Fluctuating Asymmetry in relation to single housing versus group housing in three inbred mouse strains

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Summary

Fluctuating asymmetry (FA) reflects small, random deviations from symmetry in otherwise bilaterally symmetrical characters and has been used to detect harmful conditions such as environmental and genomic stress in growing animals and humans. The development of FA may be related to the balance between canalization (the ability of the genotype to develop a constant phenotype under changing environments) and plasticity (the ability of the genotype to change phenotype dependent on the environment) of the individual. Different mouse strains differ in coping strategies in stress situations, and these coping strategies may be related to this balance. In this study, development of FA was studied in female mice of three different inbred strains, 129s6/Sv, C57BL/6J, and BALB/c, during a 6 week period. Besides the comparison of different strains, single housing was compared to group housing conditions. Overall, FA did not differ between strains. After six weeks, single-housed mice had higher FA than those that were group housed ($P<0.001$), which may indicate that single housing causes a higher degree of environmental stress than group housing does.

Introduction

Fluctuating asymmetry (FA) reflects small, random deviations from symmetry in otherwise bilaterally symmetrical characters *(Wilson & Manning, 1996)* and has been used to detect harmful conditions such as environmental and genomic stress in growing animals and humans *(Parsons, 1992)*. The mechanism behind the development of FA is not yet known. FA is a measurement of developmental instability, which reflects the ability of stable body ´the development under changing conditions. A high capability of canalization, i.e. the ability of the genotype to develop a constant phenotype under changing environments, implies a high resistance to developmental perturbations. This will result in a low degree of FA, independent of the level of developmental perturbations. In contrast, a high degree of plasticity, i.e. the ability of the genotype to change phenotype dependent on the environment, results in a low resistance to developmental perturbations. Developmental perturbations will then result in a changed adapted phenotype, associated with a high or a low degree of FA depending on whether the organism has been influenced by many or few developmental perturbations, respectively. Developmental stability, i.e. the ability to undergo a stable and symmetrical development of the body, refers to the balance between canalization and plasticity of the individual *(Debat & David, 2001)*. In

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humans, some genetic diseases, such as Down´s syndrome *(Katznelson et al., 1999)*, as well as some environmental factors, such as social stress and birth order, are found to affect FA *(Valetsky et al., 1997; Lalumiere et al., 1999)*. It is possible to measure FA reproducibly in rats and mice *(Stub et al., 2002)*, and in rats it may also be used to demonstrate the expression of stress related to genetic *(Stub et al., 2004b)* or environmental conditions *(Stub et al., 2004a)*. A group-housed animal that is to be housed singly, will be affected by this event. It may induce stress-related changes in the animal, at least for a period of time after its separation from the group. Many studies have shown that single-housed mice and rats react differently, both physiologically and behaviourally, compared to animals that are housed in groups *(Krohn et al., 2004)*. In contrast to group- housed rats, single-housed rats were unable to decrease their FA over a period of 11 weeks *(Stub et al., 2004a)*. Also in genetically modified mice, FA can be found, which may be related to the impact of the transgenetically induced disease *(Naver et al., 2003; Stub et al., 2003)*. So far we have not used FA for demonstrating possible environmental impact on mice. Different mouse strains differ in how they react to stress. *(Koolhaas et al., 2000)*. C57BL/6J and BALB/c mice were found to react differently to the same kind of environmental enrichment *(Van de Weerd et al., 1994)* and housing conditions *(Krohn & Hansen, 2002),* when tested in behavioural tests, such as the Open Field Test. These different coping strategies may be related to the degree of canalization and plasticity of a certain strain. In case the coping strategies would be related to the degree of canalization and plasticity, FA development patterns may differ between strains. Therefore, we studied three different mouse strains, 129s6/SvEv, C57BL/6J, and BALB/c, during a 6 week period, and evaluated also whether FA in single housing as compared to group housing conditions would be different.

Materials and Methods

Fifteen females of each of the three strains 129s6/Sv/Bom, C57Bl/6J/Bom, and BALB/c/Bom, microbiologically defined according to FELASA guidelines *(Nicklas et al. 2002)*, were housed with lights on from 6.30 to 18.30, a room temperature of 21 +/- 2˚C, a relative humidity of 55 +/- 15%, and 15 air changes per hour. They were fed Altromin 1324 (Altromin Denmark, Chr. Petersen A/S, DK-4100 Ringsted, Denmark) and tap water *ad libitum,* and they were ear marked. After one week of acclimatization in Macrolon Type III cages with

Table 1. Relative fluctuating asymmetry (mean ± s.d.) of three different mouse strains before and six weeks after splitting up in group and single housed animals.

Strain	Housed	n	Baseline	Six weeks
129s6/Sv	Group Single	8 7	0.034 ± 0.015 0.029 ± 0.018	0.033 ± 0.022 0.046 ± 0.019
C57BI/6J	Group Single	8 7	0.047 ± 0.031 0.039 ± 0.020	0.014 ± 0.005 0.055 ± 0.012**
BALB/c	Group Single	7* 7	0.031 ± 0.016 0.038 ± 0.015	0.017 ± 0.016 0.055 ± 0.012**

* Data from one mouse missing.

** Significant differences (Mann Whitney U-test; $P<0.01$) between the group housed and the single housed mice of each strain.

bedding and nest material from Tapvei (Vaikkojoentie 33 Fin-73620, Kortteinen), baseline FA was measured under anaesthesia: SC injection with 0.75 ml/100 g body weight of a mixture of 25% Hypnorm® (Janssen-Cilag; fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml), 25% Dormicum® (Roche; midazolam 5 mg/ml) and 50% sterile water. After recovery from anaesthesia, the 45 animals were devided into 7 single-housed mice and two groups of 4 mice, for each of the three stains (as in Table 1). Single-housed mice were housed in Macrolon Type II cages, and group housed mice were housed in Macrolon Type III cages. After six weeks, FA was measured in the mice, which were then anaesthetised as described above and killed before recovery.

FA was measured using a digital caliber with constant pressure to the nearest 0.01 mm (Mitutoyo, Mitutoyo Corporation, Japan). Each site was measured twice. Trait size was determined by calculating the mean of the left and right traits. Absolute FA was defined as right-minus-left trait size. Relative FA of a trait was defined as absolute FA divided by trait size (relative FA=absolute FA/($^1/_2$ x size of right side + $^1/_2$ x size of left side)). Mean relative asymmetry was the mean relative asymmetry of the two different traits, here the mean of the numeric values of the relative FA of the individual traits. For examining the type of asymmetry, the absolute asymmetry (not the numeric value) of each trait was used. The width of the carpal bones (outside the animal) and the width of the joint between tibia and tarsal bones (outside the animal) were measured, as previously validated to express FA in mice and rats *(Stub et al., 2002)*. The raw data were square-root transformed in order to approximate a normal distribution. We performed a 3-way analysis of variance (ANOVA) in order to determine whether the asymmetry of the animals was affected significantly by any of the experimental factors: mouse strain, housing condition, and test time point (start versus 6 weeks) (α= 0.05). Single-and group-housed mice of each strain were compared using the Mann Whitney U-test.

Results

All traits measured fulfilled the demands of FA, as absolute asymmetry followed a normal distribution and had a mean of zero. Group housed C57BL/6J and BALB/c decreased their FA over the six weeks,

Table 2. Results of the 3-way ANOVA calculation.

Source	Df	F	P
Strain	2	0.361	0.79
Housing condition	1	14.257	<0.001
Test time	1	0.010	0.81
Strain x Housing	2	1.994	0.07
Strain x Test time	2	1.499	0.22
Housing x Test time	1	18.547	<0.001
Strain x Housing x Testtime	2	1.332	0.37
Error	76		

Df: degree of freedom, F: F-value, P: P-value

while this was not the case for 129s6/SvEv mice. All single-housed mice increased their FA over the six weeks, i.e. after six weeks there was a significant difference between the single and group-housed C57BL/6J as well as BALB/c mice ($P < 0.01$), but no significant differences between single-and group-housed 129s6/SvEv mice (Table 1). Overall the baseline FA of the different strains did not differ from each other, neither for group-housed nor for single-housed animals. After six weeks, the single-housed mice had a higher FA than the group-housed animals (ANOVA on housing condition: $P<0.001$, Table 2). ANOVA showed a significant interaction between housing condition and test time ($P<0.001$) (Table 2): FA increases over time under single housing conditions and decreases under group housing conditions, except in the 129s6/Sv strain (Table 1).

Discussion

In this study, development of FA in single- as compared to group-housed female mice was studied in three different mouse strains, 129s6/Sv, C57BL/6J, and BALB/c over a 6-week period. In agreement with our previous study performed in rats *(Stub et al., 2004a)*, FA decreased in group-housed animals in two strains and increased in single-housed animals in all three strains after six weeks. The present study supports other studies indicating that housing conditions, e.g. single housing vs. group housing, may influence rodent physiology being expressed e.g. in an increased level of FA under single housing conditions. This may imply that single housing is a stressor, resulting in a higher degree of FA, whereas group housing works in the opposite direction in two strains. As the FA decreased under group housing conditions in only two of the three tested strains, these effects are not absolute. Furthermore, the results of this study indicate that, in addition to our previous experience *(Stub et al., 2002; Naver et al., 2003; Stub et al., 2003; Stub et al., 2004a; Stub et al., 2004b)*, this method can be used as a supplement to other methods to obtain an indication of animal welfare.

We expected to find different results from the various mouse strains as these show differences in coping strategies. The 129s6/Sv mice may have a slightly lower degree of plasticity, whereas C57BL/6J and BALB/c mice reacted in a similar way, thereby probably having the same degree of canalization and plasticity. A significant difference between mice from the 129s6/Sv strain versus the two other strains might have been shown when a larger sample size has been used. A difference between the means of 0.019, as shown between 129s6/Sv and C57BL/6J, would with a s.d. of 0.02 demand a sample size of 25 to achieve a power of 90 %. This difference of 0.019 could well be a true difference. Strain differences are a common finding when studying other parameters related to welfare research. E.g. BALB/c mice were significantly less active in an Open Field Test and had significantly higher levels of corticosterone as compared to C57BL/6J mice *(Krohn & Hansen, 2002)*. Further studies are needed to be able to accept or reject the theory that strains differ in canalization and plasticity.

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