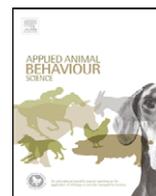




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## Leaving home: A study of laboratory mouse pup independence

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### ABSTRACT

Juvenile wild house mice leave their mothers at 8 weeks (+). In contrast, laboratory strains of mice (lab mice) are typically 'weaned' at postnatal day (PND) 21. Lab mice might mature faster than their wild forebears; but if they do not, standard laboratory weaning likely involves maternal deprivation. We therefore investigated when lab mice voluntarily leave their mothers. C57BL/6J families were housed in home cages (HC) each attached via a tunnel to an identical 'dispersal cage' (DC); tunnel-widths allowed pups but not dams to pass. For generality, we used two common cage-types: Ancare 'shoe-boxes' (28 cm × 19 cm × 13 cm) and Thoren 'duplexes' (30 cm × 14 cm × 14 cm). Measures of pup independence were recorded PND 16–35. Pups first visited the DC at PND 21.5 ± 0.35; utilised its food/nesting material at PND 22.6 ± 1.5; and DC-use increased up to PND 35. However, maternal care continued and even at PND 35, pups spent at least 50% of their time with mothers. This differed between cage-types: 'shoe-box' mice matured faster ( $P < 0.01$ ), becoming indifferent between the HC and DC by PND 35; while same aged 'duplex' mice preferred the HC, and received more maternal care (perhaps because they weighed less). Thus, similar to wild house mice, lab mouse independence occurs weeks after PND 21; independence is also plastic, affected by the local environment. 'Weaning' lab mouse pups at PND 21 therefore deprives them of maternal care, to an extent varying between different widely used cage-types. The impact this has on stress, abnormal behaviour, brain development, and inter-lab variation now needs investigating.

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### 1. Introduction

Laboratory strains of mice (lab mice) are arguably the most important model organisms in biomedical research today and tens of millions are bred and used annually worldwide (Malakoff, 2000). Despite this, and in contrast to rats (Berdoy, 2002), few empirical studies have compared their behaviour with their wild forebears, to identify whether discrepancies between laboratory housing and the life of wild mice cause welfare problems. This

is important because a fundamental understanding of the evolved behavioural biology of research animals can not only improve welfare (Latham and Mason, 2004), but also improve the validity with which they are used in the laboratory (Berdoy and Drickamer, 2007). Lab mice have been domesticated from wild "house mice"—largely the species variously termed '*Mus musculus*' or '*Mus domesticus*' (Berry and Bronson, 1992), with C57 strains also having small contributions from another closely related "house mouse", *Mus spretus* (Rikke et al., 1995). Ethological data on wild house mice can therefore be used to generate testable hypotheses regarding optimal husbandry procedures for lab mice, and factors that might impair normal behavioural biology in the lab (Latham and Mason, 2004).

One discrepancy between the lives of wild house mice and lab mice is the age at which young animals become permanently separated from their mothers. Lab mice are

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routinely separated from their mothers ('weaned') at 21 days. However, if permitted, wild house mice display mother–young interactions for days or even weeks beyond this age. For example, mother–young interactions, including suckling/nursing, can continue until 27–28 days (König and Markl, 1987; Mendl and Paul, 1990). The earliest estimated dispersal age for house mice in the wild is 40 days (see Fig. 6 in Berry and Bronson, 1992; Berdoy and Drickamer, 2007), although for wild house mice in the laboratory the earliest age was 52 days for males (mean: 80 days) and 56 for females (mean: 86 days; Gerlach, 1996). Furthermore, some young wild house mice, especially females, do not leave the mother at all, but stay in the natal territory long into adulthood (Gerlach, 1990, 1996; Pocock et al., 2005). Mouse development is very plastic (e.g. Latham and Mason, 2004), and so it could be that laboratory conditions, along with genetic changes via domestication, accelerates lab mouse maturation to enable leaving the mother much earlier than occurs in nature. However, two findings argue against this. First, lab mice are 'weaned' when they are c. 35% of their adult weight (A. Bechard, J. Roder, G. Mason, unpublished data), while for wild mice dispersing in experimental set-ups, emigrating young males average 75% of an adult male's weight (Gerlach, 1996). Second, lab mice are weaned before sexual maturity (which occurs at 4–7 weeks; Yoon, 1955; Berry and Bronson, 1992) while young house mice do not leave their mothers permanently until several weeks after sexual maturity (Gerlach, 1990, 1996). This suggests that standard laboratory 'weaning' may involve premature maternal deprivation.

We therefore investigated the preferred social environment of the maturing lab mouse pup. No previous research has been conducted on the natural independence of lab mice. To do this, we used a novel housing system that allowed young lab mice to choose between spending time in the home cage (HC) with the mother, or an identical distant cage (a 'dispersal cage': DC) which the mother could not access. Our objective was to determine the age at which young lab mice prefer to spend time in a distant cage not containing their mother. We also wanted to assess whether similar factors affect maturation and independence as they do in wild house mice. Previous studies of house mice suggest that nursing ceases when young are c. 9 g (by day 23; König and Markl, 1987; or a few days later in another study: Mendl and Paul, 1990), and mothers wean slow-growing (large) litters at a later age than fast-growing (small) litters (König and Markl, 1987). Thus we expected smaller pups (e.g. those in larger litters) to receive more maternal contact for longer. Coinciding with the decline of suckling, young house mice begin to leave with their siblings on short exploratory trips outside of the nest, running back to the safety of the nest between each progressively longer trip (Crowcroft, 1966; Pocock et al., 2005). These 'excursions', or temporary ventures away from the home site, are a type of 'quasi dispersal' (Pocock et al., 2005), and precede complete dispersal (the one-way movement of an individual to a new, non-overlapping home range: Stenseth and Lidicker, 1992). We therefore expected short visits away to the DC to occur in mice in their fourth week of age, with these visits gradually increasing in number and length as pups

matured. Finally, we also wanted to see if males spent more time away from the HC (or left it entirely) earlier than their sisters, as occurs in dispersing wild house mice (Gerlach, 1990, 1996).

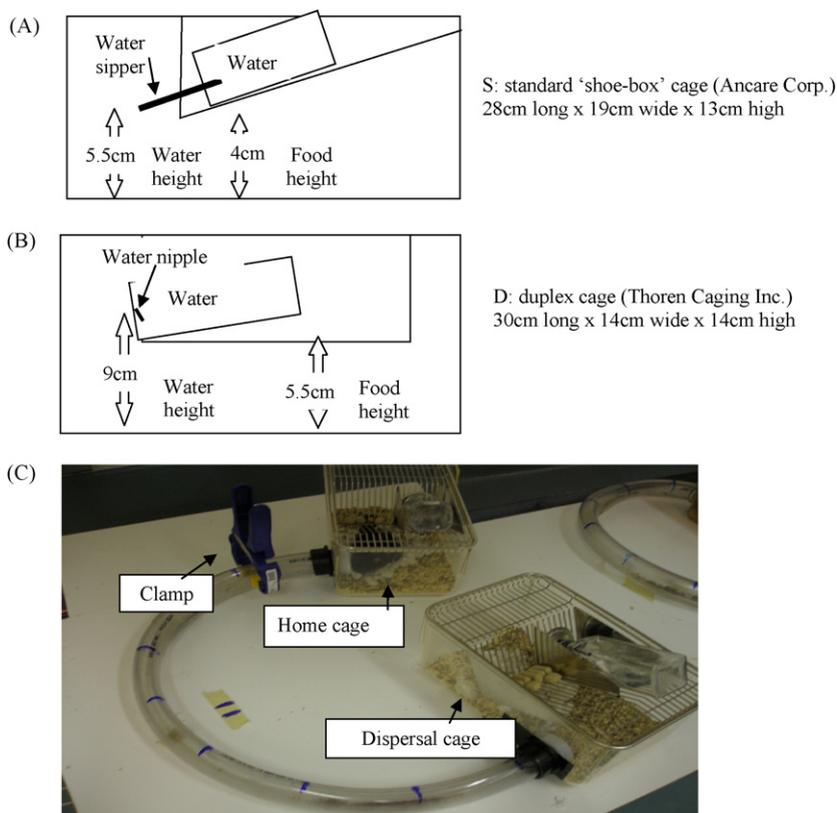
## 2. Materials and methods

### 2.1. Animals and weighing regime

C57BL/6J (B6) mice were chosen due to their widespread use in behavioural research and as a genetic background strain, and maintained inside a single temperature – ( $20 \pm 1^\circ\text{C}$ ) and humidity – (50–60%) controlled room, under a 12 h diurnal cycle. Virgin adult males and females were mated, and at first sign of pregnancy, individually re-housed. The day of birth was recorded as postnatal day (PND) 1. To allocate litters between treatments, litters were classified as small ( $N \leq 6$ ), medium ( $N = 7-8$ ), or large ( $N \geq 9$ ; cf. König and Markl, 1987). At PND 14, families were moved into a new housing system (details below). One male and female from each litter were selected as focal pups to investigate sex differences in pup behaviour, and identified by hair clipping and inked tail marks beginning at PND 14 and maintained at weekly intervals. Weights of all animals (including dams) were taken weekly across the observation period and, when possible, offspring were re-weighed as adults (aged over 4 months; adult sample size was reduced as some offspring became the subjects of another study). Identification of focal pups was no longer possible as adults, since by this time, marks had worn off.

### 2.2. Caging

Litters were initially bred in standard laboratory 'shoebox'-style (S) polycarbonate mouse cages (Ancare Corp., model: N10HT, Bellmore, NY, USA; see Fig. 1a for dimensions), which always contained bedding, nesting material, food, and water, replaced weekly. At PND 14, the approximate age of first eye opening (Sale et al., 2004), all families were moved into experimental dispersal caging, comprised either of S-cages, or duplex (D)-cages (Thoren Caging Systems Inc., model #3, Hazleton, PA, USA; see Fig. 1b for dimensions), counterbalanced by litter size. We used different cages which are typical of those in widespread use, since the designs of different cages (especially food hopper and drinker location) may cause variation in the onset of nutritional independence. This thus allowed us to investigate whether widely used cage designs can impact pup behaviour and physical development, our hypothesis was that the standard cages would accelerate growth because food and water were easier for pups to access. A dispersal cage (DC) was connected to each home cage (HC) by a tunnel (91.4 cm  $\times$  5 cm, made of PVC clear tubing; Fig. 1c), and offered offspring unlimited access to distant resources in a cage identical to the home cage, with bedding, nesting material, food and water, replaced weekly. An adjustable clamp positioned just outside of the HC was constricted to a point that pilot data determined would prevent the mother from leaving; however, despite this our sample size was still reduced from 20 to 17 litters ( $S = 9, D = 8$ ) as a result of improper clamp set-up. By PND 35, offspring had reached



**Fig. 1.** Cage design and dimensions. (a) Standard 'shoe-box' (S) cages had a triangular food hopper, a water sipper extending into the cage. (b) In duplex (D) cages, the food hopper was rectangular and higher, and the water nipple did not extend into the cage. (c) Dispersal caging comprised of standard (S) cages.

adult size, so the clamp no longer could allow pup entry while excluding the dams. Therefore, the experiment was terminated, and pups were weaned into single-sex cages ( $N=2-3$  per cage).

### 2.3. Methods used to investigate pup use of the dispersal cage (DC)

Every morning prior to the start of live observations on PND 16–35, the presence of faeces in the DC was checked for (note: statistical analyses use only the age at which faeces were first noticed in the DC), the DC food weighed, and the amount of food consumed there over 24 h calculated based on the previous day's weight. The percentage of the nestlet chewed in the DC (visually assessed from 100%: intact square to 0%: entirely shredded) was also recorded as a further "around the clock" indicator of offspring presence in and use of the DC.

Families were directly observed daily under dim red light from PND 16 until PND 35, at which time pups were separated by sex and placed in new cages. The observation period spanned the initial 2 h of the active (dark) period. Each family was observed once every 10 min, for a total of 12 observations per day, and final scores calculated as a daily percentage of observations. Behaviours were identified using a mixture of scan and focal sampling: a family was scanned initially to identify the mouse of interest

(e.g. dam), followed by focal instantaneous observation of that individual. The HC was watched for the behaviours described in Table 1. Dam presence on pups was measured when the dam was the focal animal, and analysed together with nursing and pup licking/grooming as one measure termed 'dam on pups'. The dam was scored as on top of her pups even if some of the pups were active outside of the nest. The activity and location of the entire litter were additionally recorded as a percentage of pups per cage. Mice spend the majority of time inactive, and so the place of rest is suggested to be the 'preferred location' (Blom et al., 1992) of a healthy animal. We therefore also assessed the amount of time pups spent inactive in the DC relative to the total amount of time spent inactive, as a measure of preference for the environment.

### 2.4. Assessing pup growth and initial use of the dispersal cage (DC)

Proc Mixed models, blocking by cage-type and interactions, with dam (a random factor) as the unit of replication, and age as the independent variable, were used to assess differences in weekly mean pup weight. The potential for litter size to interact with these factors was then assessed by re-running the model, including litter size as a covariate, and its interactions. General Linear Models (GLMs; SAS, 9.1) blocking for age, sex, and cage-type, were used

**Table 1**  
List of behaviours.

Behaviour	Description
Resting with pups	Resting (remains in a hunched posture, with eyes closed or hidden, for at least 3 s) in contact with pups (bodies touching, tails do not count)
Resting alone	Resting without contacting pups
Rest/avoid	Resting in active attempt to evade pups (e.g. nipples pressed to cage wall)
Active	General movement and maintenance behaviour (e.g. walking, feeding, and drinking)
<sup>a</sup> Active on pups	Dam is active while remaining on pups
Pup retrieval	Dam carries pup in her mouth
Nest building	Dam is moving or shredding nest material
<sup>a</sup> Licking pup	Dam's nose touches pup and head moves forward
<sup>a</sup> AGL	Anogenital licking: dam licks pup's anogenital region
Grooming off	Dam grooms while off pups
<sup>a</sup> Grooming on	Dam grooms while on pups
<sup>a</sup> Grooming pup	Dam grooms pup
<sup>a</sup> Nursing	Dam on pups extended dorsal side up, or pups observed attached to nipples (includes all nursing postures e.g. arched-back, blanket, supine)
Nursing gather	Dam gathers pups for nursing bout
Walk off	Dam walks off pups while they are attached to her nipples
Tunnel try	An unsuccessful attempt to pass through clamp into tunnel
Stereotypic	Invariant, repetitive (occurring three times without interruption) behaviour with no apparent function (e.g. bar-gnawing)
Suckling	Pup attached to nipple
Trying to suckle	Pup unsuccessfully attempts to attach to nipple
Other	Other behaviour: e.g. aggression, mounting

<sup>a</sup> Denotes behaviours that were included in the category termed 'dam on pups'.

to assess factors affecting the age at which pups initially used the DC, as indicated by presence of faeces, and first food and nestlet consumption. Additionally, these measures served as 'snap-shot' images of pup development to investigate effects of litter size, and so were re-analysed with litter size as a covariate; any significant interactions with cage-type were subjected to regression analyses split by cage-type.

### 2.5. Assessing pup use of the dispersal cage (DC) over time

The relationship between pup development and use of the DC over the ensuing days was assessed using Proc Mixed models, blocking by cage-type and interactions, with dam (a random factor) as the unit of replication, and age as the independent variable. Daily values were meaned over adjacent days to smooth data. Dependent variables included: % of observations active in the DC, % resting time in the DC, and % of DC food and nestlet use. To investigate results showing a significant interactive effect of cage-type by age (e.g. % of observations in the DC), age was replaced by pup weight as the independent variable indexing development. Two families were not weighed on all designated days, and were thus removed from relevant analyses. The data for the focal male and female from each litter were also analysed for sex differences in the total amount of time active in the DC, using GLMs blocking by treatment and sex. The model was split by sex, and then re-used to investigate whether focal animals were spending a similar amount of time in the DC as the whole litter, by including whole litter data as a covariate. Post hoc Tukey tests were used as supporting analyses for any significant interactions. Data that did not meet the model assumptions of normality were compared using non-parametric (Kruskal–Wallis) tests.

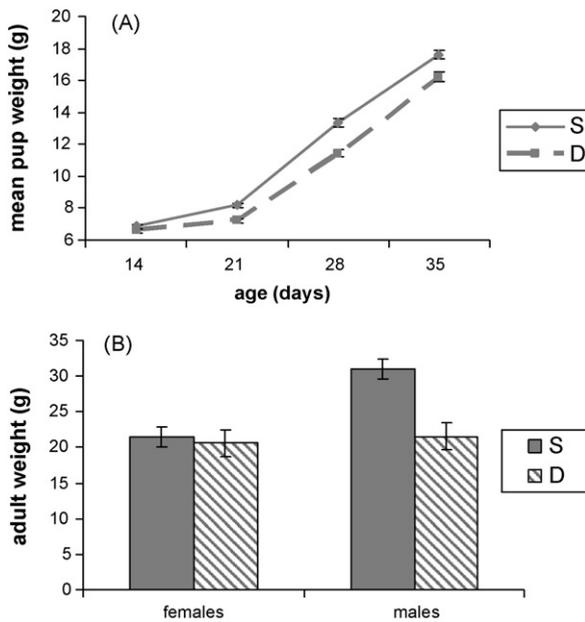
### 2.6. Assessing maternal care

Maternal behavioural changes with litter development were analysed using Proc Mixed models, as above. Maternal care was assessed both for changes over time (pup age); also for effects on this due to litter size and cage-type. To investigate the influence of litter size, data were re-analysed, now with litter size as a covariate. The total amount of maternal care was calculated (GLM), with litter size as a covariate, and blocking for cage-type and its interactions. Dam weight loss over time was analysed using repeated measures, again blocking by cage-type. All methods adhere to the guidelines of the [Canadian Council on Animal Care \(1993\)](#), and were approved by the University of Guelph Animal Care Committee.

## 3. Results

### 3.1. Pup growth and development

Overall, pups grew rapidly between PND 14–35: their body weight doubled between PND 21–35. Across PND 14–35, S-caged pups tended to be heavier than mice from D-cages, with effects for males persisting into adulthood ([Fig. 2](#)). As pups aged, litter size affected mean pup weight, such that as age increased, pups from smaller litters were heavier (Proc Mixed:  $P=0.031$ ,  $F_{3,87}=3.09$ ). The ratio of females to males within a litter ( $S=1.6\pm 0.13$  cf.  $D=1.68\pm 0.59$ ) and the total mean litter size ( $S=7.1\pm 0.45$  cf.  $D=7.0\pm 0.59$ ) did not differ significantly by cage-type (GLM: F:M:  $P=0.72$ ,  $F_{1,15}=0.13$ ; litter size:  $P=0.88$ ,  $F_{1,15}=0.02$ ) and so could not explain this effect. Regardless of location, mice younger than PND 21 spent less than 20% of their time engaged in active behaviour (e.g. ambulation, eating, drinking, grooming), instead the bulk of their time was spent huddled in the nest. Activity increased

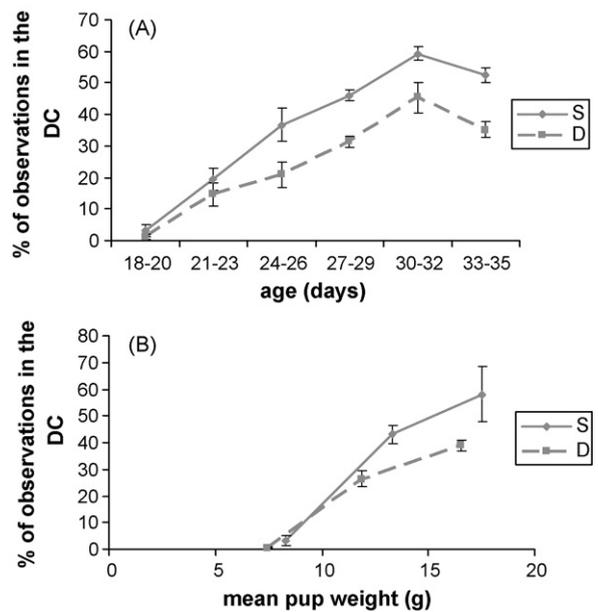


**Fig. 2.** Mean weights of offspring over time separated by cage-type (standard 'shoe-box': S; duplex: D). (a) Offspring weights taken weekly between days 14 and 35 suggest a slight reduction in mean pup growth due to cage design (Proc Mixed: age  $\times$  cage:  $P=0.054$ ,  $F_{3,101}=2.60$ ). (b) As adults ( $F$ :  $151 \pm 11$  days;  $M$ :  $148 \pm 11$  days), males raised in S-cages were heavier than males raised in D-cages (cage  $\times$  sex:  $P=0.006$ ,  $F_{1,5}=19.6$ , Tukey: S-M > D-M:  $P=0.033$ ). Plotted values are means  $\pm$  SEs.

with age (Kruskal–Wallis:  $P < 0.0001$ ,  $H_{5,83} = 50.8$ ), but was not affected by cage-type ( $P = 0.63$ ,  $H_{1,17} = 0.23$ ). Sexes were indistinguishable when assessing overall activity levels.

### 3.2. First use of the dispersal cage (DC)

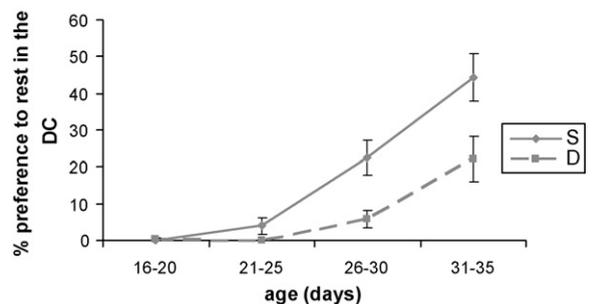
Initial pup exploration of the DC, as indicated by the presence of faeces, occurred at PND  $21.6 \pm 0.36$ . This was not influenced by cage-type (GLM:  $P = 0.70$ ,  $F_{1,15} = 0.15$ ). The age offspring began consuming food and nestlets in the DC was PND  $22.3 \pm 0.34$  and  $22.9 \pm 0.35$ , respectively; again this did not depend on cage-type (food:  $P = 0.69$ ,  $F_{1,15} = 0.16$ ; nestlet:  $P = 0.76$ ,  $F_{1,15} = 0.09$ ). Litter size did affect the ages at which these key stages were reached. As indicated by the presence of faeces, litter size interacted with cage-type to influence the age pups first visited the DC ( $P = 0.007$ ,  $F_{1,13} = 10.46$ ): S-caged mice from smaller litters visited the DC later (regression:  $P = 0.02$ ,  $F_{1,7} = 8.9$ ), although the same is not evident in D-caged mice. A similar interaction was found for the first food consumed in the DC (GLM:  $P = 0.033$ ,  $F_{1,13} = 5.71$ ), although no significant effect of litter size was found on the shredding of nestlets ( $P = 0.10$ ,  $F_{1,13} = 3.06$ ). Plotting litter size by cage-type for first faeces and food consumed revealed negative slopes in S-cages only (regression:  $P = 0.015$ ,  $F_{1,7} = 10.4$ ), such that smaller litters are later to visit/use the DC; with no such effects in D-cages.



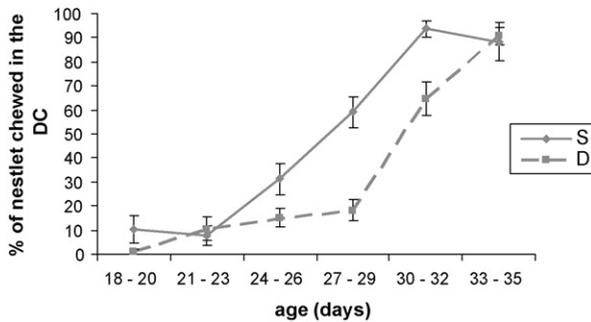
**Fig. 3.** Offspring total use of the dispersal cage (DC) shown as a percentage of observations. (a) Age and cage-type significantly influenced offspring use of the DC (Proc Mixed:  $P = 0.005$ ,  $F_{5,25} = 4.33$ ), with significant differences on days 24–26 (Tukey: S > D:  $P = 0.002$ ). (b) Pup weight predicted active time in the DC ( $P < 0.0001$ ,  $F_{1,29} = 77.6$ ), and using weight to predict behaviour also removed the significant cage-type influence (age  $\times$  cage:  $P = 0.06$ ,  $F_{1,29} = 3.82$ ; cage:  $P = 0.21$ ,  $F_{1,15} = 1.71$ ). Plotted values are means  $\pm$  SEs.

### 3.3. Behavioural measures of pup independence: effects of pup age, weight, cage-type, and litter size

Pups began spending an equal amount of active time in the DC as in the HC during their fourth or fifth week of life. However, the rate at which this increased as pups aged was affected by cage-type; as age increased, S-caged mice spent more time away (Fig. 3a). This cage-type effect was explained by pup weight: repeating the analysis using mean pup weight, instead of age, predicted time in the DC, and removed the significance of cage-type (Fig. 3b). All litters increased their preference to rest away from their mother with age, but S-caged mice spent more time resting in the dispersal cage (Fig. 4). The results for selected



**Fig. 4.** The amount of time spent inactive in the dispersal cage (DC), as a percentage of all time spent inactive. All litters increased their preference to rest away from their mother with age (Kruskal–Wallis:  $P < 0.0001$ ,  $H_{5,84} = 43.2$ ), however, S-caged mice spent more inactive time in the DC ( $P = 0.027$ ,  $H_{1,17} = 4.9$ ). Plotted values are means  $\pm$  SEs.



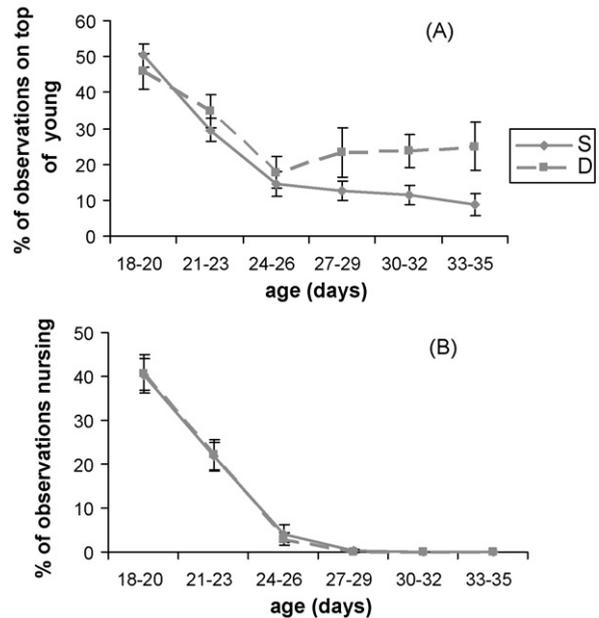
**Fig. 5.** ‘Consumption’ measures of independence. Nesting material in the form of a square nestlet was shredded in the dispersal cage (DC) increasingly with pup age (Kruskal–Wallis:  $P < 0.0001$ ,  $H_{1,83} = 50.5$ ). S-caged mice tended to shred more of the nestlet than D-caged mice ( $P = 0.083$ ,  $H_{1,17} = 3.0$ ). Plotted values are means  $\pm$  SEs.

female and male focal pups were similar to the picture for whole litters, in that they showed a very similar age by cage interaction (GLM: females:  $P = 0.005$ ,  $F_{1,14} = 11.2$ ; males:  $P < 0.0001$ ,  $F_{1,14} = 25.07$ ). This suggests that when 50% of the litter is in the DC, it really does mean that pups are spending 50% of their time there (rather than half spending all of their time there, and half spending none of their time there). Comparing focal females and males to each other revealed no sex differences in the active use of the DC ( $P = 0.72$ ,  $F_{1,30} = 0.12$ ). Litter size did however significantly interact with cage-type to influence pup resting behaviour in the DC across PND 33–35 ( $P = 0.031$ ,  $F_{1,11} = 6.14$ ). Further investigation showed a tendency for D-caged mice in larger litters to rest more in the DC ( $P = 0.082$ ,  $F_{1,6} = 4.36$ ), but this time, there was no litter size effect in the S-caged mice.

Overall, by PND 35, S-caged mice were away from their mother for 50% of the observations; D-caged mice were away only 35% of the time (see Fig. 3a). S-caged mice were spending an equal amount of time in the DC as the HC when mean pup weight was c. 17.0 g (S-caged mean pup  $g \pm$  SE: males =  $17.9 \pm 0.59$ , females =  $16.1 \pm 0.33$ ). In contrast, D-caged mice did not reach this weight by PND 35 (D-caged mean pup  $g \pm$  SE: males =  $16.3 \pm 0.63$ , females =  $14.5 \pm 0.50$ ).

#### 3.4. DC food and nestlet use as ‘24 h around the clock’ measures: effects of pup age, weight, cage-type, and litter size

Food and nestlet consumed in the DC served as indirect measures of offspring independence, and they validated our behavioural observations. For both cage-types, these 24 h-measures proved to be valid predictors of time measured in the DC (Regression: food: S:  $P < 0.0001$ ,  $F_{1,20} = 106.0$ ,  $R^2$  (adj.) = 83.3%; D:  $P < 0.0001$ ,  $F_{1,20} = 243.9$ ,  $R^2$  (adj.) = 92%; nestlet: S:  $P < 0.0001$ ,  $F_{1,20} = 105.7$ ,  $R^2$  (adj.) = 83.3%; D:  $P < 0.0001$ ,  $F_{1,20} = 33.5$ ,  $R^2$  (adj.) = 60.8%). In the DC, food consumed per pup always increased with age (Proc Mixed:  $P < 0.0001$ ,  $F_{5,27} = 38.2$ ), but was greater in S-cages ( $P = 0.006$ ,  $F_{1,16} = 9.76$ ). A similar result was found for the amount of nestlet shredded (Fig. 5). However, when pup weight, not age, was the predictor of resources consumed,



**Fig. 6.** Maternal care. (a) Dams raising their litters in duplex (D)-cages tended to spend more time on top of older pups than dams housed in standard (S)-cages (Proc Mixed: cage  $\times$  age:  $P = 0.090$ ,  $F_{5,24} = 2.18$ ). (b) Nursing was observed until c. 25 days ( $P < 0.0001$ ,  $F_{5,69} = 83.1$ ), and was unaffected by cage-type ( $P = 0.90$ ,  $F_{1,12} = 0.02$ ). Plotted values are means  $\pm$  SEs.

mice from S- and D-cages did not differ significantly (food:  $P = 0.74$ ,  $F_{1,13} = 0.11$ ; nestlet:  $P = 0.89$ ,  $F_{1,15} = 0.02$ ).

#### 3.5. Maternal care: effects of litter age, cage-type, and litter size

Behaviourally, at PND 21 dams still spent c. 35% of observed time on top of their litters. As pups aged, this decreased, although at a rate that tended to differ by cage-type (Fig. 6a). Regardless of cage-type, nursing was almost entirely absent by PND 25 (Fig. 6b). Mothers in S- and D-cages decreased in weight across PND 14–35, similarly (Proc Mixed:  $P = 0.72$ ,  $F_{1,14} = 0.13$ ) and significantly ( $P < 0.0001$ ,  $F_{3,33} = 115.0$ ), but weight loss was unaffected by litter size ( $P = 0.22$ ,  $F_{1,12} = 0.80$ ). Dams with larger litters provided more maternal care overall (GLM:  $P = 0.028$ ,  $F_{1,15} = 5.87$ ). No unwanted pregnancies or instances of within litter or mother-pup aggression were observed.

## 4. Discussion

Our results show that young laboratory mice mature in a similar way to wild house mice, with implications for improving their husbandry. Here we discuss how these data resemble or diverge from previous findings for wild house mice; the factors affecting lab mouse maturation; the potential implications of these findings for lab mouse welfare and behavioural normality; and finally, avenues for future research.

Our results for maternal care mirror those reported for wild house mice (König and Markl, 1987): at 21 days, C57BL/6j dams spent around one-fifth of the observation

period nursing their pups. Dams weaned their offspring gradually, without overt conflict, and by the time offspring were c. 10 g (c. PND 25), dams rarely appeared to be a nutritional resource. Mother-pup contact continued beyond this, however; thus as dams began spending less time on top of their litters, resting alongside of pups increased—just as reported by König and Markl (1987) for wild house mice, and continued until the end of the experiment at PND 35. As predicted, dams also devoted more time to maternal care if pups were small for their age (König and Markl, 1987; Mendl and Paul, 1990): thus in larger litters, and also in the 'duplex' (D)-cages. The mean age pups entered the dispersal cage (DC) was PND 22; however, the earliest family visited the DC at PND 18, around the same time as similar accounts of independent forays in wild house mice (17 days: König and Markl, 1987; 20 days: Mendl and Paul, 1990). Pups first started eating and using the nestlet there a day later, and as previously reviewed for wild house mice (Crowcroft, 1966), offspring excursions then increased in number and/or length (our data do not speak to which) such that the proportion of both active and resting time spent away from the mother increased. However, even by PND 35, when the pups were about twice the weight they were at conventional lab weaning age (PND 21), they still did not spend most of their time away from their dams. At this age (and a mean female pup weight of 16.5 g, a mean male pup weight of 18.5 g), young in the standard (S)-cages spent 50% of the observed time in the DC, suggesting indifference between this and the HC, but young mice in the D-cages, not yet at these bodyweights, spent only about one-third of their time in the DC.

The physical environment, in the form of cage-type, was thus clearly influential—one of several factors expected to affect how rapidly pups transition to independence. Litters diverged in body weight within a week of being moved to the two different cage-types, we suspect because food and water were harder for pups to reach in the D-cages. Thus, like wild house mouse development, laboratory mouse pup development was plastic and apparently affected by resource availability (reviewed by Latham and Mason, 2004). By PND 35, pups raised in D-cages were about 5 days behind S-caged mice in weight, about 10 days behind in terms of degree of maternal contact, and still showed a preference to be in the HC. Cage-type effects on behaviour generally became non-significant when pup weight replaced age, showing that behavioural differences between cages were typically 'side effects' of the growth rate differences. Litter size also affected pup independence, but in a manner often interacting with cage-type. As predicted by König and Markl (1987), larger litters received more maternal care over the course of the experiment, but they did not necessarily become independent later; in fact if anything the opposite was true. Thus larger litters first visited the DC at younger ages, at least amongst S-caged pups. This may reflect a greater competition for milk in larger litters, combined with the better accessibility of water and solid food in the S-style cages. During later observations, litter size also tended to positively correlate with the amount of time spent resting in the DC, at least for D-caged animals—perhaps because large litters were more crowded in the HC in these cages with smaller floor

areas. Pup sex, in contrast, had no detectable effects. We had predicted focal males would disperse earlier than focal females, but saw no such sex difference. However, early male dispersal has been suggested to be driven by agonistic interactions with the dominant male (Gerlach, 1996), and in our study, no adult males were present.

Overall, our results show that just as in wild mice, C57BL/6J mice are still dependent on their mothers at 21 days: conventional laboratory 'weaning' age. This is important because in many other mammals, premature maternal separation causes acute stress and predisposes animals to more anxiety and abnormal behaviour in adulthood (reviewed by Latham and Mason, 2008). In addition, nutritional deficiencies in developing offspring can affect growth and behaviour (Latham and Mason, 2004). Weaning mice at PND 21 thus probably compromises mouse welfare. Given the links between stereotypic behaviour and impaired functioning of the forebrain (Garner and Mason, 2002; Garner, 2005), it probably causes CNS dysfunction too.

Our data suggest that lasting behavioural differences may be found between adult mice weaned at 3 weeks and those weaned when older. Indeed the few studies to investigate this support this idea: compared to mice weaned at 21 days, Adriani and Laviola (2002) found that CD-1 mice weaned at 27 were less anxious adults, Curley et al. (2009) found decreased social neophobia in 28 day-weaned C57 mice, and Bechard (2007) found both lower adult anxiety and abnormal behaviour in 35 day-weaned lab mice. Future work using the dispersal apparatus which includes 21 day-weaned control mice could directly test for such behavioural differences. Our findings here also have implications for the reproducibility of research, by showing that pup development varies between two commonly used cage-types. This suggests that optimal weaning ages likely vary from facility to facility; thus pups in S-cages appeared indifferent as to where to be at PND 35, suggesting weaning at this age may be acceptable, but mice in D-cages were not. This also correspondingly suggests that the impact of weaning at a given age will similarly vary between facilities. Previous studies have already shown that pups that are lighter at weaning develop into more stereotypic adults (Würbel and Stauffacher, 1997, 1998). Our own work elsewhere (A. Bechard, J. Roder, A., Nicholson, G. Mason, unpublished data) also indicates that delaying weaning until PND 35 only benefits pups in a facility where they had successfully reached a mean weight of 17 g. These hypotheses now need direct test.

There are many other avenues for future work. One is to replicate this experiment using a more 'high-tech' approach, perhaps using implanted radio frequency identification (RFID) tags in an apparatus where 'chipped' individuals can open a door to the DC. This would allow the experiment to continue beyond PND 35, and thus to ascertain when individuals complete dispersal (i.e. do not return to the natal territory). The individual identification would allow us to see if early dispersers are heavier than their siblings (Gerlach, 1996), and also avoid potential effects on pup behaviour arising from repeated coat-clipping (or similar forms of marking). Other research questions pertain to the effects of the early social and phys-

ical environment, especially the means by which cage-type affects development; the effects of the presence of a dominant male (Gerlach, 1996); and the potential influence of a second litter being present, since in some commercial mouse breeding set-ups, females whelp every 3 weeks (for interesting studies of overlapping litters in gerbils, see Waiblinger and König, 2004; in rats, see Uriarte et al., 2008). Pups may have returned to the dam for thermoregulatory purposes; thus, future studies which provide a ready-made nest in the DC for pups to use may encourage earlier independence. Future work could also include systematically varying food and water accessibility, to address the potential underlying causes of our cage-type effects. Finally, this work should be repeated using different strains, since genotype can affect natural weaning age in wild mice (Mendl and Paul, 1990), lab mouse strains mature sexually at different ages (Berry and Bronson, 1992), and strains vary in how close they are to adult weight at conventional weaning age (see Fig. 18 in Cunliffe-Beamer and Les, 1987).

## 5. Conclusion

An investigation into the behaviour of the maturing lab mouse pup reveals that just as in wild mice, C57BL/6J mouse pups are still dependent on their mothers at 21 days: conventional laboratory 'weaning' age. Weaning at this age is thus likely a mild form of maternal deprivation, and may compromise mouse welfare. In addition, lab mouse pup independence is affected by the local environment, and so the extent of maternal deprivation may vary between different widely used cage-types. The impact of this deprivation on stress, abnormal behaviour, brain development, and inter-lab variation now needs investigating.

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