements detected but not determining the location of the mouse. This phenomenon was not encountered during instrumentation development, and its cause is still uncertain. We believe that power fluctuations in the facility affected communication between the computer and data acquisition system, but we cannot test this hypothesis because the chamber has been relocated. This phenomenon should be further explored in subsequent experiments, followed by appropriate adjustments, if needed.

The behavior of individual mice was not recorded in the preference test. The illumination-intensity–sensitive photosensor did not yield accurate information on individual identity due to the grooming behavior of the mice, which led to fading or disappearance of the artificial coloring of the fur. We are unaware of a superior dye or marker. Further refinements to the ATS may be made, perhaps based on implanted radio-transmitting tags. Marking the mice and collecting individual behavior data by digital image analysis may be another option, although keeping the marking on the mice over an extended period will remain a difficult task, sensitivity to intensity will be reduced. Human errors during video viewing are inevitable, given the inclusion of cage furnishings and obscured viewing, and automated tracking potentially will reduce errors.

Responses of mice in the ammonia preference tests. This experiment represents our first attempt to determine the preferences of laboratory mice for atmospheric NH_3 at concentrations typically encountered in laboratory animal facilities. This study included only 4 replications with the limited exposure duration of 2 d. Therefore, the results must be considered preliminary. Nonetheless, for the final 24 h of the treatments, the results surprisingly indicated 2 motivations acting on mouse behavior: a preference for the upper tier compartments and no preference for, or aversion to, atmospheric NH_3.

Clearly, the mice were familiar with the chamber, because they explored and regularly patrolled all the compartments, averaging 13 moves hourly per mouse. Although the mice made full use of the chamber and may have valued the spatial enrichment provided by its unique 3-dimensional design, the reason they showed a clear preference for the upper tier of compartments remains to be investigated. The uniformity of several variables (noise, airflow, illumination) within the compartments was tested, but no differences were observed. The preference for the upper tier may have been due to an unknown environmental heterogeneity or simply an attraction for an elevated location. The location of tunnel openings may explain this apparent preference, perhaps due to a perceived threat from an overhead opening, or an energetic cost of traveling vertically *downward* as compared with the cost of traveling horizontally or vertically *upward*. These alternative explanations could be tested experimentally with the EPC.

Presence of atmospheric NH_3 increased activity of the mice. Mice entered and explored every compartment, even those with the highest NH_3 concentrations. This apparent lack of aversion to NH_3 persisted throughout the second 24-h period, especially in the upper-tier compartments in which the higher NH_3 concentrations were present in several trials. Therefore a strong compartmental preference seemed to override any preference for fresh air over an ammoniated atmosphere or at least any aversion to ammonia. Even when both compartments

Table 3. Use of a preference chamber by mice when given a choice between ammoniated atmospheres during the final 24 h of a 48-h preference test.

	Nominal NH_3 concentration (actual mean concentration ± SE)				*P*
	0 (4 ± 2)	25 (30 ± 2)	50 (56 ± 4)	100 (110 ± 6)	Analysis A / B
Total occupancy, h	27.4 ± 3.7	21.9 ± 3.7	28.1 ± 3.7	16.1 ± 3.7	0.19 / 0.79
% Time in compartment	29.6 ± 3.7	23.0 ± 3.7	29.6 ± 3.7	17.8 ± 3.7	0.17 / 0.81
No. of moves into	337 ± 20	332 ± 20	310 ± 20	305 ± 20	0.61 / 0.67
Duration in, min	4.5 ± 0.7	3.6 ± 0.7	4.7 ± 0.7	2.9 ± 0.7	0.29 / 0.82
No. of mice per compartment	1.2 ± 0.2	0.9 ± 0.2	1.2 ± 0.2	0.7 ± 0.2	0.16 / 0.81

Analysis A	Compartment				
	I	II	III	IV	*P*
Total occupancy, h	57.4 ± 3.7[a]	21.8 ± 3.7[b]	7.4 ± 3.7[c]	7.0 ± 3.7[c]	0.0002
% Time in compartment	61.8 ± 3.7[a]	23.0 ± 3.7[b]	7.8 ± 3.7[c]	7.4 ± 3.7[c]	0.0001
No. of moves into	412 ± 20[a]	409 ± 20[a]	258 ± 20[b]	205 ± 20[c]	0.0006
Duration in, min	8.7 ± 0.7[a]	3.2 ± 0.7[b]	1.8 ± 0.7[b]	2.1 ± 0.7[b]	0.001
No. of mice per compartment	2.5 ± 0.2[a]	0.9 ± 0.2[b]	0.3 ± 0.2[c]	0.3 ± 0.2[c]	0.0001

Analysis B	Chamber level		
	Upper	Lower	*P*
Total occupancy, h	39.6 ± 6.7[a]	7.2 ± 6.7[b]	0.0091
% Time in compartment	42.4 ± 7.2[a]	7.6 ± 7.2[b]	0.0093
Number moves into	410 ± 15[a]	231 ± 15[b]	0.0001
Duration in compartment, min	5.9 ± 1.1[a]	1.9 ± 1.1[b]	0.0288
No. of mice per compartment	1.7 ± 0.3[a]	0.3 ± 0.3[b]	0.0093

Analysis completed for effect of ammonia concentration, compartment location, and trial. Compartments I and II are on the upper tier, III and IV on the lower tier. Data are given as mean ± SE.
[a, b, c]denotes statistically different ($P \leq 0.001$) for the given variable

on the upper tier contained ammonia (trials 2 and 4), the mice showed no clear preference for the lower NH_3 concentration: the mice preferred the upper tier despite the presence of ammonia at a concentration equal to or higher than the currently recommended exposure limit of 25 ppm.

In similar preference experiments, pigs and chickens showed a strong but delayed aversion to NH_3 at concentrations of 20 ppm and higher.[8-10] The preference (or aversion) of mice may be related to their reliance on olfaction as the dominant sensory modality: olfactory cues are particularly important for sexual, social, and maternal behavior.[11,15] In particular, ammonia may be associated with urinary odors used in territorial scent marking.

The results of this preliminary experiment require confirmation but potentially have implications for the design and management of mouse facilities. Human exposure limits may not be appropriate for mice, which apparently tolerate higher concentrations of NH_3 for several days with little adverse effects. Future studies should include a period sufficient to establish baseline preferences after the mice have been trained to use the chamber. This design would allow determination of any shift from the baseline due to addition of an environmental treatment. In the case of studies with NH_3, the treatment period should be extended beyond 2 d, to assess the possibility of a delayed aversion to NH_3 that could not be observed in this study. Testing a wider range of NH_3 concentrations may be advisable, because the mouse's tolerance for NH_3 may be higher than previously suggested. In addition to the behavioral responses of mice, assessing the physiologic effects of NH_3 on laboratory mice may enhance the determination of the exposure threshold. Moreover, olfactory perceptions may vary with age and strain; therefore, the chamber should be adapted for tests with older, larger mice and those of other strains.

This study provides a system for future research on the environmental preferences and motivations of laboratory rodents. The applications are manifold and include other gases, lighting, thermal conditions, physical resources, and social factors such as group size. The goal is to define a physical environment for mice that accommodates their preferences, provides high standards of care, and does not interfere with the validity of scientific tests.

Acknowledgments

The authors wish to express appreciation and gratitude to the following persons for their contributions to the project: Siobhan Abeyesinghe and Morven McLeman for insight into animal behavior; Len Burgess and Elia Nigro for assistance with chamber construction and experimental setup; Rodger White and Philip Dixon for statistical consultation; and Paul Berry for conceptual input. BioZoneGlobal provided the ventilated cage rack. Financial support for the study was provided in part by the National Science Foundation through a Graduate Fellowship (awarded to ARG).

References

1. **American Conference of Governmental Industrial Hygienists.** 2000. Guide to occupational exposure values. Cincinnati (OH): ACGIHOH. p 6.
2. **Bernard RS, Richardson ME, Diehl JR, Bridges WC.** 2000. The influence of husbandry schedules on the numbers of embryos collected from superovulated mice. Contemp Top Lab Anim Sci **39:**13–15.

3. **Broderson JR, Lindsey R, Crawford JE.** 1976. The role of environmental ammonia in respiratory mycoplasmosis of rats. Am J Pathol **85:**115–130.
4. **Clough G.** 1982. Environmental effects on animals used in biomedical research. Biol Rev Camb Philos Soc **57:**487–523.
5. **Duncan IJH.** 1992. Measuring preferences and the strength of preferences. Poult Sci **71:**658–663.
6. **Everleigh JR.** 1993. Murine cage density: cage ammonia levels during the reproductive performance of an inbred strain and 2 outbred stocks of monogamous breeding pairs of mice. Lab Anim **27:**156–160.
7. **Gaafar H, Girgis R, Hussein M, Elnemr F.** 1992. The effect of ammonia on the respiratory nasal mucosa of mice—a histological and histochemical study. Acta Otolaryngol **112:**339–342.
8. **Jones EKM, Wathes CM, Webster AJF.** 2005. Avoidance of atmospheric ammonia by domestic fowl and the effect of early experience. Appl Anim Behav Sci **90:**293–308.
9. **Jones JB, Burgess LR, Webster AJF, Wathes CM.** 1996. Behavioural responses of pigs to atmospheric ammonia in a chronic choice test. Anim Sci **63:**437–445.
10. **Kristensen HH, Burgess LR, Demmers TGM, Wathes CM.** 2000. The preferences of laying hens for different concentrations of atmospheric ammonia. Appl Anim Behav Sci **68:**307–318.
11. **Latham N, Mason G.** 2004. From house mouse to mouse house: the behavioural biology of free-living *Mus musculus* and its implication in the laboratory. Appl Anim Behav Sci **86:**261–289.
12. **Lipman NS.** 1992. Microenvironmental conditions in isolator cages: an important research variable. Lab Anim **21:**23–27.
13. **Lipman NS.** 1999. Isolator rodent caging systems (state of the art): a critical review. Contemp Top Lab Anim Sci **38:**9–17.
14. **Nevison CM, Hurst JL, Barnard CJ.** 1999. Why do male ICR (CD-1) mice perform bar-related (stereotypic) behaviour? Contemp Top Lab Anim Sci **47:**95–111.
15. **Olsson IAS, Nevison CM, Patterson-Kane EG, Sherwin CM, Van de Weerd HA, Wurbel H.** 2003. Understanding behavior: the relevance of ethological approaches in laboratory animal science. Appl Anim Behav Sci **81:**245–264.
16. **Reeb CK, Jones RB, Bearg DW, Bedigian H, Myers DD, Paigen B.** 1998. Microenvironment in ventilated animal cages with differing ventilation rates, mice populations, and frequency of bedding changes. Contemp Top Lab Anim Sci **37:**43–49.
17. **Reeb-Whitaker CK, Paigen B, Beamer WG, Bronson RT, Churchill GA, Schweitzer IB, Myers DD.** 2001. The impact of reduced frequency of cage changes on the health of mice housed in ventilated cages. Lab Anim **35:**58–73.
18. **SAS.** 2007. User's guide, version 8. Gary (NC): SAS Institute.
19. **Schoeb TR, Davidson MK, Lindsey R.** 1982. Intracage ammonia promotes growth of *Mycoplasma pulmonis* in the respiratory tract of rats. Infect Immun **38:**212–217.
20. **Targowski SP, Klucinski W, Babiker S, Nonnecke BJ.** 1984. Effect of ammonia on in vivo and in vitro immune responses. Infect Immun **43:**289–293.
21. **United Kingdom Home Office.** 1986. The Animals (Scientific Procedures) Act (1986). London: The Stationery Office.