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Individually ventilated cages cause chronic low-grade hypoxia impacting mice hematologically and behaviorally ☆

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ABSTRACT

Use of individually ventilated caging (IVC) systems for mouse-based laboratory investigation has dramatically increased. We found that without mice present, intra-cage oxygen concentration was comparable (21%) between IVC housing and ambient environment caging (AEC) that used wire top lids. However, when mice were housed 4-to-a-cage for 1 week, intra-cage oxygen dropped to 20.5% in IVC housing as compared to 21% for AEC housing. IVC intra-cage humidity was also elevated relative to AEC housing. Mice raised in IVC housing as compared to mice raised in AEC housing had higher RBC mass, hematocrit and hemoglobin concentrations. They also had elevated platelet counts but lower white blood cell counts. IVC mice, relative to AEC mice, had increased saccharin preference and increased fluid consumption but similar locomotion, food intake, social exploration and novel object recognition when tested in an AEC environment. Taken together, these data indicate that ventilated caging systems can have a 0.5% reduction from ambient oxygen concentration that is coupled to mouse red blood cell indices indicative of chronic exposure to a hypoxia. Importantly, IVC housing can impact behavioral testing for depressive-like behavior.

1. Introduction

An important trend in laboratory rodent housing is the use of individually ventilated caging (IVC) systems. Purported advantages of IVC systems over conventional, ambient environment caging (AEC) (i.e. wire top, open air caging) are allergen and volatile organic compound (VOCs) reduction and the ability to increase animal population densities (Höglund and Renström, 2001; Mineu and Crusio, 2009; Silverman et al., 2008). In animal care/research personnel, allergy to laboratory animals can be as high as 44%, with a median time to allergy onset of less than 2 years (Fisher et al., 1998; Hunskaar and Fosse, 1990). Allergen exposure can originate from sources such as urine, fur/pelt, saliva and serum proteins (Gordon, 1997), and these allergens can contaminate the animal facility in both airborne particulate and fomite forms (Gordon and Preece, 2003; Kaliste et al., 2004). VOCs such as ammonia have been identified as causative agents of "sick building syndrome", with animal care/research personnel reporting headache, nausea and fatigue (Kacergis et al., 1996). Educational training programs focused on personal hygiene and the use of personal protective equipment have reduced the incidence of laboratory animal-associated allergies, but with AEC the impact of such interventions has been modest (up to 22% of staff still developing allergies) (Fisher et al., 1998). On the other hand, IVC housing has been shown to significantly reduce the important mouse-derived human allergen, murine urinary protein (Gordon and Preece, 2003).

As we (York et al., 2012) and others (Jennings et al., 1998) have reviewed, pre-experimental conditions are critical to rodent-based behavioral testing outcomes. The methodology by which mice are fed, handled and housed can dramatically impact a host of behaviors and like-behaviors including those reliant on locomotion, food intake, learning/memory, social interaction, anxiety and depression (Hrabé de Angelis et al., 2004). Most rodent behavioral testing is conducted outside the home cage and in macro-environments removed from where the animal was reared. This is especially true of mice bred and raised with commercial suppliers. A critical assumption made is that once rodents of a particular strain are

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acclimatized to their new surroundings, whether it is a new cage, intra/inter-facility room or institution, that they behave equivalently. This notion has encompassed micro-environmental concerns as well because sophisticated behavioral testing requires specialized equipment and tasks that are unable to fit inside a standard shoebox sized cage. Thus, rodent behavioral testing is almost exclusively performed in an open air environment.

Little is known concerning how pre-experimental IVC housing affects mouse behaviors when compared to AEC housing. The single study published to date showed no effects of IVC housing in mice during plus maze, open field, radial arm maze, acoustic startle or resident intruder tests (Mineu and Crusio, 2009). While others have investigated the impact of IVC housing on mouse behavior, these studies either used IVC system cages outside of the ventilation unit (Kallnik et al., 2007) or were comparing different IVC systems to one another (Höglund and Renström, 2001). Importantly, no clear differences in IVC vs. AEC housing were seen or mechanism for behavioral change presented (Höglund and Renström, 2001; Kallnik et al., 2007; Mineu and Crusio, 2009). In contrast, a cornucopia of data exists on the intra-cage microenvironmental differences between IVC and AEC housing with special attention paid to carbon dioxide, ammonia vapor and relative humidity (Höglund and Renström, 2001; Kacergis et al., 1996; Krohn and Hansen, 2002; Rosenbaum et al., 2010; Silverman et al., 2008). Surprisingly, intra-cage oxygen concentration in IVC housing has been ignored, although it is well known that in confined spaces with sealed ventilation systems like commercial airplanes (Rushkin et al., 2008), submarines (Luria and Morris, 1988) and space stations (Stewart et al., 2007), oxygen concentrations can easily fall below 21%. In turn, hypoxia impacts a variety of physiologic functions and bioactives including behavior, as we have shown (Johnson et al., 2007) and reviewed (Johnson et al., 2008). In sum, no studies have reported on intra-cage oxygen concentration in IVC housing. Therefore, we examined intra-cage oxygen in IVC housing to determine its potential relevance to pre-experimental mouse physiology.

2. Materials and methods

2.1. Materials

All reagents and chemicals were purchased from Sigma–Aldrich (St. Louis, MO) except as noted.

2.2. Animals and housing

Animal use was conducted in accordance with institutional guidelines for the care and use of laboratory mice. All experimental procedures were approved by the University of Illinois at Urbana-Champaign IACUC. All animals were housed in an AAALAC accredited laboratory facility as outlined in the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research., 2011). Male C57BL/6J mice, 3 weeks of age, were obtained from The Jackson Laboratory (Bar Harbor, MN). Since IVC systems/care varies (pressure, air exchange, animal density, bed changes), 3-week old mice were used to maximize time spent within our IVC housing system. Prior to shipping, mice had been housed in either IVC or AEC conditions by The Jackson Laboratory. Mice were group housed (4/cage except for oxygen and humidity studies where 2 and 3 mice/cage were also examined) in standard shoebox cages (28 cm × 17 cm × 12.5 cm) with wire top cage lids that were either open to the ambient environment (AEC) or attached to a positive-pressure Micro-VENT Mouse individually ventilated caging (IVC) system (Allentown, Inc., Allentown, NJ). All mice were allowed water and standard rodent chow (NIH-31 7013, Harlan Laboratories, Inc., Indianapolis, IN) *ad libitum*. Regardless of housing method used, the room in which the mice resided was environmentally controlled on a 12:12 h dark:light cycle (2000–0800 h) at a temperature of 72° F, relative humidity of 26–48% and 10–15 hourly air changes. The total number of mice used was 250.

2.3. Oxygen, carbon dioxide, ammonia and humidity

Room and intra-cage air oxygen and carbon dioxide were measured using ProOx oxygen and $ProCO_2$ carbon dioxide sensors, respectively (Biospherix, Lacona, NY). Humidity was measured using a digital hygrometer (Cat. No. 11-661-18, Fisher Scientific, Pittsburgh, PA). Ammonia was measured using a Kwik–Draw Sampling Pump (Cat. No. 488543, MSA, Pittsburgh, PA) with 5–700 ppm Ammonia-specific sampling tubes (Cat. No. CH20501, Dräger, Germany). Room and intra-cage air and humidity measurements were performed at 1400 h daily for three consecutive days. For intra-cage measurements, the sensors were located in the food hopper. Measurements were randomized in regard to sensor placement within the room and to cage location within the cage racks.

2.4. Treatments and testing

Mice examined were between 8–12 weeks of age and had spent 5–9 weeks in AEC or IVC conditions. AEC mice were housed in AEC conditions (10–15 air changes/h) and then treated and/or tested in AEC conditions. IVC mice were housed in IVC conditions (60 air changes/h) and then treated and/or tested in AEC conditions.

2.5. Hematology

After being housed in either AEC or IVC housing, mice were euthanized using carbon dioxide. Blood was drawn using post-mortem intracardiac puncture. A total approximate volume of 0.6–0.8 mL of blood was obtained from each mouse and placed into two separate (0.3–0.5 mL) pediatric EDTA anticoagulation microtainer tubes (Cat. No. 365974, Becton Dickinson, Franklin Lakes, NJ). Complete blood counts (CBC) and differentials were performed at University of Illinois Veterinary Diagnostic Laboratory (Urbana, IL) on an Abbott Diagnostics Cell Dyn 3700 automated hematology cell counter (Abbott Park, IL).

2.6. Body mass and food and water consumption

Immediately prior to testing, mice were individually housed in AEC. Body mass and food and water consumption were measured daily at 1000 h by weight. Food and water consumption were determined from the weight of the water bottle plus water and the weight of the food container plus food before and after each 24 h data collection period by methods we have previously described (Sherry et al., 2010). Briefly, the daily mass of the food or water in their respective containers were subtracted from the previous days mass, to determine amount consumed. Cage floors and bedding were carefully checked to account for food spillage and potential hoarding.

2.7. Group housed water consumption

Similar to the above, water consumption was determined from the weight of the water bottle plus water from AEC and IVC group housed mice. After 1 week, water loss was recorded. Briefly, final mass was subtracted from initial mass to determine amount consumed. Grams of water consumed per gram of mouse (total water consumed/total cage mouse weight) was calculated.