

Effects of Housing Social Context on Emotional Behaviour and Physiological Responses in Female Mice

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Summary

In laboratory breeding procedures, mice are usually housed in single-sex unfamiliar groups since weaning, while individual housing is widely employed in many experimental settings. While there is a considerable amount of evidence on the behavioural and physiological effects of various social contexts in male mice and rats, few data are available on female mice. We examined short-term modulation of social context in the housing environment on exploratory and emotional behaviours in response to novelty (i.e., free-exploratory open field) and on physiology (i.e. organs and body weight, and basal corticosterone level) of female CD1 mice, taking into account the estrous phase as an additional variable. Living alone or grouped with siblings or with unfamiliar females for a short period (7 days) did not affect any physiological indexes of stress in female house mice and had marginal effects on emotional behaviour. When challenged with a free choice between a novel environment and their home cage, female mice housed with siblings did not differ on any behavioural parameter from females housed with same-aged unfamiliar mice, while individually housed females showed higher propensity to enter the novel arena but no differences in activity or in anxiety as compared to grouped mice. Information about sex specifics under standard housing conditions as well as in response to common laboratory procedures could be important for the understanding of sex differences in vulnerability to psychiatric disorders and response to drug treatment.

Introduction

It is a common practice in the laboratory environment, to house animals, particularly mice and rats, under identical conditions, in order to maintain experimental control and reduce any effects of environmental differences. However, housing animals under particular social conditions, such as unfamiliar groups or isolation, may differentially affect males' and females' behavior and physiology (*Palanza, 2001; Palanza et al., 2001; Bartolomucci et al., 2004*). Much interest has been shown, and welfare guidelines devised, to define the optimal cage

size, physical environment and number of animals per cage according a species and age (*Poole & Robinson, 1987; Rodent Refinement Working Party, 1998*). Improvements have been suggested for environmental enrichment (e.g. *Wurbel, 2001*), but despite evidence of marked sex differences in social and aggressive behavior (*Parmigiani et al., 1989; 1999*), no specific comments have been provided by the Rodent Refinement Working Party (*1998*) on the issue of sex-specific social settings. In laboratory breeding procedures, mice are usually housed in single-sex unfamiliar groups since weaning, while individual housing is widely employed in many experimental settings.

There is a considerable amount of evidences describing in detail the behavioural and physiological effects of various social contexts in mice and rats, mostly focused on males (for review: *Bartolomucci et al., 2003; 2005*). Higher aggression and increased

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stress-related parameters between caged unfamiliar animals have been amply reported in rats and mice (for review see *Bartolomucci et al., 2005; Palanza, 2001; Sgoifo et al., 2005*). Reports on the possible stressfulness of isolation in rodents are ambiguous; there is little evidence of stressfulness of isolation *per se*, as many studies showed no endocrine changes in isolated animals as compared with group-housed ones (*Holson et al., 1991; Misslin et al., 1982*). We have shown that individual housing in itself does not change immunocompetence and corticosterone level of male mice, but does affect reactivity to a stressor (*Bartolomucci et al. 2003*). In fact, individually housed male mice showed high behavioral arousal, as well as altered immuno-endocrine parameters, when challenged with mild psychological novelty-stress. However, both anxiogenic-like (*Ferrari et al., 1998*) and anxiolytic-like (*Hilakivi et al., 1989; Rodgers & Cole, 1993*) effects of isolation have been reported in male mice. These contrasting findings are likely due to differences among different studies in the species, the laboratory strain and/or the sex utilized, as well as the duration and timing of isolation and handling procedures (e.g., *Brown & Gunenberg, 1995; Holson et al., 1991; Misslin et al., 1982; Brain & Benton, 1979*). However, psychosocial effects of isolation and/or grouping may be interpreted only by taking into account the “natural” social behavior of the species examined and inter-individual variability, such as sex- and/or age-related differences and individual experience.

Sexual selection theory (*Darwin 1871*) predicts that the behavioral strategies in coping with social and environment challenges would differ in males and females when a discrepancy in parental investment exists - as it is in all mammalian species. Therefore, it is reasonable to postulate that male and female mice would reveal sex differences in response to social environment and risk-taking behaviors. Although strain differences exist, male and female house mice show generally clear differences in their social behavior (*Berry & Bronson, 1992, for a review; Palanza et al., 1993*). Male CD1 mice are indeed territorial and aggressive to other males; while

females are generally tolerant and socially oriented to other females (*Parmigiani et al., 1989*). When male mice are grouped together, aggressive interactions between cage-mates are generally observed and a social structure develops after a short time, a single male being identified as dominant and the others as subordinates. This is utilized as the colony model for social stress, as the subordinate animals are exposed to continuous social stress (*Blanchard & Blanchard, 1990*). On the other hand, for a male mouse, a short period of isolation as an adult can mimic the establishment of a territory, which is aggressively defended against conspecific intruders, and only dominant territorial males have high reproductive success (*Brain & Benton, 1977; Berry & Bronson, 1992*). On the contrary, female mice show very low or no aggression when grouped with unfamiliar same-sex conspecifics (except during periparturition stages), and do not develop a detectable social structure. When individually housed and non-reproductively active, female mice do not show territory defence towards conspecific intruders (*Palanza et al., 1994; 2005*). This striking gender difference in social behaviour would require an in-depth analysis of the differential response to altered social context. However, only an extremely small proportion of the experiments on the relationships between social context in the housing environment and behavioral and physiological responses involve females. Female rodents as subjects in behavioural and stress-related research are often neglected because they are thought to be too “variable”, and it is easier and cheaper to use only males (*Blanchard et al., 1995*). Ovarian hormone fluctuations may indeed lead to behavioural changes that may be related to emotionality or anxiety (*Gray & Levine, 1964; Mora et al., 1997*).

In a previous study, we have shown that living alone for a short period or with same-sex siblings (brothers or sisters) may have a different psychosocial relevance for the two genders. When challenged to explore an unfamiliar area, female mice housed individually showed decreased exploratory behaviour and increased anxiety relative to females housed in

groups. In contrast, for males, being individually housed for a 7-day period induced higher exploration of the unfamiliar area compared to grouped males (*Palanza et al., 2001*). Establishing unisexual groups of mice at different age (before or after puberty) induced several behavioral and physiological alterations in males but not in females, with the exception of lower corticosterone level in both male and female housed together with unrelated conspecifics after weaning or as subadults (*Bartolomucci et al., 2004*).

In the present study we examined short-term modulation of social context in the housing environment, as a possible source of stress, on behavior (i.e. exploratory behavior and emotional responses to novelty) and physiology (i.e. organs and body weight, and basal corticosterone level) of female mice, taking into account the estrous phase as an additional variable.

Materials and Methods

Animals and procedures

Mice were derived from male and female Swiss CD-1 mice purchased from Charles River Italia (Calco, Lecco, Italy) but reared and bred in a colony room at the University of Parma at 22 ± 2 °C and using a 12-hr light–dark cycle (lights on at 0700 and off at 1900). Food (Mucedola, Italy) and water were available *ad libitum*. Mice were weaned at post-natal day (PND) 26-28 and housed in unisexual sibling groups and tested when 2-3 months old.

One-hundred-forty females were assigned to one of three experimental groups for 7 days: individually housed (IND, n=20); housed in a group of three non-familiar, same-aged adult females (UNFAM, n=60); housed in groups of three siblings (FAM, n=60). Animals were housed in 40 x 25 x 15 cm² polycarbonate cages with an opening on one side which can be connected to a small tunnel to be connected to an open arena.

Mice remained undisturbed in their home cage for seven days, with body weight being measured before and on the last day of, testing. On day 7 the mice underwent the free-exploratory open-field test as de-

tailed below. The following morning (between 9:00 and 10:00) all individually housed and 2 *per* group females were sacrificed upon brief CO₂ exposure by decapitation. Blood was collected in eparinized tubes and assayed for total corticosterone level as detailed below. At autopsy ovaries, spleen, thymus and adrenals were dissected and weighed.

All animal experimentation was conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/EEC) and approved by the Italian Institute of Health.

Estrous cycle determination

Estrous cycle was determined every single day and soon after the free exploratory paradigm and at autopsy by vaginal smears (*Palanza et al., 2001*). Accordingly, estrous and diestrous females were categorized. The analysis of body and internal organs weight and behavior was carried out on one estrous and one diestrous female per each 3-females cage (n= 10 per each experimental group) and on all the individually-housed females.

Free exploratory paradigm

For all individually-housed females and for each 3-sibling group, one estrous or one diestrous female were tested (n= 10/estrous phase/group). Five minutes before testing, all females but one were removed from the grouped cages. The test was conducted as previously described (*Palanza et al., 2001*). Briefly, the home cage was connected to an open-field (OF), in which a bright and a dark zone were created, by means of a small opening that remained closed with a removable barrier until testing. Once the barrier was removed, a cut-off of 10 min was used for animals that did not emerge on the surface of the OF. These animals were included in the statistical analysis with a latency to enter the open field of 10 min and zero seconds spent in the OF, while being excluded from further behavioral analysis. The test was considered to have started after the first entry into the unfamiliar OF (with the four paws) and lasted 5 min. Latency to enter the OF (the time from first approach to the opening of the

home cage to actual entrance into the OF with all four paws), Stretch-Attend-Posture (SAP: defined as forward elongation of the head and shoulders and scanning the unfamiliar OF occurring from the home cage), Walk, Rearing (standing with the fore-paws raised), Sniff, Time in the home cage and Time in the OF were scored by means of the specific software The Observer (Noldus, The Netherlands) by a trained observer.

Corticosterone assay

Trunk blood was collected in heparinized tubes, centrifuged and the supernatant frozen at -20°C until analyzed. Circulating levels of corticosterone were directly measured in the plasma. The measurements were done in duplicate and in a single assay by a commercially available radioimmunoassay kit (RPA 548, Amersham, USA). The sensitivity was 0.06 ng/tube; the intra-assay coefficient of variation was 3.4%.

Statistical Analysis

All parameters were analyzed with 2 or 3 way ANOVA (for repeated measures in case of body weight) followed by Duncan's post hoc test.

Results

Physiological parameters

Neither seven days of individual housing nor group reorganization with unfamiliar adult females determined any effect in several physiological parameters including body weight, basal corticosterone level and weight of reproductive and/or stress-related internal organs related to such as spleen, ovaries, adrenals and thymus (Table 1).

Behavior

There was a significant difference in the number of females who did not exit the home cage. Indeed, all IND-females (20/20) entered the arena while only 15/20 ($\chi_2 = 4.5$, $p < 0.05$) and 17/20 ($\chi_2 = 2.8$, $p < 0.1$) UNFAM and FAM, respectively, did enter the arena. This effect was paralleled by a tendency for IND housed females to enter the arena earlier than UNFAM group ($p < 0.1$; Figure 1 upper panel). This result was not affected by estrous cycle (data not shown).

Beside this clear difference induced by differential housing, the detailed behavioral analysis only showed small differences between groups revealing that individual housing and housing with unrelated females tend to neutralize estrous-cycle related differences. Namely, estrous FAM females showed a lower frequency of sniff within the arena when compared with diestrous FAM (housing x estrous cycle (Figure 1 lower panel; $F(2, 45) = 5.8$, $p < 0.01$) while this difference was not present in both IND and UNFAM groups. Neither housing nor estrous cycle affected the time spent in the arena and the amount of locomotion there.

Discussion

Living alone or grouped with sibling or with unfamiliar females for a short period (7 days) had marginal effects on emotional behaviour and did not affect any physiological indexes of stress in female house mice. When challenged with a free choice between a novel environment and their home cage, female mice housed with siblings since birth did not differ in any behavioural parameter from females housed with same-aged unfamiliar mice. Compared

Table 1. Physiological parameters of female mice in different housing conditions.

Parameter	Group - Familiar	Group - Unfamiliar	Individually housed
Body weight gain (g)	-0.13 (0.3)	0.17 (0.4)	-0.025 (0.3)
Corticosterone (ng/ml)	36.6 (3.4)	35.5 (3.4)	41.0 (4.7)
Spleen (g)	0.11 (0.005)	0.12 (0.005)	0.11 (0.004)
Ovaries (g)	0.02 (0.001)	0.02 (0.001)	0.02 (0.0009)
Adrenals (g)	0.011 (0.0007)	0.01 (0.0005)	0.011 (0.0006)
Thymus (g)	0.05 (0.003)	0.05 (0.003)	0.047 (0.002)

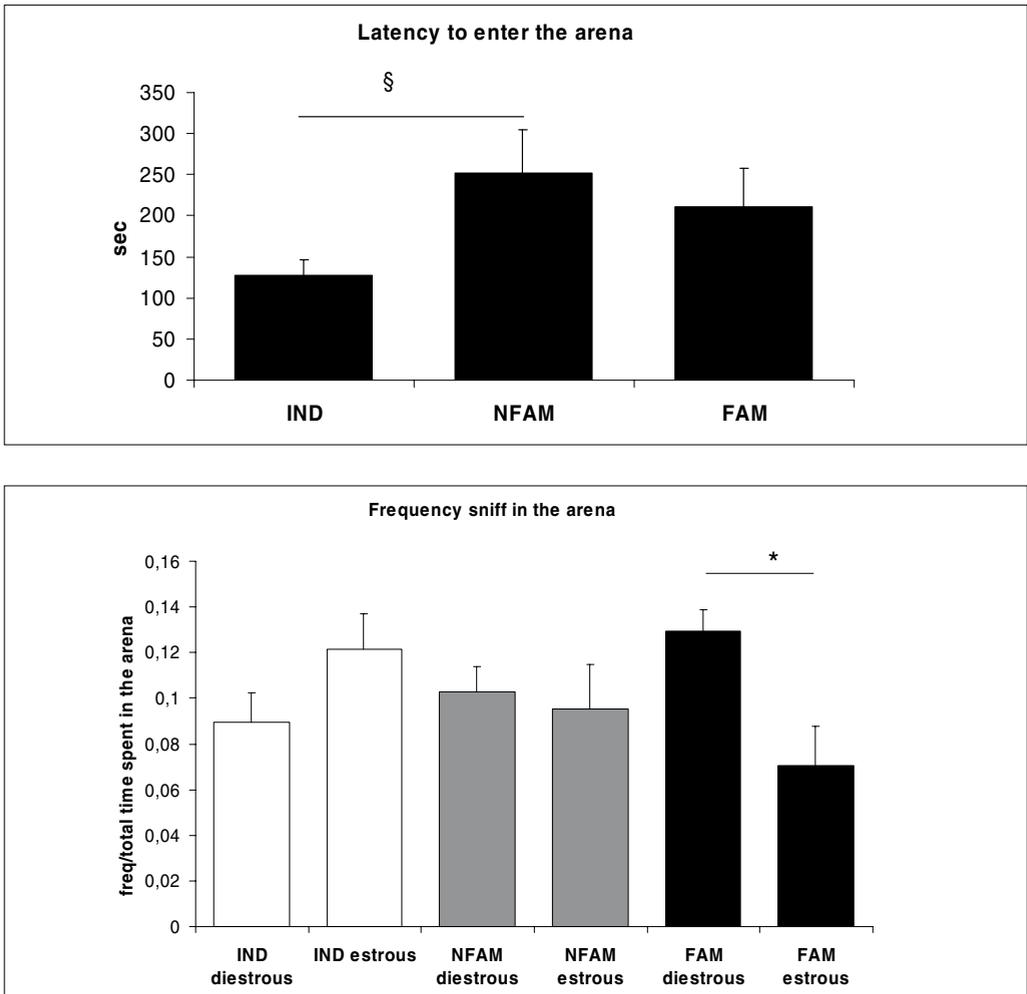


Figure 1. Behavior in the free exploratory paradigm. Upper panel, latency to enter the arena as measured on all tested females. Lower panel, normalized frequency (frequency/time spent in the arena) of sniff behavior in the arena. IND, individually housed, UNFAM, unfamiliar, FAM, familiar. §= $p < 0.1$; *= $p < 0.05$.

to grouped females, individually housed females showed a higher propensity to enter the novel arena but did not differ in exploratory activity or in anxiety responses. This finding suggests that a short period of individual housing in itself does not change corticosterone level and emotional responses, but can affect reactivity to a stressor. In fact, individually housed females showed lower neophobic re-

sponse when challenged with mild psychological novelty-stress. Increased behavioural arousal following short-lasting social deprivation has also been reported for male mice, though more pronounced (Bartolomucci *et al.*, 2003). Although social isolation certainly cannot be considered as a housing condition of choice for a social species like mice (Brain, 1975; Kim & Kirkpatrick, 1996), present

data together with previous studies (*Bartolomucci et al., 2003; Siegfried et al., 1981; Hilakivi et al., 1989; Rilke et al., 1998*) indicate that isolated mice can display increased activity, reactivity and excitability, thus suggesting that individually housed mice tend to be hyperresponsive when they are faced with a novel, potentially stressful situation. In the same way, socially isolated subjects have been reported to be hypersensitive to, and highly aroused by, challenge with stressful stimuli (*Brain, 1975; Brain & Benton, 1977; Brain & Benton, 1983; Sachser, 1986*). Our present findings add to this knowledge the notion that short-lasting isolation seems to have no effects on female mice endocrine status and a marginal effect on their exploratory propensity.

This finding is slightly different from our previous report that singly housed female mice showed lower exploration and higher anxiety when compared to group-housed females or to singly housed males (*Palanza, 2001; Palanza et al., 2001*). However, some methodological differences exist between these studies. In our previous studies, females were moved into the experimental apparatus 24-h prior to testing and, when group-housed, were left in sensory contact with their cage-mates separated by a wire net partition. In the present experiment, females were kept in the same home cage till testing and grouped females were separated from cage-mates by removing them from the cage just before testing. It is possible that different handling procedures were stressful *per se*, and influenced the animal responses to the novel arena differentially in relation to social context.

Present data confirm that the identity of the components of a given group does not affect female house mice, while males seem much more sensitive to familiarity and relatedness with their cage-mates (*Bartolomucci et al., 2004*). While crowding induces social stress in male rats, female rats are not strongly affected by this condition but showed higher levels of corticosterone (a biochemical index of sustained stress response), when individually housed for long periods (*Brown & Grunenberg, 1995*). In addition, Haller and coworkers (*1999*)

have reported that social defeat (loss of status) is a major stress (high corticosterone level) for males but not for females, whereas social instability (obtained by alternating isolation and crowding phases) is more stressful for females than for males. However, even in this case, female rats did not show behavioral alterations indicative of stress (e.g., anxiety) (*Haller et al., 1999*).

Behavioral variability may be expected in relation to estrous cycle in female mice. In our study, no main effect of estrous cycle on female behavioral responses was observed, thus confirming previous findings (*Palanza et al., 2001*). However, estrus mice appear to show increased olfactory exploration of the novel arena only when housed with sibling females. Enhanced exploration and reductions in behavioral indices of anxiety across the estrous cycle have been reported in other studies in rats (*Fernandez & Picazo, 1990; Mora et al., 1997; Zimmerberg & Fairley, 1993*). It is well known that the peak of ovarian steroid hormones occurs during proestrus (*Butcher et al., 1974*), and experimental evidence has shown that the ovarian hormones estradiol and progesterone, and its metabolites (i.e., active neurosteroids) exert an anti-anxiety effect in different experimental paradigms of anxiety in both rats and mice (*Fernandez-Guasti & Picazo, 1997; Rodriguez-Sierra et al., 1984; Rodgers et al., 1998*). Estrus stage did not affect levels of serum corticosterone in females, but independently of housing condition, female mice have much higher corticosterone serum concentration than male mice (see *Bartolomucci et al., 2003*). This basal difference could be related to different stress sensitivity and adaptation ability between the sexes (*Ehlers et al., 1993*).

Laboratory housing conditions have significant physiological and psychological effects on rodents, raising both scientific and welfare concerns (*Wurber, 2001; Bartolomucci et al., 2004*). During the past decade, the ability to generate transgenic and knockout strains of mice resulted in animal models with high potential validity for the study of mechanisms underlying human brain disorders. Several recent studies revealed that genetic effects

on behaviour are sometimes negated or reversed by minor variations in environmental background (Crabbe *et al.*, 1999; Cabib *et al.*, 2000). The scientific implications of the impact of current housing standards on brain development and behaviour in rodents have not been sufficiently considered. On this background, characteristic behavioural differences between males and females are generally not taken into account in animal (and clinical) studies, where commonly only males are analysed or sex is neglected and the results obtained are generalized (for review: Blanchard *et al.*, 1996; Palanza, 2001). Data about sex specifics under standard housing conditions as well as in response to common laboratory procedures could be important for the understanding of sex differences in vulnerability to psychiatric disorders and response to drug treatment.

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