

Clustered environmental enrichments induce more aggression and stereotypic behaviour than do dispersed enrichments in female mice

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ABSTRACT

Adding environmental enrichments to a previously resource-poor cage or enclosure can sometimes cause elevated aggression in socially housed animals, due to competition over the provided resources. Here, using female C57BL/6J mice, we investigated whether the way that environmental enrichments are distributed affects the risk of negative interactions between individuals and whether familiar siblings are less likely to compete than unfamiliar, unrelated animals. Twenty adult females were each subjected to four treatments in a 2 × 2 factorial design with familiarity to partner (familiar versus unfamiliar) and resource distribution (environmental enrichments clustered versus dispersed) as the main factors. The resources used were: running wheels, nesting material of two types, and wooden chewing sticks coated with peanut butter. The behaviour of each female was observed 18 times for 5 min per treatment during the week they were housed in the treatment. In addition, stress levels were measured after each treatment by corticosterone metabolites in the faeces. The results showed increased aggression ($P=0.035$) and stereotypic behaviour ($P=0.007$), and a trend towards higher rates of displacement of one mouse by another from resources ($P=0.057$), in the clustered environment compared to the dispersed environment. However, no effects of conspecific familiarity were found. Furthermore, the elevated aggression and stereotypic behaviour co-varied in the clustered treatment in a way not seen in our distributed condition, suggesting common underlying causal factors. However, we could not detect any treatment effects in the stress level measured. Our results suggest that physically arranging valuable resources like enrichments in a dispersed way, so that they are easy to share and hard to monopolize, is better for welfare than clustering.

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1. Introduction

Making the social and physical environments of captive animals more complex or naturalistic is widely recognized as a potential means of improving welfare. As a result, in recent years, the social housing of domesticated farm and laboratory animals has become more common (e.g.

Broom and Fraser, 2007), as has the provision of environmental enrichment. However, social housing brings with it the risk of negative interactions, such as competition over potential, present or future resources. As a result, adding environmental enrichments to a previously resource-poor cage or enclosure can sometimes cause elevated aggression in socially housed animals (e.g. Nevison et al., 1999 on laboratory mice; Honess and Marin, 2006 on laboratory primates; Young, 2003 on zoo animals). Here, using laboratory female mice as a model, we investigate whether the way that enrichments are distributed affects this risk

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(especially whether or not they are easy to monopolize); whether familiar siblings are less likely to compete than unfamiliar, unrelated animals; and how these two aspects of the social and physical environment interact.

Whenever competition increases the access or utilization of a valuable resource (i.e. the resources are depleted by use, and/or are monopolizable), the potential benefits of engaging in competitive interactions will be high and aggressive interactions are expected (Arnott and Elwood, 2008; Enquist and Leimar, 1987; Grant and Guha, 1993; Maynard Smith and Parker, 1976; Milinski and Parker, 1991). For example, Grant and Guha (1993) found that dominant fish monopolized food with spatial clustered resource distribution and that the frequency of aggression increased with clustering the resources; while in farmed foxes, when the daily food is provided in a localized, monopolizable place rather than spread out in a dispersed way, higher levels of physical aggression occur around feeding time (Akre et al., 2010). Similarly, in mice (*Mus musculus*), the distribution of resources (food, water and nest-sites) within an enclosure affects 'resident intruder test' aggression in both sexes: residents are more aggressive in areas containing resources than in areas with no resources (Gray et al., 2002). These findings suggest that environmental enrichments will be least likely to induce competition and aggression in captive animals when they are non-depleting, and presented in a non-monopolizable way.

The severity of such aggression also might depend on the animals' relatedness and familiarity. According to kin selection theory, an animal should accept a kin more readily compared to a non-kin, due to the potential for inclusive fitness benefits (Hamilton, 1964; Maynard Smith, 1964), thus leading to lower aggression levels between kin. Thus female mice who whelp in a nest shared with their sister show a distinct reproductive advantage (being more successful at weaning young), compared with females who co-nest with non-sibs (Dobson et al., 2000). Furthermore, the existence of established dominance relationships between familiar individuals is also expected to reduce negative interactions (Maynard Smith and Parker, 1976). In nature, familiarity and relatedness are likely to be confounded for mice: they live in mixed-sex groups (demes), consisting of a dominant male, together with a number of related breeding and non-breeding adult females, and juveniles of both sexes (e.g. Krebs et al., 1995). As a result, one might expect less aggressive competition for resources between familiar sibs than between unfamiliar, unrelated mice.

This study therefore aimed to investigate the short-term effect of resource distribution and familiarity on different behaviours and physiological measures in caged female C57BL/6J mice. We used resources identified as highly valued in previous studies: a running wheel (Banjanin and Mrosovsky, 2000; Sherwin, 1998a,b; Sherwin and Nicol, 1996), manipulative nesting materials (Van De Weerd et al., 1997, 1998a,b; Van Loo et al., 2002), shelters (Van De Weerd et al., 1998a,b) and chewing blocks (Van De Weerd et al., 2002). We predicted that if these resources were clustered and therefore monopolizable, this would lead to more displacements of one mouse by the other, and more ago-

nistic encounters. We also predicted that these negative interactions would be more severe in mice caged with an unfamiliar, unrelated individual. Aggression, and having highly motivated behaviours frustrated, are both stressful experiences which could well diminish overall welfare; we therefore also predicted that social or physical environments that elevate competition should also increase stereotypic behaviour (e.g. Mason, 1991, 2006; Würbel, 2006) and corticosterone output (Hunt and Hambly, 2006; Möstl and Palme, 2002; Touma et al., 2003).

2. Materials and methods

2.1. Animals

Female mice C57BL/6J (*Mus musculus f. domesticus*) were housed with same-sex littermates ($N=2-3$ per cage) after weaning in standard Makrolon mouse cages (28.0 cm deep \times 18.0 cm wide \times 13.0 cm high) containing corncob bedding, a transparent red-tinted plastic nest house and shredded paper for nesting material, which was changed weekly. In three of the total 20 cages, the mice were housed in triplets until three weeks before the experiment started, where one random cage mate was removed and thereby all females were housed in sister pairs. All cages were provided with food pellets and water *ad libitum*. The mice were maintained on a reversed phase light: dark cycle of 12:12 h with light coming on at 10.30 am. During the dark period the room was illuminated with red light to allow direct observation. The mice were about 11 months at the beginning of the experiment; as pups they had all been used in a natural dispersal experiment (see Allison and Mason, 2010) and since then had been kept in standard facility conditions awaiting this experiment. All experimental procedures were approved by the Animal Care Committee of the University of Guelph and were conducted in conformity with the requirement of Ontario's Animals for Research Act, 1971 and the Canadian Council on Animal and Care (1995).

2.2. Experimental design

Two different housing environments were created based on differing resource distribution: one environment where resources were clustered and one where the resources were dispersed. Both environments consisted of two clear cages (29.0 cm deep \times 14.0 cm wide \times 15.0 cm high each) connected with a transparent PVC tube (7.0 cm long and 4.0 cm wide), see Fig. 1. Bedding, food (standard pellets in the cage lid hopper) and water were provided in both the cages. In the clustered environment, additional resources were positioned in one of the two cages; these were a running wheel attached to an igloo shelter (Fast-trac; Bioserv, USA), two treat sticks (two 1.2 cm \times 1.0 cm \times 8.0 cm wood sticks greased with peanut butter once a day), two pieces of nesting material (5.0 cm \times 5.0 cm NestletTM), and two pieces of paper tissue (6.0 cm \times 24.0 cm) provided at the cage lid. The location of the 'resource cage' in the clustered environment was always to the other cage's immediate right; however the cages were distributed evenly around the room (on three sides of a square) eliminating the possibility of system-

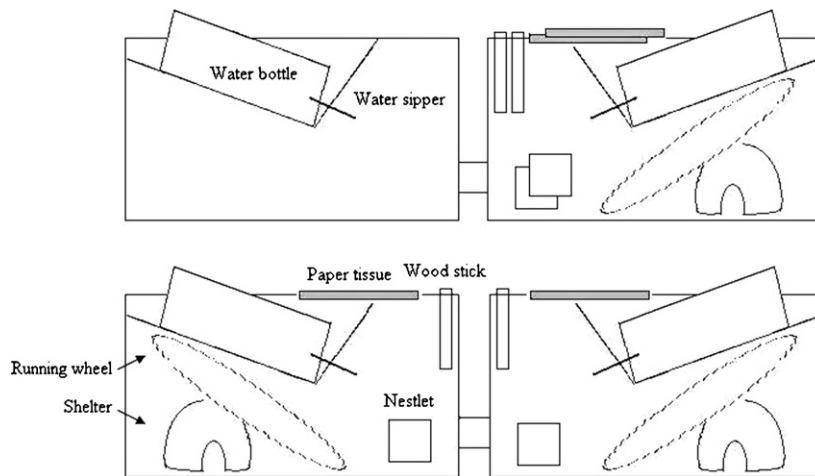


Fig. 1. Arrangement of the resources (wood stick attached to the cage lid, paper tissue provided at the lid, Nestlet and running wheel attached to a shelter) within the two types of resource distribution environment. 'Clustered' is shown in the top figure, 'dispersed' in the lower. All cages were provided with food and water *ad libitum* so that a subordinate animal could never be excluded from resources vital for survival.

atic side biases due to other external factors that might affect the cage preference. In the dispersed environment the added enrichments were distributed across both of the cages, so each cage had one treat stick, one Nestlet and one piece of paper tissue (see Fig. 1). Furthermore, both cages had a running wheel attached to an igloo shelter (Fast-trac; Bioserv, USA). Thus the amount of enrichment did not differ between the two types of housing environments, except for the running wheel/nest house which could not be divided in two for the dispersed environment, nor doubled up and placed in a single cage in the clustered environment (due to the small size of standard mouse cages). This last was a non-ideal feature of the setup (see Section 4), but this problem aside, the design of this apparatus was chosen deliberately for its potential to yield more clear cut results than might readily be measurable in a conventional cage (results which, if promising, could then be replicated in a standard cage). For one, this setup made it very clear when animals have chosen to be in the vicinity of the environmental enrichments (in a conventional cage one would have to estimate distances from enrichments to assess proximity). In addition, the narrowness of the plastic tube between the two cages could also in principle allow a single mouse to monopolize the resources, if she chose to, in the clustered environment, thus enhancing the potential effects of resource competition. Within these two housing environments the females were housed either with a familiar related female: a sib with whom they had lived with since birth; or an unfamiliar female: a new cage-mate from another litter, provided one day before each period of data collection. Thus overall there were four treatments: housed with a familiar, related female (hereafter called 'familiar' for brevity) in the clustered environment (Treatment 1), an unfamiliar, unrelated female (hereafter called 'unfamiliar' for brevity) in the clustered environment (Treatment 2), a familiar female in the dispersed environment (Treatment 3) or with an unfamiliar female in the dispersed environment (Treatment 4). Each female ($N = 20$) received all four treatments in a random order, in a new pair of cages each time. They were housed for six days in each

of the four treatments before their housing environment was changed. Before each treatment, the focal mouse was marked on the tail with a non toxic pen, to differentiate between the individuals in the pair.

2.3. Behaviours recorded

Behaviours recorded are shown in Table 1. Direct observations were made during the dark phase when the nocturnal mice are most active, on days two, three, four and five of each treatment. During these periods, behaviours were recorded by direct observations of each cage with focal animal sampling, using a one-zero sampling at 30 s

Table 1
Description of the behaviours recorded under the different treatments.

Behaviour	Descriptions
Stereotypic behaviour	Route tracing, bar mouthing, bar gnawing or gnawing at the metal grommet around the water nipple. The behaviour must be repeated at least three times in succession, or in the case of bar mouthing, sustained for at least 5 s (cf. e.g. Howerton et al., 2008). Barbering is not included, due to the difficulty of accurately observing this in our setup.
Wheel running	Running together or alone on the wheel.
Eat	Eating together or alone of the food pellets
Stick exploring/licking	Using or exploring the treat stick
Aggression	Offensive behaviours such as vigorous sniffing of head, tail or genitals of the female partner, tail rattling, chasing and fighting (e.g. Van Loo et al., 2002).
Displacement	Focal mouse displaces her female partner from a resource or another item she was investigating. Displacements from the wheel are not included, due to the extreme difficulty of accurately observing displacement from this resource.

intervals. Position in the cage was also recorded by instantaneous sampling at these 30 s intervals and the female was recorded as alone if she was alone in one of the cages and together if they were both in the same cage. The observations were recorded over 8 h (1100–1900) for 4 × 5 min on day two and day three, plus 5 × 5 min on day four and day five, resulting in a total of 90 min of observation (180 scores) per cage for each treatment. The time budgets performing each behaviour were calculated as percentage of active time (i.e. scans where the focal female mice were active and not lying down) because there were several inactive phases throughout the day. The order in which the cages were observed was randomized, with one constraint: the focal female in the cage had to be active when the observation period started.

2.4. Faecal collection for hormone assay

After each treatment, dry faecal samples (approximately forty faecal boli from one cage containing enrichments per treatment) were collected from the home cage for the measurement of glucocorticoid metabolites. This method measures the average glucocorticoid metabolites within each pair and throughout the day. This procedure was chosen as we were unable to separate between individual boli within the pairs. The dry faecal samples were then crushed and mixed, so that all the faecal pellets in each sample became a homogenous powder. From each sample, 0.05 g of this powder was mixed with 1 ml 80% methanol, shaken for approximately 1 min and then centrifuged (Touma et al., 2003). Afterwards 0.5 ml of the supernatant were transferred into a new vial and dried down. These samples were sent to the University of Veterinary Medicine in Vienna, Austria and analyzed for immunoreactive corticosterone metabolites (CM) using a 5 α -pregnane-3 β ,11 β ,21-triol-20-one enzyme-immunoassay (EIA) as described and validated for mice by Touma et al. (2003, 2004).

2.5. Statistical analyses

JMP (version 7 from SAS Institute) software was used for all statistical analyses. A General Linear Model (GLM) was performed to test the effect of treatments on the observed number of behaviours and corticosterone metabolite concentration. Mouse identity was defined as a random effect and resource distribution, familiarity and the interactions between these factors were the independent variables of interest. Homogeneity of variance and normality for all data were evaluated visually by diagnostic plots, and data was transformed where necessary. Effects were considered to be significant if $P \leq 0.05$ and results are expressed as mean values \pm SE. Results that were $0.10 < P < 0.05$ are presented as trends needing replication for confirmation. Despite our directional predictions, all P -values are two-tailed to be conservative. One focal female was excluded from the analysis of stereotypic behaviour because high frequencies of this behaviour made her a statistical outlier. To examine possible time differences in the females' position in the two available cages a paired t -test was used. The inter-relationships between stereotypic behaviour, aggres-

sion, and corticosteroid excretion were also analyzed using GLMs, with identity of mouse as a random effect, and experimental treatments as fixed effects as before, but with the addition of one continuous variable of interest and its interactions. A similar model was run to investigate relationships between time spent wheel-running and time spent stereotyping. Such models were non-orthogonal (due to the covariates), and so they were re-run using the sequential instead of the adjusted sums of squares, with the main effects in all possible orders. The same was done for the interaction terms (Grafen and Hails, 2002). Robust results from such analyses are presented as $F >$ or $F <$ rather than $F =$.

3. Results

When resources were dispersed and evenly spread over both cages, the active time the focal mice spent in each of the two cages did not differ ($t_{19} = -0.67$, $P = 0.51$). Equally unsurprising, given that we had chosen valued enrichments (see Section 1), when all the enrichments were clustered in one cage (the 'right cage'), the mice spent most of their active time in that cage ($t_{19} = -13.77$, $P < 0.001$). Presumably as a result of the resource distribution in the different environment, the focal mice spent significantly more time with their social partner (i.e. in the same cage) when housed in a clustered environment than when in a dispersed environment ($F_{1,19} = 80.84$, $P < 0.001$; see Fig. 2).

Much of their active time seemed to be spent using the running wheel; and mice with two wheels (dispersed) spent more time wheel-running ($F_{1,19} = 14.13$, $P = 0.001$), and were more likely to run alone ($F_{1,19} = 125.02$, $P < 0.001$), and less likely to run together with their social partner ($F_{1,19} = 21.76$, $P < 0.001$, see Table 2). The magnitude of this resource distribution effect on wheel-use was unaffected by whether they were with a familiar or an unfamiliar cage mate. Also, there was no main effect of familiarity to partner on running-wheel use (see Table 2). Unexpectedly,

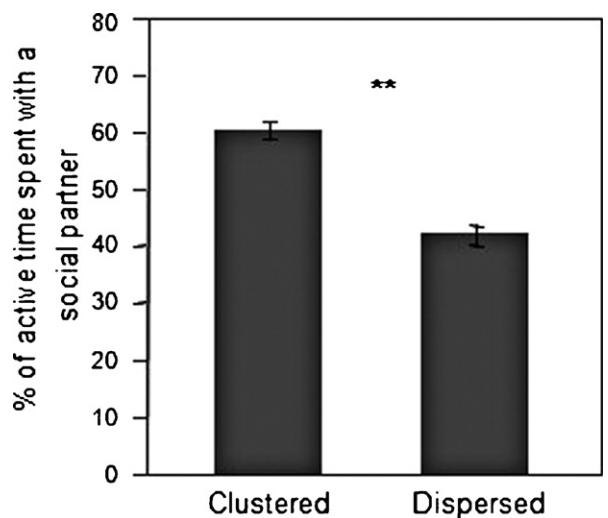


Fig. 2. Percentage of active time the focal females spent with their social partners (i.e. in the same cage) when housed in a clustered and a dispersed environment (means \pm SE), ** $P < 0.01$.

Table 2

The mean (\pm SE) percentage of active time spent on different behaviours and corticosterone metabolite levels per focal individual for the treatments during the total observation period.

	Clustered distribution		Dispersed distribution		P-value	Resource distribution	Familiarity	Interaction
	Familiar	Unfamiliar	Familiar	Unfamiliar				
Total time together	61.92 \pm 1.69	58.82 \pm 1.44	41.46 \pm 1.69	42.70 \pm 1.44	<0.001	NS	NS	
Total time on wheel	50.97 \pm 2.67	45.71 \pm 2.38	64.76 \pm 2.67	60.65 \pm 2.38	0.001	NS	NS	
Together on wheel	23.57 \pm 1.92	21.41 \pm 1.76	8.07 \pm 1.92	9.86 \pm 1.76	<0.001	NS	NS	
Alone on wheel	27.40 \pm 2.05	24.30 \pm 1.47	56.70 \pm 2.05	50.78 \pm 1.47	<0.001	NS	NS	
Stick total	2.93 \pm 0.36	3.63 \pm 0.38	2.64 \pm 0.36	2.47 \pm 0.38	NS	NS	NS	
Eat total	19.20 \pm 1.13	22.75 \pm 1.87	15.45 \pm 1.13	17.74 \pm 1.87	0.058	NS	NS	
Eat together	4.65 \pm 0.67	5.04 \pm 1.04	2.98 \pm 0.67	3.36 \pm 1.04	NS	NS	NS	
Eat alone	14.55 \pm 0.95	17.70 \pm 1.41	12.46 \pm 0.95	14.38 \pm 1.41	NS	NS	NS	
Aggression	0.55 \pm 0.07	0.41 \pm 0.10	0.08 \pm 0.07	0.30 \pm 0.10	0.035	NS	NS	
Displacement	3.19 \pm 0.34	4.36 \pm 0.60	2.34 \pm 0.34	2.76 \pm 0.60	0.057	NS	NS	
Stereotypic behaviour	3.64 \pm 0.46	4.21 \pm 0.67	0.71 \pm 0.46	1.05 \pm 0.67	0.007	NS	NS	
Cort. levels ^a	92.90 \pm 5.20	91.40 \pm 4.17	91.25 \pm 5.20	88.40 \pm 4.17	NS	NS	NS	

^a Corticosterone metabolites (ng/0.05 g).

the percentage of active time exploring/using the 'treat stick' was unaffected by whether the resources were clustered or dispersed (see Table 2); but the total time spent eating tended to be higher in the clustered environment ($F_{1,19} = 4.07$, $P = 0.058$, see Table 2).

Despite the overall greater time spent in close social proximity when in an environment with resources to compete over, and despite the decreased level of wheel running in this environment, there were no significant interaction effects of social and physical environments on competitive behaviours (see Table 2). Instead, competitive behaviours were determined by just a simple main effect of resource distribution: as predicted, clustered resources were associated with higher frequencies of aggression ($F_{1,19} = 5.18$, $P = 0.035$; see Fig. 3), as well as with a trend towards more displacement from resources of one mouse by another ($F_{1,19} = 4.12$, $P = 0.057$; see Fig. 4). There was also a corresponding main effect of resource distribution on stereotypic behaviour, with clustered resources inducing higher levels of this abnormal

behaviour ($F_{1,19} = 9.40$, $P = 0.007$, see Fig. 5). The increase in stereotypic behaviour in the clustered treatment was not an artifact of time-budgeting caused by the decrease in wheel-running: mice which showed the largest decreases in wheel-running in the clustered treatment did not show the largest increases in stereotypic behaviour (NS). There was a tendency for stereotypic behaviour to be predicted by aggression interacting with resource distribution ($F_{1,16} > 3.25$, $P < 0.091$). Splitting the dataset by resource distribution to investigate this interaction further showed that stereotypic behaviour and aggression positively co-varied in the clustered treatment ($F_{1,16} > 9.79$, $P < 0.007$) while not co-varying at all in the dispersed treatment ($F_{1,16} < 1.40$, $P > 0.25$).

Despite this effect of resource distribution on one welfare index, there were no treatment effects on corticosterone metabolite levels in the faeces (Table 2). Exploring these measures in more detail, aggression and excreted CM levels did not co-vary; nor did stereotypic behaviour co-vary with this hormonal index.

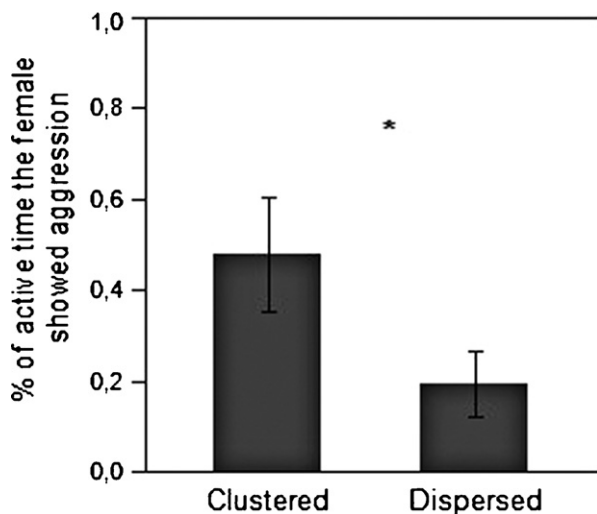


Fig. 3. Percentage of active time the focal females showed aggression when housed in a clustered and a dispersed environment (means \pm SE), * $P < 0.05$.

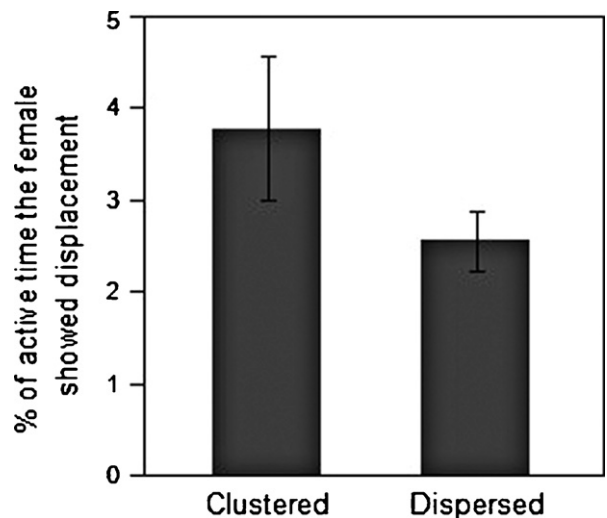


Fig. 4. Percentage of active time the focal females showed displacement when housed in a clustered and a dispersed environment (means \pm SE).

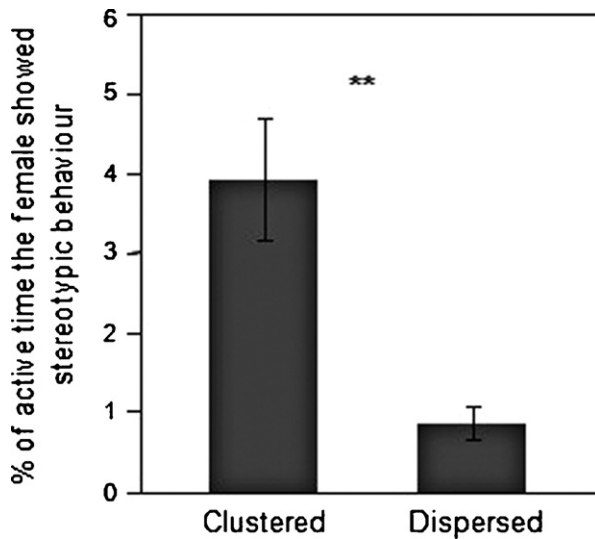


Fig. 5. Percentage of active time the focal females showed stereotypic behaviour when housed in a clustered and a dispersed environment (means \pm SE), ** $P < 0.01$.

4. Discussion

Our findings show that how environmental enrichments are distributed within a cage or enclosure can increase or reduce the risks of inducing stressful competitive interactions in socially housed animals. In particular, we found that our clustered treatment increased the rate at which mice displaced each other from resources, increased the number of aggressive interactions, and increased stereotypic behaviour. Furthermore, the elevated aggression and stereotypic behaviour co-varied in a way not seen in our distributed condition, suggesting common underlying causal factors (perhaps increased motivations to escape from the cage: see Nevison et al., 1999).

Our clustered treatment involved several attributes that could have played a role here. First, one resource was simply less available in this treatment: it involved only one running wheel, not two. As a result, females spent less time wheel-running in the clustered single-wheel treatment (cf. similar findings on the effects of wheel number on group housed mice by Vargas-Pérez, 2009), and a greater proportion of this behaviour comprised running in tandem with their cage mates. However, the increase in stereotypic behaviour observed was not simply a time-budgeting side-effect of spending less time on the wheel, and furthermore, the increased rate at which mice displaced each other was significant even though this measure did not include displacements from the wheel, only from those resources differing in distribution, not amount, across the two conditions. This suggests that resource distribution *per se* (not just numbers) did play a major role in the effects that we found. The second potentially relevant attribute of our clustered environment was that enrichments were all positioned in one cage reached by a tunnel, making the whole resource area potentially monopolizable. However, although such aspects of enclosure layout

may well be important in other set-ups, or for mice of other strains or sexes, or even in other species, in practice here this did not seem to cause the increased stereotypy and agonistic interactions observed in our experiment: our females spent more time together in the same cage in clustered treatments (not in separate cages, as would occur if one female became despotic over the resource cage), and furthermore, little aggression was seen at the tunnel connecting the two cages. We suspect that one major cause of our findings was a third attribute of the clustered treatment: that resources were more dense or spatially concentrated; this, combined with the evident preference both mice showed for being in the resource cage containing the enrichments, caused the mice to spend more time in close proximity to each other (and perhaps also caused the social facilitation of similar motivations: Clayton, 1978), so increasing the probability that both mice try to use the same enrichments at the same time. Whatever the precise underlying cause, which remains a topic for future research, these data do illustrate, for the first time, an important and little explored principle: that effects like resource monopolizability, depletability, density, number and value are all likely to modify the intensity of social competition that can be induced by adding environmental enrichments.

In terms of mouse husbandry *per se*, our data suggest that dispersed cage enrichments might better than clustered ones for socially housed female C57BL/6J mice in terms of lowering the incidence of competitive interactions. All aggressive and displacement responses observed in this study, even in the clustered treatments, were of low intensity, as was expected given that female mice largely cohabit peaceably. Nevertheless, our data show that social competition can increase stereotypic behaviour, even in a sex and strain of mouse that shows little overt aggression. Perhaps even more practically useful is that our data now suggest ways of mitigating unwanted aggressive behaviours in male mice. It is primarily male mice that fight over territories and social rank (Jennings et al., 1998; Palanza et al., 1994), and they can exhibit severe aggression when caged together (e.g. Van Loo et al., 2003), especially when provided with enrichments (e.g. see Howerton et al., 2008 on adding running wheels to cages of group-housed male CD-1 mice). Using females as our first model allowed us to conduct preliminary tests of our hypotheses without fear of morbidity, mortality or having to terminate the study due to excessive aggression. Also, the use of female mice in animal research is increasing, but males still dominate animal biomedicine studies (see Zucker and Beery, 2010). Thereby, having successfully demonstrated in principle that resource distribution affects enrichment-induced competition and its welfare implications, our results can now guide further experiments on males. It is generally recommended to house laboratory mice, even males, in groups (Council of Europe, 2007; Jennings et al., 1998), so optimizing how social and physical environments combine for best welfare is important and useful.

For future studies on this topic, on either sex, we suggest some improvements and modifications over the protocols used here. Contrary to our predictions, no treatment differences were found in corticosterone metabolites measured

in faeces – surprising since previous studies demonstrate a link between agonistic interactions and endocrine stress reactions (e.g. Haemisch et al., 1994; Marashi et al., 2003). However, we could not separate faeces from the focal female and her partner, nor could we identify the times of day at which our samples were produced (a time lag of 8–12 h occurs between corticosterone release in the bloodstream and peak concentrations of faecal CM: Touma et al., 2004). More accurate sampling, perhaps from animals temporarily separated by a divider, which allows, e.g. visual and olfactory contact (cf. Frynta et al., 2009; Latham, 2004) could solve these problems in future studies. Future studies investigating resource distribution in mice could also use bigger cages to enable large indivisible resources like, e.g. the highly valued running wheel (cf. e.g. Sherwin, 1998a,b) to be doubled up while still fitting in one cage (to standardize the number of resources available in each housing environment); and in addition, independently manipulate the properties of each enrichment (its size and/or divisibility) and how closely they are placed together, to tease out any effects of the local stocking density potentially caused by attraction to areas rich in resources.

The second aspect of our study concerned social relatedness and familiarity. Less aggression occurs between pairs of familiar female siblings than unfamiliar female pairs in wild house mice (e.g. Palanza et al., 2005), yet female mice in our experiment did not respond differently when housed with a familiar or a unfamiliar partner. One possible explanation is that abilities to discriminate between individuals have been disturbed by inbreeding (as has been shown in males: Nevison et al., 2000, 2003), or that familiarity is less important when competing for access to important resources.

Overall, this is the first study to our knowledge demonstrating that how environmental enrichments are distributed is important for socially housed mice. It appears that there is less effect of familiarity on behaviour in female mice, at least in this strain. Given this, one can conclude that clustering valuable resources in the home environment potentially causes some welfare concerns compared to environments with dispersed resources, due to more frequent negative interactions and more intense performance of stereotypic behaviour. It is perhaps ironic from a welfare perspective that the most valuable resources (thus most important for individual welfare) are the ones that are more likely to be fought over. Consequently, we would suggest that when valuable resources are given to socially housed female mice – or indeed other animals – they should be provided with a dispersed distribution, and in sufficient number, to reduce competition.

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