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# BEHAVIOURAL CONSEQUENCES OF IVC CAGES ON MALE AND FEMALE C57BL/6J MICE

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**Abstract—Recent developments in the technology to breed and house laboratory rodents for medical research has produced individually ventilated cage (IVC) systems. These IVC systems produce a cage environment significantly different to conventional cages. As it is not known in detail whether housing mice in IVCs impacts on their baseline and drug-induced behaviours compared to mice of conventional filter-top cages a comprehensive multi-tiered phenotyping strategy was used to test the behavioural consequences of IVC housing in male and female C57BL/6JArc mice. IVC had anxiety-like effects in the elevated plus maze, which were more pronounced in female mice whereas cognition and locomotion of all test mice were not modified by IVC housing. Mice raised in IVC cage systems were socially more active than mice of filter-top systems. Furthermore, males raised in IVC exhibited an increased sensitivity to the locomotor-stimulating effects of acute MK-801 treatment compared to males in conventional cages. In summary, this is the first study revealing the longer-term effects of IVC housing on social behaviours and the locomotor response to an acute MK-801 challenge. In conclusion, researchers upgrading their holding facilities to IVC housing may encounter a shift in experimental outcomes (e.g. post pharmacological challenges) and the behavioural phenotype of test mice. Furthermore, differences between the housing conditions of breeding facilities and test facilities must carefully be considered. Finally, researchers should clarify in detail the type of housing test animals have been exposed to when publishing experimental animal research data.**


**Key words: mouse, C57BL/6J, sex, individually ventilated cage, filter-top cage, behaviour.**

## INTRODUCTION

Over the past decade, technology for housing laboratory animals has continued to evolve. Initially, to protect mice and workers from infections, filter covers (i.e. micro-isolators) were introduced on static conventional cages (i.e. filter-top cages: FILTER). However, as $CO_2$ and $NH_3$ levels rise significantly in these cages within a short time (Lipman et al., 1992; Krohn and Hansen, 2002), more recent developments have resulted in the employment of individually ventilated cage (IVC) systems. These IVC systems produce a cage environment different to FILTER in regard to airflow, noise levels within the cage, and frequency of cage changes (Mineur and Crusio, 2009). For example, air changes ranging from 25 to 120 times per hour (Huerkamp and Lehner, 1994; Perkins and Lipman, 1996) and air speeds of at minimum 0.2 m/s at the animal level (Wu et al., 1985; Corning and Lipman, 1992; Lipman, 1999) have been reported for IVC cages. Furthermore, IVC designs vary significantly in terms of cage size, shape, internal structural complexity and the way the air is forced or drawn through the cage. Variations such as inlet vent size and position may impact significantly on air speeds within cages and the location of fans in positively ventilated IVCs can impact on sound in the cages. These differences across IVC systems are significant: Krohn et al. (2003) found that rats kept in cages with high air changing rates developed a place aversion to those cage environments. Furthermore, IVC systems limit the interchange of olfactory and acoustic cues between rodents across cages. This restricted sensory input from the outside potentially represents a form of isolation housing (Hawkins et al., 2003). Most IVCs also provide less climbing opportunities than conventional cages (Kallnik et al., 2007), although such differences could be eliminated by adding environmental enrichment. Finally, IVC systems could be a source of cage rack vibrations (Mineur and Crusio, 2009). This is dependent on the type of air supply and exhaust ventilation system used. Some IVC rack types use the central heat ventilation air conditioning system of the animal room to supply and exhaust air from the cages (passive ventilation system), whereas fans of other IVC systems are positioned on

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*Abbreviations:* ANOVA, analysis of variance; CS, conditioned stimulus; EPM, elevated plus maze; FC, fear conditioning; FILTER, filter-top cages; IVC, individually ventilated cage; MK-801, dizocilpine; NeuRA, Neuroscience Research Australia; NMDA, *N*-methyl-D-aspartate; OF, open field; PPI, prepulse inhibition; RM, repeated measures; SI, social interaction; US, unconditioned stimulus; YM, Y-maze.

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the IVC rack itself or are located next to the rack (active ventilation systems).

Importantly, it is not known in detail whether housing mice in IVCs to maintain optimal hygienic conditions impacts on baseline and drug-induced behaviours compared to mice kept in FILTER. To our knowledge, only two studies have investigated the consequences of IVC systems on mouse behaviours in some detail and reported strain- and sex-specific effects on motor activity, anxiety-related behaviours, (fear-potentiated) startle response, spatial memory and anhedonia (Kallnik et al., 2007; Mineur and Crusio, 2009). Despite these initial findings and the knowledge that physiological, neurological and behavioural phenotypes are affected by even minor environmental factors (Crabbe et al., 1999; Bohannon, 2002; Benaroya-Milshtein et al., 2004; Champy et al., 2004; Karl et al., 2007), IVCs have become an alternative animal husbandry system for an increasing number of commercial animal suppliers as well as large research institutes and universities. This development is probably based on the advantage of IVC systems over more conventional housing solutions in regard to hygienic standards and holding capacities (Hoglund and Renstrom, 2001). Researchers even use IVC systems as the control housing condition when investigating the impact of particular housing factors on experimental animal models (Blottner et al., 2009). Unfortunately, most experimental mouse studies fail to indicate what cage type was used. This is problematic for data comparisons across institutes where different cage systems (i.e. IVC or FILTER) are found. Furthermore, experimental mice might have been housed in both IVC and FILTER cages before testing, as large animal suppliers nowadays commonly breed and raise commercially available mouse strains/lines in IVC racks whereas the holding facilities of the particular research institute might only facilitate FILTER (Hoglund and Renstrom, 2001).

Thus, understanding the differences between IVC and FILTER housing more comprehensively is important to enable comparability and reproducibility of data across research facilities. We decided to use a comprehensive multi-tiered phenotyping strategy to test the behavioural consequences of IVC and FILTER housing in male and female C57BL/6JArc mice. We also included an acute drug challenge to clarify for the first time if IVC systems influence the response of mice to a pharmacological challenge. Importantly, the potential change in housing conditions between commercial breeding facilities (i.e. IVC) and small research institutes (i.e. conventional housing) has been portrayed in the current study.

## EXPERIMENTAL PROCEDURES

### Animals

Test mice were age-matched adult (5 months) male and female mice on C57BL6/JArc background. Animals were Specific Pathogen Free animals and were screened on a quarterly basis using a dirty bedding sentinel system. Mice were bred and group-housed (2–4 animals per cage) at the Australian BioResources (Moss Vale, Australia) in either FILTER [Type 1144B; Tecniplast, Rydalmere, Australia; dimensions: 33 (L) × 15 (W) × 13 cm (H)] or IVC [Type Mouse Version 1; Airlaw, Smithfield, Australia; dimensions: 37 (L) × 11 (W) × 15 cm (H); air change: 90–120 times per hour averaged across all cages within one rack system; air speed: 0.12 m/s; passive exhaust ventilation system using the central heat ventilation air conditioning system of the animal room; air was drawn through a filter located on the lower part of the front wall of the cage and was exhausted by a filter located on the top of the cage near the rear of the cage]. IVC cages contained no wire lid but a wire hopper, which was suspended from the lid (giving the animals some limited vertical climbing opportunities). Both cage systems were located within the same holding room and the same animal caretaker changed all cages once a week. Importantly, this housing strategy ruled out differences in holding room characteristics and animal maintenance as confounding factors. Two weeks before behavioural testing commenced all animals ($N$ = 12 mice per housing condition and sex) were transported to Neuroscience Research Australia (NeuRA) and group-housed (2–3 animals per cage) in conventional cages with a white opaque base and a wire lid (18M5; Mascot Wire Works Pty Ltd., Homebush, Australia). This guaranteed that all mice had to habituate to a new cage environment. Occasionally, male test animals had to be isolated due to high levels of intermale aggression (occurrence of fighting was not cage-specific). For animal welfare reasons, all cages at NeuRA were minimally enriched with certified polycarbonate mouse igloos (Bioserv, Frenchtown, NJ, USA), tissues as nesting material and a steel ring (Mascot Wireworks; diameter: 3 cm) in the cage lid. Mice were kept under a 12:12-h light: dark schedule [light phase: white light (illumination: 124 lx) – dark phase: red light (illumination: < 2 lx)]. Food (gamma-irradiated mouse breeding diet; Gordon's Specialty Stockfeeds, Yanderra, Australia) and water (filtered and treated with UV light and acidified to a pH of 2.5–2.8 using hydrochloric acid) were available *ad libitum*. Environmental temperature was automatically regulated at 21 ± 1 °C and relative humidity was 40–60%. Age-matched male A/JArc mice (Animal Resources Centre, Canning Vale, Australia) were used as standard opponents in the social interaction (SI) paradigm. All research and animal care procedures were approved by the University of New South Wales Animal Care and Ethics Committee and in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

### Behavioural phenotyping

Animals were tested in a comprehensive battery of behavioural tasks relevant to locomotion, exploration, anxiety, cognition, sensorimotor gating, and social behaviours (Crawley and Paylor, 1997; Crawley, 1999; Karl et al., 2003). The least aversive/disruptive tasks were carried out first (inter-test interval of at least 3 days): elevated plus maze (EPM), Y maze, SI, fear conditioning (FC), prepulse inhibition (PPI), and open field (OF) [baseline and following acute treatment with the non-competitive *N*-methyl-D-aspartate (NMDA) antagonist MK-801]. All devices were cleaned thoroughly with 70% ethanol in between trials and sessions. Testing occurred during the light phase (within 1–5 h of light onset) with the exemption of the EPM, which was performed during the dark phase (2 h prior to light onset).

*EPM.* The EPM measures locomotion, exploration, and anxiety-related behaviours. Mice were allowed to explore the EPM apparatus freely for 5 min (as described previously Boucher et al., 2007; Karl et al., 2008). Arm entries (when the mouse entered an arm with all four paws), time spent in arms, and the frequency of *head dipping* were scored for open and enclosed arms. Anxiety-related behaviours were examined by recording the time spent on open arms (open time) and open