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Exposure in the Shoebox: Comparison of Physical Environment of IVCs and Open Rat Cages

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Summary

New caging and innovative items for more structured environment within the cage have been introduced. Many of these innovations cannot be seen as 'pure' or individual procedures, but rather they represent a mixed exposure with a multitude of operant factors, some possibly having an impact on animals and research. One kind of new caging system is the individually ventilated cage (IVC), where each cage receives its own non-contaminated airflow, primarily designed for health status maintenance and occupational safety. Even though those cages may be the same as those used in open cage systems, the physical environment inside the cage may not identical. Comparison between cage types is difficult without characterization of the physical environment, because the change may involve alterations in several parameters in the environment. The aim of this study is to characterize and compare common physical parameters in the ordinary situation, where IVC-racks are kept in the same room with open cages. The cage type used was a polysulfone solid bottom cage. The parameters measured in this study were: illumination, temperature, relative humidity (RH) and acoustic level in both IVCs and open top cages. No animals were in the cages during light intensity, but there was bedding in the cage during acoustic measurements and both bedding as well as a half-full food hopper during the illumination measurements. The temperature and (RH) measurements were carried out with three male rats in each cage. There were differences between IVCs and open top cages in all measured parameters. The light intensity was lower in IVCs, most likely due to more compact cage placement in the rack and the additional plastic cover lid of the cage. Both maximum and minimum temperatures were 1-4 ^{O}C higher in IVCs; which suggests that their ventilation is incapable of taking away heat, produced inside the cage. Similarly, the relative humidity was higher in the IVCs. The sound level adjusted to rat's hearing with R-weighting was higher in IVCs when compared to open cages. Furthermore, the sound level was highest in the corners next to the ventilation valves. In conclusion, there may be differences between open cages with IVCs involving several physical parameters of cage environment and this may confound comparisons between results obtained in these cage systems.

Introduction

New caging and innovative items are being introduced to provide a more structured environment within the cage. Many of these innovations cannot be seen as 'pure' or single procedures, but rather as a mixed exposure with a multitude of operant factors, possibly having an impact on animals and research.

One of those new kinds of caging systems is the individually ventilated cage (IVC), where each cage receives its own filtered air flow, primarily designed for health status maintenance and occupational

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safety. Other potential benefits include: protection of small groups of animals against infections, protection of the environment from the animals and compensation for poor air change rates in the room (*Brandstetter et al*., *2004*). Even though the cages may be the same as those used in open cage systems, the physical environment inside the cage may not be identical.

Reports on IVC-systems in the scientific journals can be divided into those concerned with the design and recommendations for (*Höglund & Renström et al., 2001; Renström et al., 2001; Hawkins et al., 2003; Brandstetter et al., 2005*) and characterization of the IVC environment (*Krohn et al., 2003; Clough et al., 1995*).

The move from traditional open cages to IVCs is bound to change the physical environment of the animals living in; what we have called, the ”shoebox”. It could be anticipated that at least temperature, relative humidity (RH), acoustic environment and light intensity may change in this transition.

Traditionally in biomedical research, attempts are made to assess the individual effects of compounds and procedures and, usually, evaluation of many simultaneous events and their combinations are avoided. The term used here is standardization, and emphasis is on the fact that all other items are exactly the same between study and control groups. Comparison of open-top cages and IVCs without characterization of the physical environment may not reveal a single causative feature, because the change inevitably involves a mixed exposure. The aim of this study is to characterize and compare physical parameters in a common situation, where IVC-racks are housed in the same room with open cages.

Materials and Methods

Animal room

All the cages were kept in the same room (length x width x height; 5.5 x 3.5 x 3.0 m) along opposite walls. The locations of cage racks, room furniture, fluorescent tubes, air inlet and outlet are illustrated in Figure 1. The IVC-rack (Figure 2) included 20, while the open cages (Figure 3) were in two racks, ten cages each. The height of the open cage rack was 176 cm and that of IVC-rack was 186 cm.

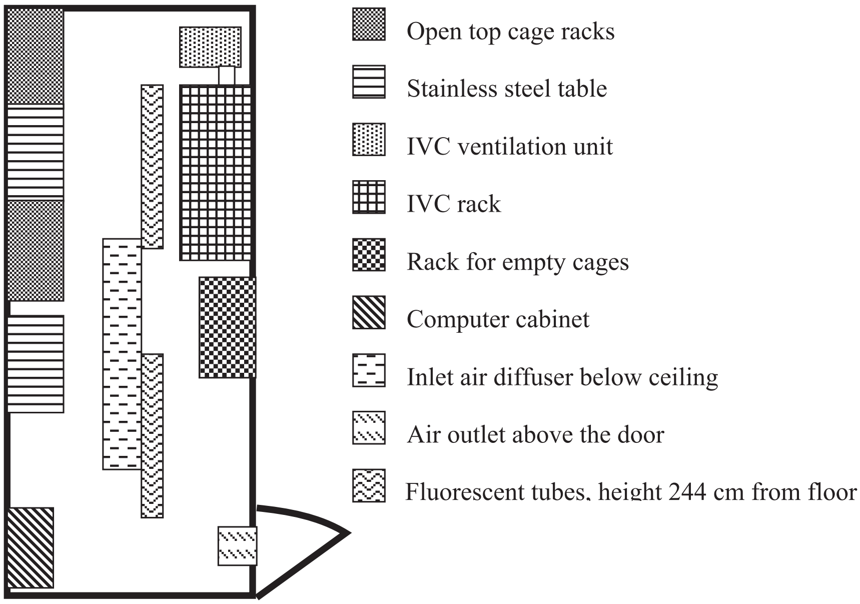

Figure 1. Layout of the animal room.

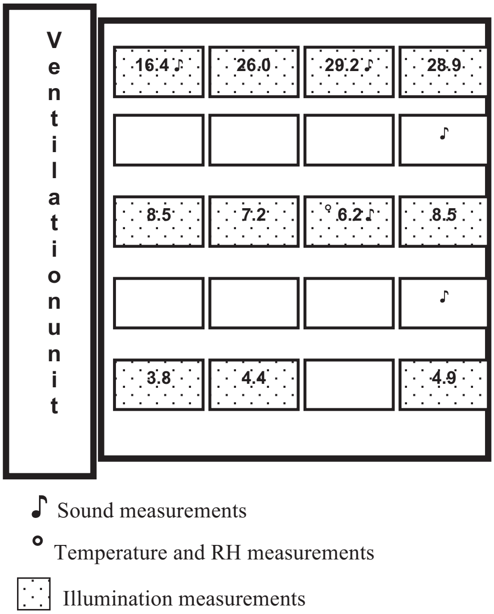

Figure 2. Frontal view of cage and ventilation unit location in IVC-cage rack with cage-specific illumination values (lux) and cages where acoustic, temperature and humidity measurements were done.

Cage types

The measurements were done from two different caging systems: open top cages and individually ventilated cages (IVC). Cages made of polysulfone (Tecniplast, Buguggiate, Italy, type1500U, dimensions 48.0 x 37.5 x 21.0 cm) with a solid bottom were used. Both cage types had a stainless steel wire lid, while in the IVCs there was an additional polysulfone cover, which contained the air supply and the exhaust air valves and a passive filter at the top of the cover. This filter allows gas exchange for a short period, when the cage is not docked to the IVC-system.

The IVC-system consisted of a ventilation module, which had both supply and exhaust units (Slim Line™, Tecniplast, Buguggiate, Italy) and the individually ventilated cage rack (Sealsafe™, Tecniplast, Buguggiate, Italy). The supply unit delivered HEPA (High Efficiency Particulate Air) filtered air into the cage, taken from the room itself, separately through each cage's supply valve, at the cage cover end. The exhaust air was taken out from the cages, also at the same end, through the exhaust valve and voided back to the room through a three filter set down to HEPA level.

Illumination

Artificial lights with two fluorescent tubes (light color warm white) were on from 06.00 to 18.00; their location in the room (106 cm below the ceiling) is illustrated in Figure 1. The IVC-rack had a stainless steel shield on top, and the open cages were covered with black plastic sheeting to prevent direct light entering the cages. No animals were in the cages during the light intensity measurements, but there was bedding in the cage and the food hopper was half full. The illuminometer (Roline digital lux meter RO 1332, Rotronic AG, Bassersdorf, Switzerland) was placed at the center of the cage floor on the bedding layer and single measurements were taken from both IVCs (Figure 2) and open cages (Figure 3).

Acoustic environment

A sound analyzer (Norsonic 121, Norsonic AS, Lierskogen, Norway.) was used for noise measurement. The measurement system was calibrated using a sound level calibrator (Wärtsilä model 5274, MIP Electronics Oy, Kerava, Finland). Four cages from both cage types were measured four times, once from each corner. The cages had bedding, but no animals were inside the cages during the measurements. Measurements were taken from cages marked with ♪ as shown in Figures 2 and 3. Equal sound pressure levels for one minute (Leq, 1min) in third-octave bands between 31.5 Hz and 20 000 Hz were measured with ½ an inch condenser microphone (Norsonic 1225, Norsonic AS, Lierskogen, Norway.). The microphone was placed about 5 cm from the walls and the bottom of the cage, and directed towards the corner of the cage.

In the weighted equal sound pressure level calculations, R-weighting and A-weighting were used. The

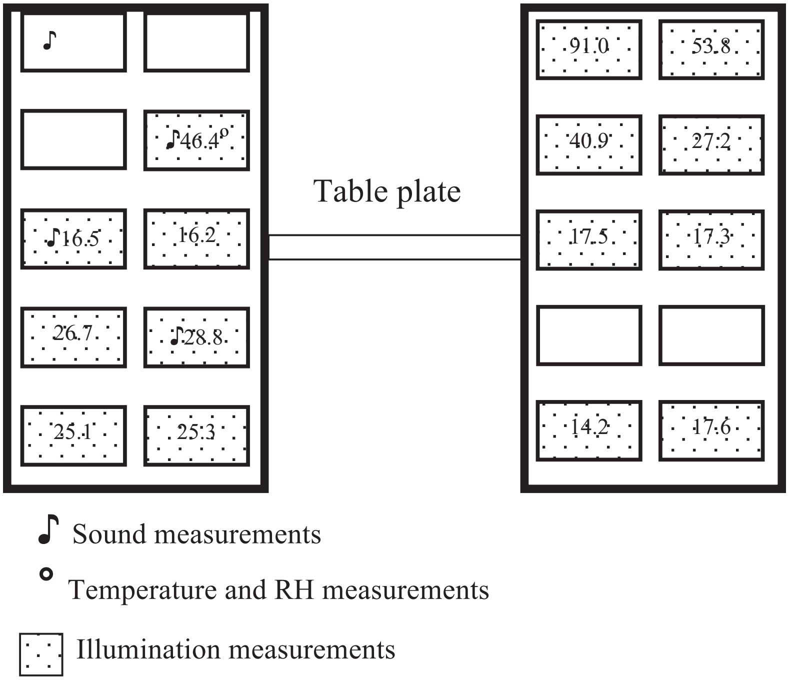

Figure 3. Frontal view of cages in open cage racks with cage-specific illumination values (lux) and cages where acoustic, temperature and humidity measurements were done.

basis of the R-weighting for rat hearing sensitivity and the numerical values are described in detail by Björk *et al*., (*2000*) and Voipio (*1997*). The A-weighting is commonly used in human sound experiments. The total weighted equal sound pressure levels were computed summing the weighted third-octave band levels on the energy bases. The weighted equal sound pressure level in each cage was computed as the mean value in the four corners on the energy bases.

Temperature and RH

Temperature and (RH) measurements were carried out with animals in the cages. Fischer344 (F344/NHsd, Harlan, Horst, The Netherlands) male rats were used in this study. The rats were 40 weeks old and weighed 380 - 400 g, three animals per cage. The cage floors were covered with 3.0 l aspen chip bedding (size 4 x 4 x 1 mm, 4HP, Tapvei Oy, Kaavi, Finland).

Municipal tap water was provided in polycarbonate bottles with stainless steel drinking nipples, changed once a week and refilled once in between. Irradiated pelleted (25 kGy) feed (2016 Global Rodent Maintenance, Teklad, Bicester, UK) was given *ad libitum*, added once a week.

Temperature and RH were measured with Besser® 7009032 Wireless Weather Station and with two Techno line TX4 433 MHz sensors (Besser, Borken, Germany). Measurements were taken simultaneously from both cage types (IVC and open top) and from the room for 7 days. The sensors were placed inside the feed hopper next to the pellets. Readings were taken once a day to provide minimum and maximum values over the previous 24 h period for

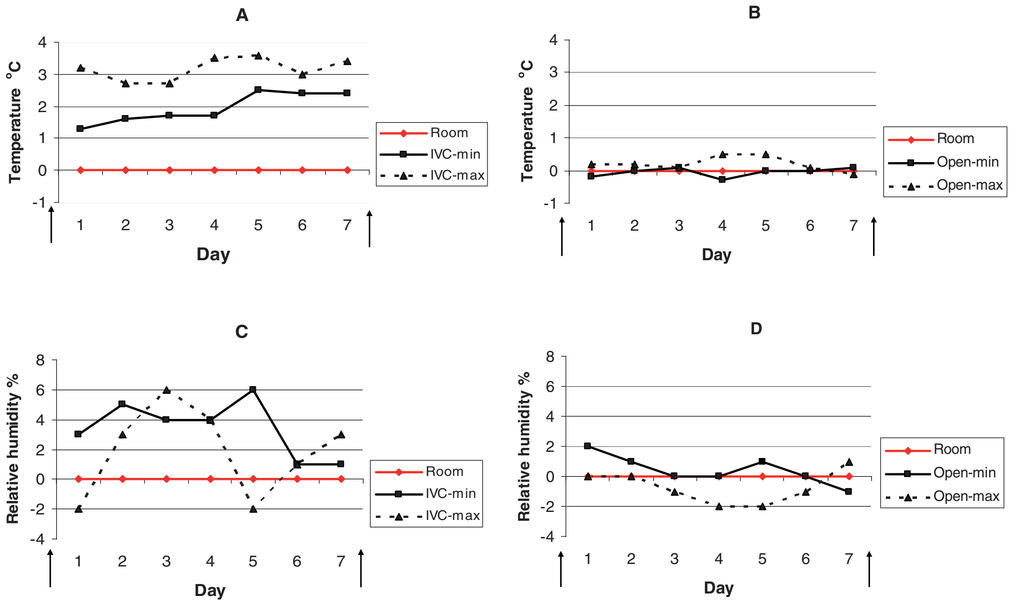

Figure 4. Deviation in the 24 hourly single cage maximum and minimum values inside the cage for temperature and relative humidity from the corresponding room values during the week and between cage changes. A and C are values for IVC-cages and B and D for open cages. Cage changes are marked with an arrow.

temperature and RH. Measurements were taken from cages as marked in Figures 2 and 3.

Results

Illumination

The light intensity at 1 m above floor in the open cages was 16-18 lux compared to 6–9 lux in the IVC´s, with upper cage rows showing considerably higher values. The light intensities of IVC-racks were lower than those measured in the open cages at corresponding levels. More detailed, cage specific, values are shown in Figures 2 and 3.

Acoustic environment

The sound level adjusted with R-weighting in empty IVC cages was 20-25 dB(R) compared to 12-18 dB(R) in the empty open cages, with the corresponding adjusted A-weighting being 45-47 dB(A) and 46-49 dB(A), respectively. The sound level was less in the open cages in the lower shelves of the rack, while in the IVCs the front of the cages showed higher sound levels compared to the back corners in the vicinity of the air valves. The sound frequency in both cage types was 16 – 16000 Hz. The mean sound pressure levels in the third-octave bands between 31.5 Hz and 20000 Hz on the energy bases of both cage types with R- and A-weighting and un-weighted (lin) are shown in Figures 5a and 5b.

Temperature and RH

There was a marked difference in temperature and RH between inside the IVC's and open top cages when compared to both open top cage and room values. In the IVCs, the maximum and minimum temperature values in the IVCs were 1-4 °C higher than the room temperature. In the open top cages the temperatures were at the same level as the room temperatures. There was a similar tendency noted in RH as shown in Figure 4.

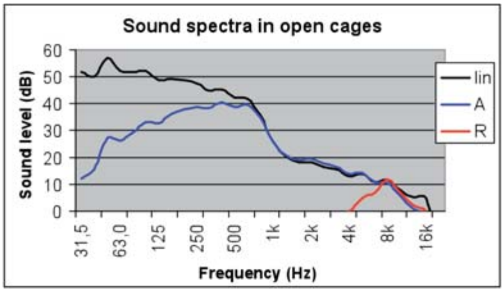

Figure 5 a. Sound spectra in open cages.

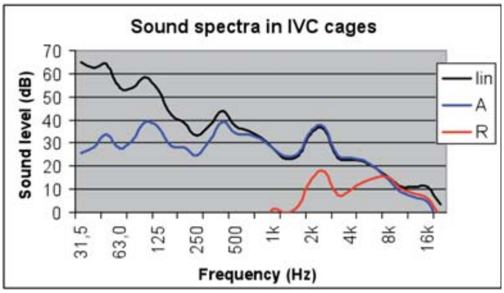

Figure 5 b. Sound spectra in IVC cages.

Discussion

IVCs are a new isolation system being installed in a large number of laboratory animal facilities. The system provides protection to the animals against infections, and has clear occupational benefits to personnel, especially in leading to a reduction in the levels of airborne allergens (*Renström et al., 2001*).

It is often assumed that the physical environment is the same in open cages and IVCs, when they are kept in the same room. Surprisingly it was found that this is not the case, and furthermore that the magnitude of the changes in physical environment is great enough that it could have an impact on the animals housed in these cages. Therefore it was decided to assess a set of easily measurable physical parameters in both caging systems.

The light intensity in IVCs was 10-60 lux lower than the corresponding values in open top cages. In the IVCs, the illumination varied between 3.8-28.9 lux and in the open top cages between 14.2-91.0 lux. The brightest cages were on the top row of both racks and the dimmest cages on the bottom row. Clough *et al.,* (*1995*) has shown similar results in the transparent, polycarbonate positive individually ventilated (PIV)-cages but in the translucent, polypropylene control cages the illumination was much brighter than used in our experiment. This difference in results may be due to the black plastic sheeting that was placed on top of the open top cage racks to equalize the lighting in both cage types. It appears that in some open top cages at the highest level, in contrast to the situation in the IVCs, the lighting was too bright, and even exceeded values shown to cause retinal damage in albino rats (*Stotzer et al., 1970; Weisse et al., 1974*).

Sound spectra (Figures 5a and 5b) show decreasing sound levels when approaching 16000 Hz and consequently it can be assumed that no ultrasound exists in either cage type. Accordingly the measured R-weighted sound pressure levels depict the correct sound level which would be heard by the rats. In IVCs, the R-weighted sound levels were about 7 dB(R) higher than in open top cages; energywise the difference was five fold and in terms of loudness almost twice as great. Although the difference in sound levels audible to the rat (R-weighted) is large, it remains to be determined whether the levels measured (< 25 dB(R)) have any major impact on the animals.

Scheer *et al.,* state the obvious: The climatic conditions in the cage are dependent on those of the surrounding room as well as the air supply of the cage-rack. In this study the temperature and RH in IVCs were the same as in the animal room when measured without animals, but placement of animals into the IVCs increased the temperature by 3-4 °C and RH by about 6 % in these cages.

In the open top cages, the temperature and RH were at the same level as in the animal room. This is in agreement with the results of Clough *et al*. (*1995*) who have shown similar results in PIV-cages. It appears that IVC-ventilation is unable to remove all the heat produced in the cage. Potential sources are heat from the animals as well as heat generated from urine-feces-bedding fermentation. Heat emission for three animals in a cage is estimated to be about 12 W (*Heine, 1998*). However, fermentation reactions are unlikely to occur because ventilation tends to keep bedding too dry.

It appears that the transition from traditional open top cages to IVCs may lead to changes in the physical environment. This makes any comparisons of these caging systems problematic without characterization of the physical parameters *e.g.* lighting intensity, sounds, temperature and RH. In conclusion, comparison of open cages with IVCs involves several physical parameters in the cage environment, which may confound straightforward comparisons.

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