Fluctuating asymmetry of teeth is not a reliable indicator for assessing stress in rats

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Summary
Fluctuating asymmetry (FA) reflects small, random deviations from symmetry in otherwise bilaterally symmetrical characteristics and has been used to detect harmful conditions such as environmental and genomic stress in growing animals and humans. Teeth are often used for measuring FA, especially in humans. The aim of the present study was to measure FA in teeth as a possible stress indicator in laboratory rats. Rats housed individually or on a grid floor were compared to rats housed on bedding with or without environmental enrichment. FA of leg traits had been found to increase by housing on a grid floor or individually as compared to housing on bedding and socially. In this study dental FA was found not to be influenced by housing conditions, probably because wear may influence FA of the teeth independent of housing conditions. Incisors in rodents grow continuously throughout life, whereas this growth stops in adult humans. The conclusion of this study is that FA measurements of teeth do not appear suitable as parameters to evaluate stress in rats.

Introduction
Fluctuating asymmetry (FA) reflects small, random deviations from symmetry in otherwise bilaterally symmetrical characteristies (*Wilson & Manning, 1996*) and has been used to detect harmful conditions such as environmental and genomic stress in growing animals and humans (*Parsons, 1992*). Various studies have shown that grid floor and individual housing cause stress in rats, (*Krohn et al., 2003; Heidbreder et al., 2000; McCormick et al., 1998*). Meanwhile FA of different leg traits found to correlate with stress in rats, as FA in these traits was increased in rats housed on a grid floor or in isolation as compared to rats housed on bedding and socially (*Stub et al., in prep*).

In humans, FA is often measured in the teeth (*Hershkovitz et al., 1993; Liversidge & Molleson, 1999*), and dental FA is found to be increased in individuals with Down´s syndrome (*Barden, 1980; Townsend, 1983*). However, these results could not be reproduced in mice with the mouse variant of Down´s syndrome (*Auffray et al., 2001*).

The aim of this study was to measure FA in teeth as a possible stress indicator in rats. Grid-floor and individual housing were used as stressors. In earlier studies these conditions were found to increase FA of leg traits in rats (*Stub et al., in prep.*).

Materials and Methods

Animals and housing

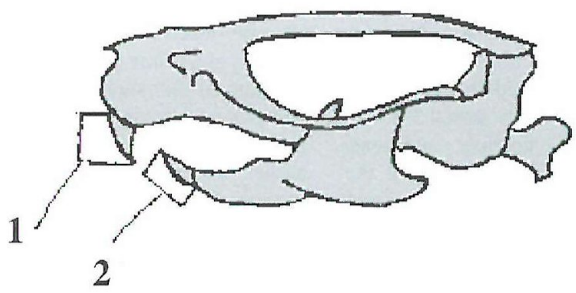
72 Lewis/Mol male rats, microbiologically defined according to the FELASA guidelines (*Nicklas et al., 2002*) were housed with lights on from 10 pm to 10 am, at a room temperature of 20-22 °C, a relative humidity of 55-80% and with ten to fifteen air changes per hour. The rats were housed in Macrolon Type III cages with 2 rats in each cage, with aspen wood shavings for bedding, aspen nest material and an aspen brick (about 5x1x1 cm) from Tapvei (Tapvei, Vaikkojoentie 33 Fin-73620,

Kortteinen). They had food (Altromin 1324 from Altromin Denmark, Chr. Petersen A/S, DK-4100 Ringsted, Denmark) and tap water (pH=6) *ad libitum*. After one week of acclimatization, the rats were split up into four groups: 18 rats were pair housed on grid floors, 18 rats were pair housed in an enriched environment with bedding, aspen nest material and a brick (Tapvei, Vaikkojoentie 33 Fin-73620, Kortteinen), whereas the control group of 18 rats were pair housed with bedding (Tapvei, Vaikkojoentie 33 Fin-73620, Kortteinen). The individually housed rats, comprising 18 animals, were maintained on bedding during the acclimatization as well as the test period, but only in the acclimatization period did they have a brick and aspen nest material. The reason for that was, that for none of the rats should social groups be changed after the acclimatization. The rats were killed at eleven weeks of age, followed by measurement of body weight and FA of tooth traits.

Fluctuating asymmetry

Measurements were made 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 the 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/(½ x size of right side + ½ x size of left side)). Mean relative asymmetry was the mean relative asymmetry of different traits using the mean of the numeric values of the relative FA of the individual traits. Absolute FA follows a normal distribution and has a mean of zero, and for testing that, the absolute asymmetry (not the numeric value) of each trait was used. FA was calculated for each trait. The traits measured were the length of the upper jaw incisor tooth from the gum (1) and the length of the lower incisor tooth from the gum (2) (Figure 1).

Figure 1



Teeth traits measured for fluctuating asymmetry: the length of the incisor tooth at the top, from the gum (1), and the length of the incisor tooth at the bottom, from the gum (2).

These traits were found in earlier studies to fulfil the statistical demands to FA *(Stub et al., 2002)*.

Statistical Analysis

All statistics were made in MINITAB 12.1 (Minitab Inc.). The individual traits were tested for fulfilling the demands to FA by testing the absolute asymmetries for normality using the Kolmogorov-Smirnov test for normality ($\alpha<0.05$), and ANOVA was used to test whether the mean of absolute asymmetry differed from zero. Absolute FA must follow a normal distribution and have a mean of zero. Numeric values of fluctuating asymmetry do not follow a normal distribution, so FA of rats housed under different housing conditions were compared using the Kruskal-Wallis test. It was calculated that with the actual number of animals in each group, the actual variation and a power of 0.9, it would be possible to detect a difference in FA of 0.017, which was similar to the difference found under the same experimental conditions in other traits (*Stub et al., in prep.)*. Body weight was tested for normality using the Kolmogorov-Smirnov test for normality ($\alpha<0.05$), and it was found to be normally distributed. Body weight was then compared between treatments using ANOVA.

Results

Both traits measured fulfilled the statistical demands to FA, as absolute asymmetry follow a normal distribution and have a mean of zero.

Development of body weight did not differ between the groups (data not shown).

The numerical value of FA of the combined as well as the individual tooth traits did not differ significantly between groups (Figure 2).

Discussion

The difference in dental FA between the groups was non-significant and in general less than the possible detectable difference of 0.017 units (see Figure 2). In addition, the direction of the change in FA as a result of changed housing conditions is different in the two dental traits measured (Figure 2), indicating that the asymmetry is random. This is expected to occur as a result of tooth wear. So dental FA measurements could not be shown to be influenced by environmental stress in rats as was, in contrast, found in FA of different leg traits (*Stub et al., in prep.)*. This result is not surprising, as the wearing due to gnawing and eating may influence the tooth length and thus dental FA independent of other environmental stress factors. Tooth wear may also influence FA measurements on teeth in humans, in which dental FA is often used (*Hershkovitz et al., 1993; Liversidge & Molleson, 1999*). However, as teeth do not grow throughout life in humans as they do in rodents, the effect of wear may be less in humans than in rodents. This physiological difference may also explain, that increased dental FA in individuals with Down's syndrome (*Barden, 1980; Townsend, 1983*) could not be reproduced in mice (*Auffray et al., 2001*), as the influence of the disease on dental FA may be blurred more by a more intensive use of teeth in rodents than in humans.

So we conclude that FA of teeth seems not to be a usable parameter of stress in rats.

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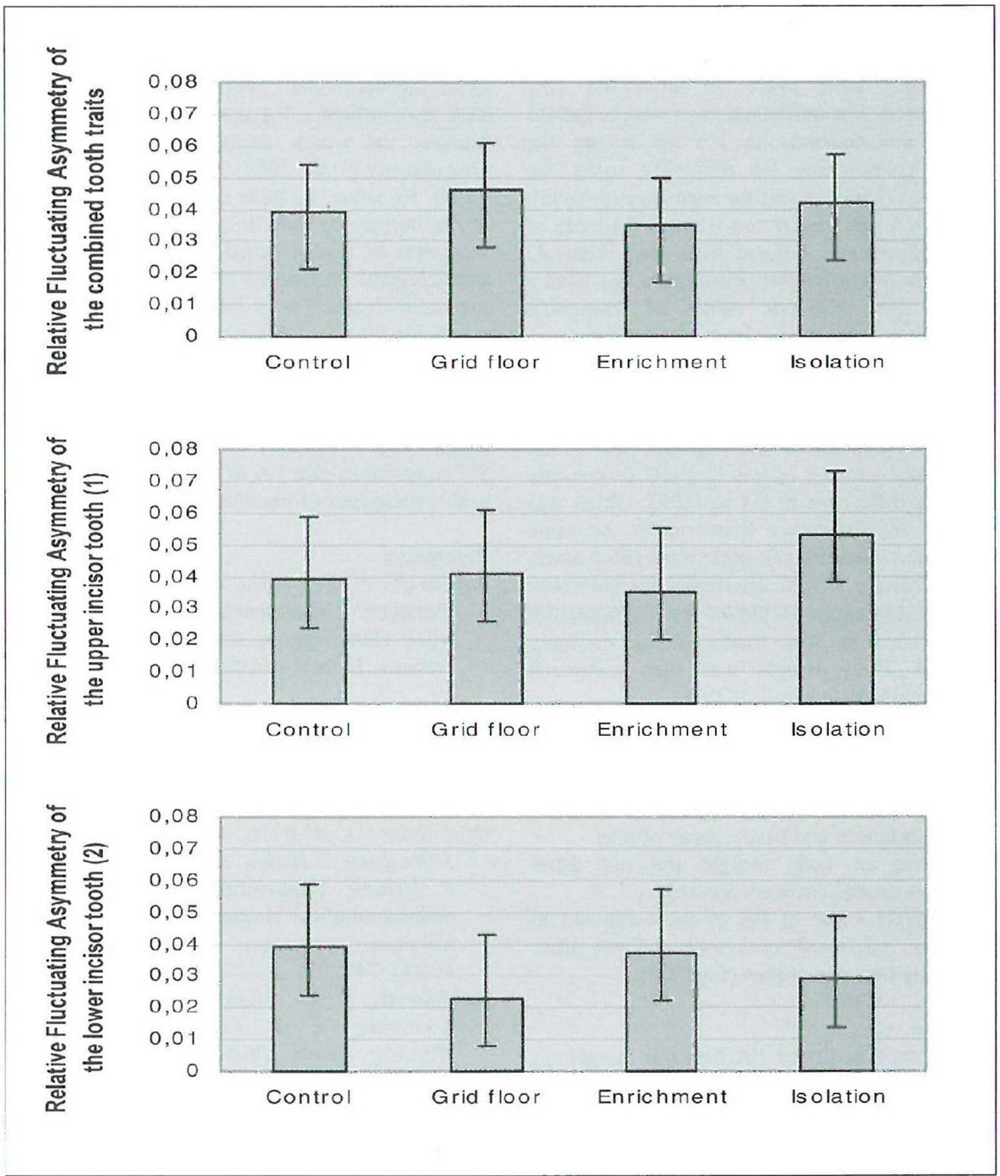
Figure 2



Relative fluctuating asymmetry (medians) of the combined and individual tooth traits. No significant differences were found.

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