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Do individually ventilated cage systems generate a problem for genetic mouse model research?

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Technological developments over recent decades have produced a novel housing system for laboratory mice, so-called 'individually ventilated cage' (IVC) systems. IVCs present a cage environment which is different to conventional filter-top cages (FILTER). Nothing is known about the consequences of IVC housing on genetic mouse models, despite studies reporting IVC-mediated changes to the phenotypes of inbred mouse strains. Thus, in this study, we systematically compared the established behavioural phenotype of a validated mouse model for the schizophrenia risk gene *neuregulin 1* (TM *Nrg1* HET) kept in FILTER housing with *Nrg1* mutant mice raised in IVC systems. We found that particular schizophrenia-relevant endophenotypes of TM *Nrg1* HETs which had been established and widely published using FILTER housing were altered when mice were raised in IVC housing. IVCs diminished the schizophrenia-relevant prepulse inhibition deficit of *Nrg1* mutant males. Furthermore, IVC housing had a sex-dependent moderate effect on the locomotive phenotype of *Nrg1* mice across test paradigms. Behavioural effects of IVC housing were less prominent in female mice. Thus, transferring the breeding colony of mouse mutants from FILTER to IVC systems can shift disease-relevant behaviours and therefore challenge the face validity of these mice. Researchers facing an upgrade of their mouse breeding or holding facilities to IVC systems must be aware of the potential impact this upgrade might have on their genetic mouse models. Future publications should provide more details on the cage system used to allow appropriate data comparison across research sites.

Keywords: Behaviour, data reliability, filter-top cages, genetic mouse model, individually ventilated cage system, laboratory housing, mouse mutant phenotype, neuregulin 1, schizophrenia, validity of mouse models

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Laboratory housing conditions of rodents used for research have evolved over the past decades to address work safety and animal welfare requirements. Furthermore, the dramatic rise in the use of genetic mouse models in medical research has put pressure on large animal facilities to provide space-efficient, cost-effective and safe (from a work health and safety perspective) housing solutions to an ever increasing number of genetic mouse lines. Technological advancements have produced individually ventilated cage (IVC) systems. Individually ventilated cage systems present a housing environment significantly different to the traditional filter-top cage (FILTER) housing, which is commonly found in smaller animal research facilities. In particular, the airflow in IVCs is increased (Baumans *et al.* 2002), noise levels (Mineur & Crusio 2009) and climbing opportunities (Kallnik *et al.* 2007) are decreased, and IVC systems using active ventilation can generate rack vibrations with unknown effects on laboratory mice (Kostomitsopoulos *et al.* 2012; Mineur & Crusio 2009). In addition, IVC systems limit the interchange of olfactory and acoustic cues between mice across cages, which may represent a form of isolation (Hawkins *et al.* 2003).

Importantly, mouse phenotypes are affected by even minor differences in environment or handling procedures (Bohannon 2002; Champy *et al.* 2004; Crabbe *et al.* 1999; Hurst & West 2010). Consequently, it is not that surprising that IVC systems change a number of phenotypes, including cognition and anxiety, in wild type-like inbred mouse strains compared to animals raised in FILTER housing (Kallnik *et al.* 2007; Mineur & Crusio 2009). Our own work has found that IVC housing also modifies the behavioural sensitivity of C57BL/6JArc mice to pharmacological manipulations (i.e. acute MK-801 treatment) (Logge *et al.* 2013). Thus, it appears very likely that IVC housing can also alter the phenotype of genetic mouse models, which have originally been established in mouse mutants kept in FILTER housing. Surprisingly, no study to date has examined the effects of IVC housing on genetically modified mice.

Here, our team systematically determined for the first time the behavioural consequences of IVC and FILTER housing systems in one of the best established and validated mouse model for the schizophrenia risk gene *neuregulin 1* (i.e. the heterozygous transmembrane domain *neuregulin 1* mutant mouse: TM *Nrg1* HET) (Duffy *et al.* 2010; Karl *et al.* 2007; Stefansson *et al.* 2002). *Nrg1* mutant mice were chosen based on our initial observation that upgrading our breeding facility to IVC systems seemed to impact on the reliability of particular endophenotypes of this model. We compared the behavioural phenotype of age-matched *Nrg1* mutant and wild type-like control male and female mice (TM *Nrg1* HET mice exhibit a sex-specific phenotype with the strongest *Nrg1*-environment interactions found in male

Table 1: Body weight [g] prior to OF testing, vertical activity (i.e. frequency of *rearing*) [*n*] in the peripheral area of the OF, time spent freezing [seconds] in the first 2 min of the conditioning trial of the FC task, and percentage distance travelled [%] in the novel arm of the YM in male and female *Nrg1* mutant mice (i.e. TM *Nrg1* HET) and control (WT) mice raised in FILTER vs. IVC housing

	FILTER		IVC	
	WT	TM *Nrg1* HET	WT	TM *Nrg1* HET
Body weight prior to OF				
Males	30.7 ± 0.4	30.0 ± 0.5	28.6 ± 0.5$^{\#\#}$	28.2 ± 0.6$^{\#}$
Females	23.3 ± 0.7	22.5 ± 0.4	22.4 ± 0.2	21.6 ± 0.4
OF: Vertical activity				
Males	28.2 ± 1.0	29.9 ± 1.4	29.1 ± 0.7	29.7 ± 1.1
Females	31.2 ± 1.0	29.0 ± 1.5	31.0 ± 1.1	32.0 ± 1.1
FC: Baseline *freezing* time				
Males	1.9 ± 0.6	0.4 ± 0.2	9.8 ± 4.3	4.7 ± 2.5^{+}
Females	1.3 ± 0.8	9.8 ± 8.5	0.3 ± 0.2	0.4 ± 0.3
YM: Percentage distance				
Males	46.6 ± 2.1	44.4 ± 2.6	44.2 ± 2.0	38.2 ± 2.0
Females	42.8 ± 3.7	45.4 ± 2.3	42.2 ± 2.6	43.7 ± 3.1

Significant Fisher-PLSD post hoc 'cage' effects vs. FILTER of the corresponding genotype are indicated by '#' ($^{\#}P < 0.05$ and $^{\#\#}P < 0.01$), a trend for a 'cage' effect in FC is indicated by '+' ($P = 0.09$).

mice only; Chesworth *et al.* 2012; Karl 2013; Long *et al.* 2010; O'tuathaigh *et al.* 2006), which were raised in either FILTER or IVC systems. All animals were kept in the same holding room and maintained by the same animal care taker. We used test protocols identical to our earlier published work in TM *Nrg1* HET mice (Duffy *et al.* 2010; Karl *et al.* 2011, 2007, 2003; Logge *et al.* 2013; Long *et al.* 2012a,2012b; Van Den Buuse *et al.* 2009).

Materials and methods

Animals and housing

Test mice were age-matched adult male and female heterozygous transmembrane domain *Nrg1* mutant mice (*Nrg1* HET) and wild type-like littermate control (WT) mice on C57BL6/JArc background (backcrossed for >10 generations; Stefansson *et al.* 2002). Mice were bred and group-housed (2–4 animals per cage) at the Australian BioResources (ABR: Moss Vale, Australia) in either FILTER (Type 1144B; Tecniplast, Rydalmere, Australia) or IVCs (Type Mouse Version 1; Airlaw, Smithfield, Australia; air change: 90–120 times per hour averaged; air speed: 0.12 m/second; passive exhaust ventilation system). Individually ventilated cage cages contained no wire lid but a wire hopper, giving the animals some limited vertical climbing opportunities. Both cage systems were located within the same holding room and the same animal care taker changed all cages once a week. Two weeks before behavioural testing commenced [testing commenced at postnatal day (PND): 178 ± 8 days] all animals ($N = 11–12$ mice for males and $N = 9–14$ mice for females per housing condition and genotype) 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) so that all mice had to habituate to a new cage environment. It is possible that mice kept in IVC cages at ABR experienced the change in housing conditions when being re-located to NeuRA in a different way compared to FILTER-housed animals (the latter cohort had to adjust to less dramatic changes to their housing environment). For animal welfare reasons, all cages at ABR had nesting material and NeuRA cages were minimally enriched with certified polycarbonate mouse igloos (Bioserv, Frenchtown, NJ, USA), tissues as nesting material and a steel ring (Mascot Wire-works, Homebush West, Australia; diameter: 3 cm) in the cage lid. Mice were kept under a 12:12h light:dark schedule [light phase: white light (illumination: 124 lx) – dark phase: red light (illumination: <2 lx)]; light phase 0830 h–2030 h. Food and water were available *ad libitum*. 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 limited number of behavioural tasks with relevance to schizophrenia (i.e. locomotion, exploration, response to a psychotropic drug, cognition, sensorimotor gating and social behaviours) and which have been shown to detect behavioural abnormalities in FILTER-raised *Nrg1* mutant mice (Duffy *et al.* 2010; Karl 2013; Karl *et al.* 2011, 2007; Long *et al.* 2012b; Powell & Miyakawa 2006; Van Den Buuse *et al.* 2009) (Table 1). The least aversive/disruptive tasks were carried out first (inter-test interval of at least 72 h): Y-maze (YM), SI, fear conditioning (FC), prepulse inhibition (PPI) and open field (OF) [prior and post 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; 0930 h–1430 h).

Y-maze

The YM assesses locomotor activity and short-term memory of context familiarity (i.e. recognition memory). The arms of the maze were equipped with different internal visual cues (Chesworth *et al.* 2012; Duffy *et al.* 2010). The YM test consisted of two trials with a 30-min inter-trial interval (ITI). The trial duration for training and test was 10 and 5 min, respectively. During training, one arm was blocked off (novel arm). In the test trial, all arms were accessible and mice were allowed to explore the apparatus freely. Entries were recorded for each arm using Any-Maze video tracking software, version 4.5 (Stoelting Co., Wood Dale, IL, USA). The total number of arm entries (i.e. locomotion) and the percentage of arm entries into in the novel arm were calculated.

Contextual fear conditioning

FC is a form of associative learning that occurs when a previously neutral stimulus (e.g. tone/context) elicits a fear response after it

has been paired with an aversive stimulus. On conditioning day, animals were placed in the test chamber (Model H10-11R-TC; Coulbourn Instruments, Whitehall, OH, USA). After 120 seconds, an 80-dB conditioned stimulus (CS) was presented twice for 30 seconds with a co-terminating 0.4 mA foot shock (unconditioned stimulus; US) of 2 seconds duration with an inter-pairing interval of 120 seconds. The test concluded 120 seconds later. On day 2 (context test), the animals were returned to the apparatus for 7 min. Time spent *freezing* was measured using Any-Maze (Chesworth *et al.* 2012; Duffy *et al.* 2010).

Social interaction

Observing a pair of unfamiliar rodents in a novel test environment can be used to measure social behaviours as well as anxiety levels. Test mice and age-matched A/JArc standard opponents of the same sex were placed in opposite corners of a grey perspex arena ($35 \times 35 \times 30$ cm^3) and allowed to explore the arena and each other freely for 10 min (Boucher *et al.* 2007; Long *et al.* 2012b). Tests were recorded using Any-Maze, and frequency and duration of the active sociopositive behaviours *sniffing*, *anogenital sniffing*, *allo-grooming*, *following* and *crawling over* the test mouse were scored manually by an observer blind to the test conditions.

Prepulse inhibition

PPI is an operational measure of sensorimotor gating, which is impaired in schizophrenia patients (Geyer & Braff 1987). Startle reactivity was measured using SR-LAB startle chambers (San Diego Instruments, San Diego, CA, USA). Animals were habituated to the chamber with a 70-dB background noise for 5 min, for 3 consecutive days prior to testing. The PPI test protocol consisted of 5 min acclimatization to 70 dB background noise, followed by 121 trials in a pseudorandom order using trials of 70 dB, 90 dB and 120 dB startle pulse trials and 96 prepulse (i.e. 74, 82 or 86 dB) presented at a variable interstimulus interval [ISI: 32, 64, 128 or 256 milliseconds prior to a startling pulse of 120 dB (PPI response)] to avoid ISI-specific effects. Percentage PPI (% PPI) was calculated as [(mean startle response – PPI response)/mean startle response] × 100. % PPI was averaged across ISIs to produce a mean % PPI for each prepulse intensity (Karl *et al.* 2011; Van Den Buuse *et al.* 2009).

Open field

General motor activity was evaluated as described previously (Karl *et al.* 2007) using an infrared photobeam-controlled open-field activity test chamber (MED Associates Inc., St Albans, WV, USA). The animal's 'horizontal activity' (i.e. distance travelled), ambulatory frequency, 'vertical activity' (i.e. *rearing*), time spent in ambulation and resting behaviour (no infrared photobeam-detectable movements) in the periphery and centre zone as well as the overall velocity were recorded automatically. Mice were tested for 15 min (baseline) before an acute i.p. injection of MK-801 (0.25 mg/kg body weight; injection volume: 10 ml/kg body weight) dissolved in saline (Livingstone Int Pty Ltd, Rosebery, Australia) (Van Den Buuse *et al.* 2009). Following the injection, animals were put back into the test chambers for another 45 min (MK treatment). Distance travelled (cm) and *rearing* frequency were measured.

Statistical analysis

Results were analysed using two-way analysis of variance (ANOVA: between factors: 'cage' and 'genotype') followed by Fisher-PLSD post hoc testing where appropriate (Karl *et al.* 2007; Long *et al.* 2012b). Data were separated for sex as previous research has shown a sex-specific phenotype of *Nrg1* mutant mice, in particular, when considering *Nrg1*-environment interactions (e.g. cannabis) (Boucher *et al.* 2007; Chesworth *et al.* 2012; Duffy *et al.* 2010; Karl 2013; Long *et al.* 2010; O'tuathaigh *et al.* 2006). Repeated measures (RMs) ANOVAs (FC: '1 min block') were used to control for successful learning over time and for locomotor effects over time (OF: '5 min block') and the *t*-test was used for the YM (to test for 'novel arm' preference). Analyses were conducted using STATVIEW 5.0. Differences were regarded as significant if $P < 0.05$. All data are presented as means ± SEM. Significant post hoc 'genotype' effects vs. WT mice of the same sex and corresponding housing condition are indicated by '*' ($^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$) whereas 'cage' effects vs. FILTER of the same sex and corresponding genotype are indicated by '#' ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$).

Results

All animals appeared to develop normally and no differences in breeding rate or litter size were detected (data not shown). However, the body weight prior to OF testing of male [$F_{1,39} = 15.5$, $P < 0.001$] and female [$F_{1,40} = 4.7$, $P < 0.05$] mice was affected by 'cage', as IVC mice had significantly reduced body weights (Table 1).

Locomotion and exploration

Male *Nrg1* HET mice showed increased locomotor activity (i.e. distance travelled across 5 min blocks) compared to WT mice during the 15 min baseline (i.e. drug-free) testing in the OF ($F_{1,43} = 18.3$, $P < 0.001$) and this hyper-locomotion was evident in both FILTER ($F_{1,21} = 8.4$, $P < 0.01$) and IVC ($F_{1,22} = 10.0$, $P < 0.01$) conditions (Fig. 1a). Repeated measure ANOVA detected a 'genotype' ($F_{1,40} = 6.1$, $P < 0.05$) as well as a 'genotype' by 'cage' interaction ($F_{1,40} = 9.6$, $P < 0.01$) for female mice. Hyper-locomotion was only evident in female mutants kept in IVC cages ($F_{1,19} = 12.6$, $P < 0.01$; Fig. 1b). At baseline, no effects of 'genotype' or 'cage' on vertical activity (i.e. *rearing*) were found for either sex (all P's > 0.05; Table 1).

The hyper-locomotive phenotype was not consistent across test paradigms, as the main effect of 'genotype' on total distance travelled of males in the YM ($F_{1,40} = 4.6$, $P < 0.05$) was only evident in mice raised in FILTER but not in IVC systems (trend for a 'genotype' by 'cage' interaction: $F_{1,40} = 3.1$, $P = 0.09$) (Fig. 1c). Females did not show a hyper-locomotive phenotype in the YM ($P > 0.05$; no interaction; Fig. 1c).

Cognition

Baseline *freezing* levels in the first 2 min of the conditioning for both male and female mice were not significantly different between genotypes (all P's > 0.05; Table 1). There was a significant two-way ANOVA effect of 'cage' in male mice: males raised in IVCs spent significantly more time *freezing* than mice of FILTER housing (males: $F_{1,42} = 5.5$, $P = 0.02$ – females: $P > 0.05$; Table 1). In the contextual FC paradigm, two-way ANOVA detected a significant 'genotype' effect on total time *freezing* for male mice ($F_{1,42} = 5.4$, $P < 0.05$) and a trend for 'cage' ($F_{1,39} = 3.5$, $P = 0.07$) and 'genotype' ($F_{1,39} = 3.2$, $P = 0.08$) effects for female mice. Male *Nrg1* HET mice spent less time *freezing* than WT littermates, but only when animals had been raised in FILTER housing (Fig. 2a). Repeated measure ANOVA detected a significant '1 min block' by 'cage' interaction for male mice ($F_{6,252} = 4.0$, $P < 0.001$). Further analyses showed that IVCs increased the *freezing* response of male WT ($F_{6,126} = 3.1$, $P < 0.01$) but not *Nrg1* HET mice ($P > 0.05$) to the conditioned context over time (Fig. 2b). In females, a trend for a significant '1 min block' by 'genotype' interaction was revealed ($F_{6,234} = 1.9$, $P = 0.08$) (Fig. 2c).

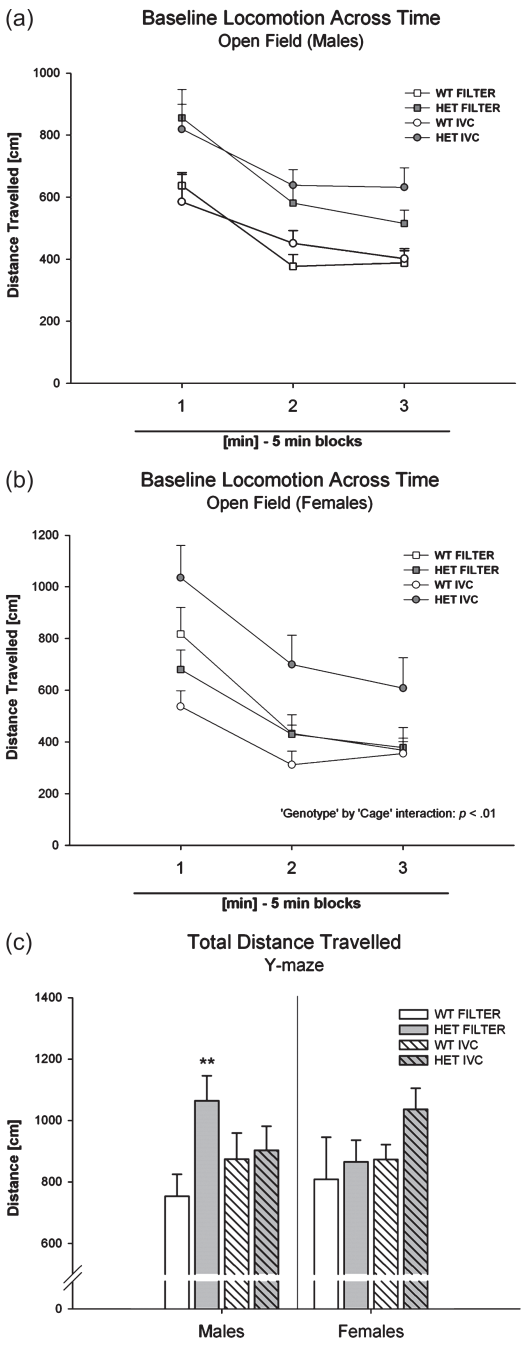

Figure 1: Locomotion in the OF and the YM. (a and b) Distance travelled [cm] in 5 min blocks across the first 15 min of OF testing (i.e. drug-free) for (a) male and (b) female mice and (c) total distance travelled [cm] of male and female mice in the YM are presented for *Nrg1* mutant (TM *Nrg1* HET: HET) and control (WT) mice raised in IVC or FILTER systems. Data are shown as means ± SEM. Fisher-PLSD post hoc effects of '*' vs. WT of the corresponding housing condition are indicated by '*' (** $P < 0.01$). Two-way ANOVA detected a trend for a 'genotype' by 'cage' interaction for male mice in the YM ($P = 0.09$).

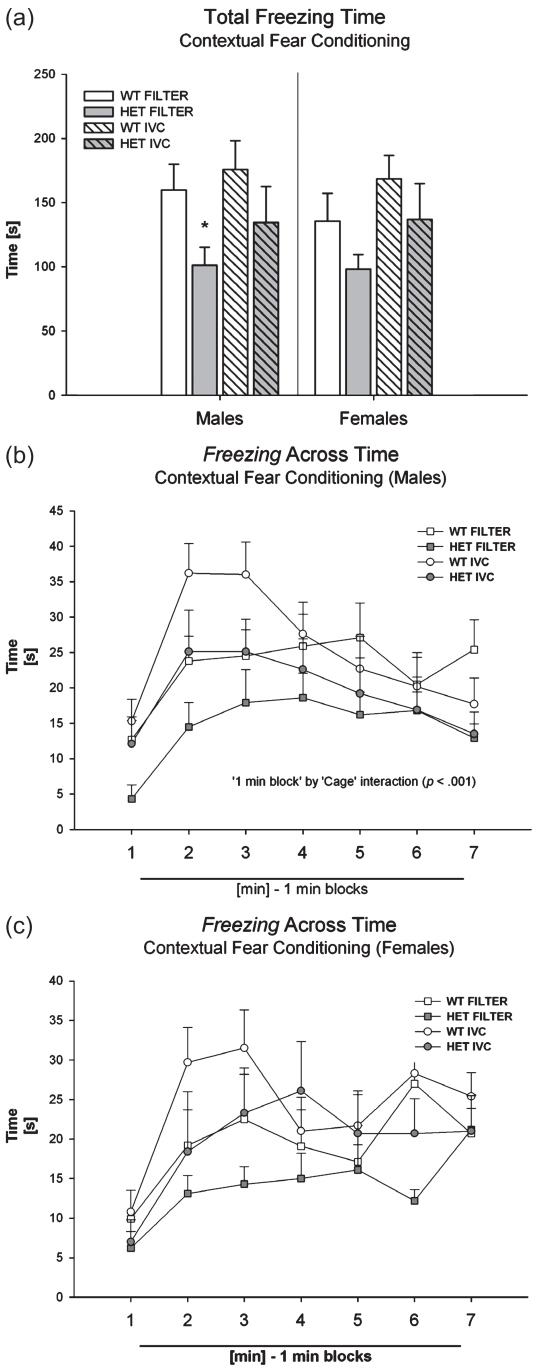

Figure 2: Contextual FC. (a) Total time spent *freezing* [seconds] and (b and c) total time spent *freezing* across 1 min blocks during context trial (context *freezing*) are presented for (b) male and (c) female *Nrg1* mutant (TM *Nrg1* HET: HET) and control (WT) mice raised in IVC or FILTER systems. Data are shown as means ± SEM. Significant Fisher-PLSD post hoc effects of 'genotype' vs. WT of the corresponding housing condition are indicated by '*' (* $P < 0.05$). RM ANOVA detected a trend for a significant '1 min block' by 'genotype' interaction for female mice ($P = 0.08$).

In the YM, all mice developed a significant preference to explore the novel arm (i.e. percentage distance travelled above 33.3%) compared to the familiar arms of the YM [males: $t(43) = 8.8$, $P < 0.001$ – females: $t(43) = 7.5$, $P < 0.001$; Table 1]. None of the test conditions affected the short-term recognition memory of mice (i.e. no main effects: all P's > 0.05).

Social behaviours

Two-way ANOVA revealed a main effect of 'genotype' for total time spent in active SI for male ($F_{1,42} = 4.8$, $P < 0.05$; no 'cage' by 'genotype' interaction) but not female mice ($P < 0.05$). It is interesting to note that male *Nrg1* mutants of IVC cages exhibited a strong trend towards increased SI (Fig. S1, Supporting Information).

Sensorimotor gating (PPI)

Two-way ANOVA for male mice detected a significant main effect of 'genotype' on the acoustic startle response (ASR) to a 120-dB startle pulse ($F_{1,37} = 15.1$, $P < 0.001$) and a significant 'genotype' by 'cage' interaction ($F_{1,37} = 16.0$, $P < 0.001$). In female mice, there was evidence for a 'genotype' effect ($F_{1,39} = 11.8$, $P < 0.01$) but no interaction. Male TM *Nrg1* HET mice raised in FILTER housing were characterized by a reduced startle response compared to WT animals, whereas no such difference was observed in mice bred in IVCs. The opposite was evident in female mice (Fig. 3a).

Statistical analysis found a significant effect of 'genotype' on PPI (averaged across prepulse intensities; $F_{1,37} = 11.7$, $P < 0.01$) and a significant 'genotype' by 'cage' interaction ($F_{1,37} = 4.6$, $P < 0.05$). No such differences were observed for female mice (all P's > 0.05) (Fig 3b). Importantly, male *Nrg1* HET mice exhibited a PPI deficit compared to WT littermates as published previously (Karl *et al.* 2011) but only when animals had been raised in FILTER housing.

Pharmacological intervention using MK-801

In line with one of our previous studies (Logge *et al.* 2013), IVC housing altered the sensitivity of all test mice towards the locomotion-stimulating effects of an acute dose of MK-801 compared to FILTER-raised mice (Fig. S2a,b). Repeated measure ANOVA revealed that acute treatment with MK-801 increased locomotion of test mice over time in male ('5 min block': $F_{8,344} = 224.1$, $P < 0.001$) and female ('5 min block': $F_{8,320} = 78.1$, $P < 0.001$) mice. ANOVAs also detected significant '5 min block' by 'cage' interaction for male mice ($F_{8,344} = 10.5$, $P < 0.001$) with IVC males being more sensitive to the locomotor-stimulating effects of MK-801 than FILTER mice across genotypes ('5 min block' by 'cage' for WT: $F_{8,168} = 5.0$, $P < 0.001$ – '5 min block' by 'cage' for *Nrg1* HET: $F_{8,176} = 6.1$, $P < 0.001$) (Fig. S2a).

Discussion

Raising *Nrg1* mutant mice and their littermate controls in IVC systems modified schizophrenia-relevant endophenotypes, which had been established and published using

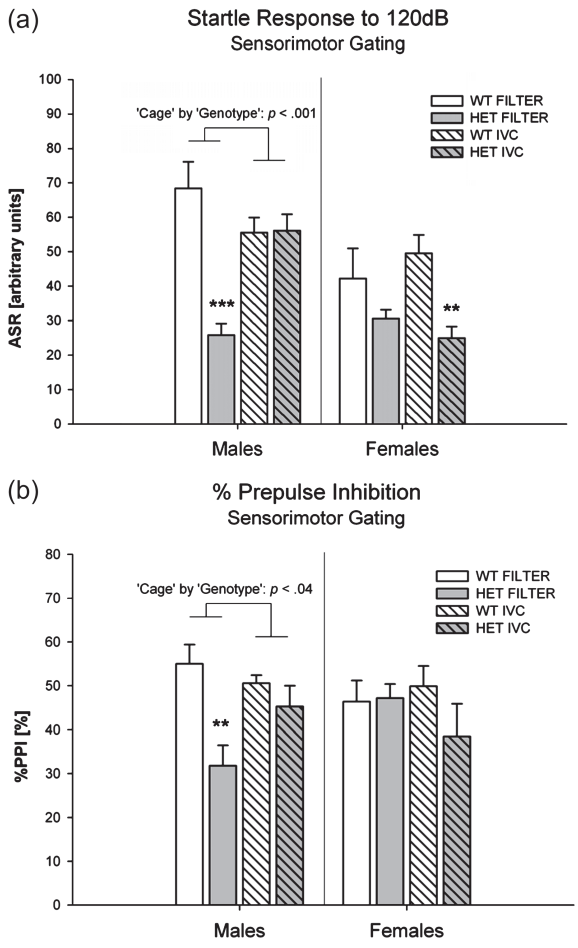

Figure 3: Startle response and sensorimotor gating (i.e. prepulse inhibition). (a) Acoustic startle response (ASR) to a 120 dB startle pulse (averaged across trials) (startle amplitude in arbitrary units) and (b) Percentage prepulse inhibition (%PPI) averaged across trials and prepulse intensities [%] are shown for *Nrg1* mutant (TM *Nrg1* HET: HET) and control (WT) mice raised in IVC or FILTER housing. Data are presented as means ± SEM. Significant one-way ANOVA 'genotype' effects vs. WT of the corresponding housing condition are indicated by '*' ($^{**}P < 0.01$, $^{***}P < 0.001$) whereas 'cage' effects vs. FILTER of the corresponding genotype are indicated by '#' ($^{\#\#\#}P < 0.001$).

TM *Nrg1* HET mice raised in FILTER systems. In particular, the hyper-locomotive phenotype of *Nrg1* mutant mice raised in IVC cages was sex-dependent. The most important finding of our study is the significant effect of IVCs on the startle response and the PPI deficit of *Nrg1* mutant males, which had been established in FILTER *Nrg1* mutants (Karl *et al.* 2011). Thus, IVC housing had a significant impact on particular schizophrenia-relevant endophenotypes and thereby challenges the face validity of this mouse model for the schizophrenia risk gene *NRG1*. However, other schizophrenia-relevant characteristics of the *Nrg1* mouse model such as cognitive and social behaviours were not severely affected by IVC housing.

Female *Nrg1* mutant mice were less sensitive to the effects of IVC housing across most behavioural domains including the behavioural response to MK-801. This phenomenon is probably related to the fact that TM *Nrg1* HET mice exhibit sex-specific phenotypes with pronounced *Nrg1*-environment interactions only found in male mice (Chesworth *et al.* 2012; Karl 2013; Long *et al.* 2010; O'tuathaigh *et al.* 2006). Nonetheless, only female *Nrg1* mutants of IVC housing exhibited a hyper-locomotive phenotype and Mineur and colleagues reported more pronounced effects of IVC housing on female behaviour as well (Mineur & Crusio 2009). These findings suggest that sex-specificity must be considered when evaluating the consequences of IVC housing on mouse model research.

Our behavioural findings are in line with the limited number or earlier studies on the effects of IVCs on mouse behaviour. Individually ventilated cage systems have been shown to modify the locomotor and anxiety response of inbred mice (Kallnik *et al.* 2007; Mineur & Crusio 2009), to alter the task acquisition in the radial arm maze (a paradigm testing spatial working memory), and to shift the fear-potentiated startle response of C57BL/6J mice (Kallnik *et al.* 2007; Mineur & Crusio 2009). The effects of IVC housing on acute MK-801 treatment reported here are identical to what our team observed in an earlier study using C57BL/6JArc mice (Logge *et al.* 2013) confirming the robustness and reliability of IVC effects in laboratory mice. Interestingly, our study is the first to discover an impact of IVCs on sensorimotor gating of mouse models. This finding is crucial for preclinical research into schizophrenia, where sensorimotor gating is one of the most commonly tested behavioural domains (Geyer & Swerdlow 1998; Karl *et al.* 2011; Van Den Buuse *et al.* 2009). Importantly, it has relevance beyond schizophrenia research, as sensorimotor gating is disrupted not only in schizophrenia but also in Tourette syndrome, bipolar, Huntington's disease and other illnesses (Geyer & Swerdlow 1998). Furthermore, other mutant mouse models of schizophrenia as well as transgenic mouse models of Alzheimer's disease and Huntingon's disease are susceptible to the effects of environmental enrichment (Lazarov *et al.* 2005; Mcomish *et al.* 2008; Van Dellen *et al.* 2000). It is likely that IVCs impact on these mouse models as well. Thus, the consideration of housing conditions is absolutely crucial for experimental animal research attempting to validate candidate genes (i.e. face validity) or new therapeutics (i.e. predictive validity) in mouse models for diseases such as schizophrenia or Alzheimer's disease (Gotz & Ittner 2008; Mazzoncini *et al.* 2009).

Past research has shown that changes to experimental procedures such as the handling of laboratory mice, which might appear of minimal importance to inexperienced animal researchers, can induce aversion and high levels of anxiety in laboratory mice and thereby impact on experimental outcomes (Crabbe *et al.* 1999; Hurst & West 2010). Furthermore, housing conditions play a crucial role in brain development, neurogenesis, synaptogenesis and epigenetic programming (Nithianantharajah & Hannan 2009; Sweatt 2009). Thus, it is not that surprising that housing laboratory mice in different cage environments (i.e. FILTER vs. IVC) can lead to significantly different behavioural phenotypes. Importantly, even body weight development and food and water intake are influenced by IVC housing (Kostomitsopoulos *et al.* 2012) suggesting that IVCs not only affect complex behavioural domains (and brain processes involved) but also modify basic physiological parameters. Thus, considering the effects of cage systems on mouse model phenotypes is therefore not only relevant for experimental mouse research attempting to validate candidate genes or new therapeutics for neuroscience, but has a much broader relevance to the field of experimental mouse research.

A number of studies have shown that environmental enrichment (i.e. environmental complexity, cognitive stimulation) can reverse genetically induced neurophysiological and behavioural alterations in laboratory mice (Nithianantharajah & Hannan 2009; Nithianantharajah *et al.* 2004). Individually ventilated cage systems are likely to represent the other end of the spectrum (less stimulation and complexity compared to FILTER housing). Depending on the level of 'deprivation' experienced by the mouse model in question, IVCs might have a negative impact on the development of genetically induced differences in brain development and mouse behaviours. Interestingly, an earlier study in our laboratory found that minimal enrichment advances the onset of a schizophrenia-like phenotype in TM *Nrg1* HET mice rather than having beneficial effects (Karl *et al.* 2007). Thus, a more complex environment might not necessarily be beneficial for mouse models of diseases which are characterised by a pronounced interaction of genes and environmental factors or only when the complexity reaches a certain threshold (i.e. complex environmental enrichment protocols; Mcomish *et al.* 2008). On the other hand, a more deprived environment could induce a less severe disease phenotype.

Individually ventilated cage cages 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 based on the advantage of IVC systems over more conventional housing solutions in regard to hygienic standards, housing costs and holding capacities (Hoglund & Renstrom 2001). However, not all commercial suppliers breed and house all their mouse lines in IVC cages. Thus, it is essential that researchers check on the history of mouse cohorts purchased from commercial suppliers in the future. In this context, it is important to mention that laboratory mice actually develop an aversion towards high intra-cage ventilation, which is a characteristic of IVC housing (Baumans *et al.* 2002) and more specifically forced-air (i.e. active ventilation) IVC systems (Kostomitsopoulos *et al.* 2012). Together with our findings, this suggests that researchers should very carefully consider the type of housing condition used for their experimental test animals. Unfortunately, mouse studies commonly fail to even indicate what cage system was used for the experimental animals. This is problematic for data comparisons across institutes where different cage systems are utilized or where mice have been housed in both IVC and FILTER cages (i.e. large animal suppliers commonly breed and raise mouse strains/lines in IVC racks whereas holding facilities of smaller research institutes facilitate FILTER cages; Hoglund & Renstrom 2001). Within this context, it should be noted that there is growing evidence that the standardization of laboratory environments might produce experimental results which are idiosyncratic

to the study in which they were obtained and that systematic variation of genetic and environmental backgrounds might be needed to generate more robust and biologically relevant data (Richter *et al.* 2010, 2009, 2011; Wurbel 2000, 2002).

In summary, this is the first study demonstrating that IVC housing can shift the phenotype of a genetic mouse model, which has been established in mutant mice raised in FILTER housing. Data comparison between research facilities is quintessential for science. Thus, factors which are known to impact on experimental outcomes and vary between laboratories (such as the type of cage system used for housing laboratory mice) must be reported in detail, and their impact on mouse models should be explored carefully (Crabbe *et al.* 1999; Paylor 2009). Otherwise, data reliability of mouse model research across institutes (using different cage systems) and across generations of test animals (where animal facilities are 'upgraded' to IVC housing) will not be guaranteed and result in only limited mouse model validity across research sites.

Our study into the effects of IVC housing in genetic mouse models demands a number of follow-up studies comparing consequences of IVC and FILTER housing in additional *Nrg1* mutant mice (e.g. *Nrg1* overexpressing mice: Yin *et al.* 2013) and in other mouse models of brain disorders (e.g. schizophrenia, Alzheimer's disease, Huntington's disease) thereby considering disease-relevant pathophysiological (e.g. expression levels of Nrg1) as well as other parameters (e.g. endocrine system). It will be important to determine whether modifications to the IVC housing environment such as providing cage enrichment, lowering the intra-cage ventilation rate or changing the location of the air valves (Baumans *et al.* 2002) can counteract the effects of IVC housing described in the current study.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Figure S1: Social interaction (SI). Duration of active SI [seconds] with an age-matched A/J standard opponent is shown for male and female *Nrg1* mutant (TM *Nrg1* HET: HET) and control (WT) mice raised in IVC or FILTER housing. Data are presented as means ± SEM. A trend for a post hoc 'genotype' effect vs. WT was detected for IVC-raised animals and is indicated by '+' ($P = 0.06$).

Figure S2: MK-801-induced locomotion in the open field (OF). Overall distance travelled [cm] after an acute challenge with MK-801 for the following 45 min are shown for (a) male and (b) female *Nrg1* mutant (TM *Nrg1* HET: HET) and control (WT) mice raised in IVC or FILTER housing. Data are presented as means ± SEM.