Modified Tapvei OÜ Stairs Induce an Anxiolytic Effect in Female C57BL/6 Mice in the Elevated Plus-Maze Test

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Summary
We evaluated the effect of modified Tapvei OÜ stairs (stairs) on the behaviour of female C57BL/6 and BALB/c mice in the elevated plus-maze (EPM) test. The mice were kept under standard conditions for 4 weeks (control) or exposed to stairs for 3 or 4 weeks, and were assessed thereafter with the EPM. The C57BL/6 mice displayed less anxiety, when compared with the BALB/c mice. Exposure to stairs had an anxiolytic effect in C57BL/6 mice, but not in BALB/c. The strain-dependent effects of stairs should be considered in the design of housing refinements and behavioural experiments.

Introduction
Environmental enrichment (EE) has been defined as any modification in the environment of captive animals that seeks to enhance their physical and psychological well-being by providing stimuli that meet the specific needs of the species (Baumans, 2000). EE is mandatory for laboratory animals in Europe (Council of Europe, 2006). Regulatory compliance is easy to achieve, but some added structures may have no value or even a negative impact (Tsai et al., 2002). In the elevated plus-maze (EPM) test, EE has produced conflicting results in mice: both anxiolytic (Friske & Gammie 2005) and anxiogenic (Kobayashi et al., 2006) effects, as well as no effect (Martínez-Cue et al., 2002), have been reported. It is difficult to compare different studies, because the structures added and cage area per mouse vary (the latter from 159 cm² to 1125 cm²) among EE-housed mice (Friske & Gammie, 2005; Kobayashi et al., 2006). In addition, the objects used were often inadequately described or used in combination with other factors, or the EE items were changed during the study. Therefore, it should be no surprise that the data obtained are often contradictory, and this prevents comparison between studies (Friske & Gammie 2005; Kobayashi et al., 2006). BALB/c and C57BL mice are among the most common inbred strains used in biomedical research (for review see Zhao et al., 2007). In the EPM test, the higher level of anxiety of BALB/c mice has been verified for both females (Augustsson et al., 2005) and males (Sunyer et al., 2007). Gender-specific results are obtained in various tests with mice (Martínez-Cue et al., 2002; Hutchinson et al., 2005), and this may well be the case with items of EE. Ideally, EE that has similar effects on the behaviour of each sex should be used. In this study, the EPM was used to assess animal welfare indirectly. Given that stress induces anxiety-like behaviour in mice in the EPM (Hsu et al., 2007; Sterlemann et al., 2008), an anxiolytic effect of EE indicates improved welfare. We evaluated the effects of a specific item of EE, Tapvei OÜ stairs (stairs), in female C57BL/6 and BALB/c mice.
Materials and Methods

Ethics
The study protocol was reviewed and approved by the committee of the Ministry of Agriculture that grants permission for conducting animal experiments in the Republic of Estonia.

Animals
Seventy-two female mice were used: C57BL/6 mice (n=36; 18.3 ± 0.2 g [mean ± SEM]) (Harlan, Horst, The Netherlands), and BALB/c mice (n=36; 19.1 ± 0.2 g) (Scanbur AB, Sollentuna, Sweden). The mice were 10 weeks old at the time of the EPM test. Animals were maintained at 21 ± 2 °C and 50 ± 5 % relative humidity, with both pelleted food (Labfor R70, Lantmännen, Södertalje, Sweden) and autoclaved water available ad libitum. They were housed in groups of six in polycarbonate cages (Tecniplast, Buguggiate, Italy) that measured 42.5 x 26.6 x 15.0 cm (Eurostandard type III), and were exposed to a 12 h:12 h light/dark cycle. The cages were illuminated from 0700 h to 1900 h. Autoclaved aspen chips (chip size 4 × 4 × 1 mm; Tapvei OÜ, Kiili, Estonia) were used as bedding.

Environmental enrichment
Stairs (Tapvei OÜ, Kiili, Estonia) that consisted of five rectangular aspen blocks (19 × 19 × 75 mm, 1 × w × h) connected by four aspen bars (diameter 7 mm; length 70 mm) were modified by removing two blocks (Figure 1). The modified stairs were autoclaved, fitted in the cages, and changed with the cage.

Groups
The following groups (which each consisted of 12 C57BL/6 and 12 BALB/c mice) were tested simultaneously:
(1) controls – without stairs;
(2) stairs provided for 3 weeks;
(3) stairs provided for 4 weeks.
The time period was chosen according to previous results, which showed that the stairs had the strongest effect during the 3rd week (Ökva et al., 2007b). The EPM test was performed during the 5th week of the study, before the weekly cage change.

The elevated plus-maze (EPM) test
The EPM test was performed according to a method modified from Lister (1987). The maze consisted of two open (8 × 17 cm) and two closed (8 × 17 × 30 cm) arms, which were connected by a central platform (8 × 8 cm) that was elevated 30 cm above the floor.
The animals were transported from their familiar animal room to the experimental room and allowed a period of acclimatization for approximately one hour, during which the EPM apparatus was not directly visible. The mice were placed on the central platform, facing an open arm. The number of entries made into the open and closed arms during a 5 minute period was counted, and the time spent in the open arms was measured. The percentage of entries made into the open arms and the percentage of time spent in the open arms were calculated. After each test, faecal boluses were collected and the EPM was cleaned (1% Virkon®; UK).

Figure 1. Two views of the modified Tapvei OÜ mouse stairs (these mice were not involved in the study)
Data analysis
The data were analysed by one-way and two-way analysis of variance (ANOVA) using the strain and stairs as factors. Further statistical analysis was performed by contrast analysis and Kruskal–Wallis test.

Results
Two-way ANOVA, supported by the central limit theorem, revealed a significant interaction between strain and stairs that influenced the number of entries made into the open arms ($F_{1,68} = 16.58; P < 0.01$) and the percentage of entries made into the open arms ($F_{1,68} = 9.61; P < 0.01$). Further statistical analysis was therefore performed on each strain separately using one-way ANOVA. In C57BL/6 mice, further ANOVA revealed a significant effect of the stairs on the number of entries made into the open arms ($F_{1,34} = 19.47; P < 0.01$), on the total number of entries ($F_{1,34} = 6.72; P < 0.05$), on the percentage of entries made into the open arms ($F_{1,34} = 23.88; P < 0.01$), and on the percentage of time spent in the open arms ($F_{1,34} = 12.71; P < 0.01–0.05$). These changes reflect an anxiolytic effect (Lister 1987). Moreover, the stairs increased the locomotor activity of C57BL/6 mice, as shown by an increase in the total number of entries. These data showed a normal distribution.

In BALB/c mice, the corresponding datasets did differ from a normal distribution, hence they were analysed using the Kruskal–Wallis test, but the stairs had no effect (Figure 2).

In female C57BL/6 mice, faecal data were not distributed normally, in contrast to the data for the BALB/c; hence ANOVA was used for BALB/c and the Kruskal–Wallis test for C57BL/6 mice. Exposure to stairs for 3–4 weeks resulted in the voiding of fewer faecal boluses by C57BL/6 mice ($0.17 \pm 0.17$ both in EE housing for 3 and 4 weeks vs. $0.67 \pm 0.23$ in standard housing ($F_{1,34} = 4.25; P < 0.05$), but not by BALB/c mice. A decrease in the number of faecal boluses voided might be associated with reduced anxiety (Calvo-Torrent et al., 1999).

Discussion
We expected stairs to induce an anxiolytic effect, as was observed in C57BL/6 mice. This study showed that exposure to EE, provided in the form of modified Tapvei OÜ mouse stairs, produced an anxiolytic effect in female C57BL/6 mice, but not in female BALB/c mice. This effect was exhibited as an increase in the number of entries into, and time spent in, the open arms of the EPM (Pellow et al., 1985; Lister 1987). Moreover, exposure to EE increased the locomotor activity, as shown by an increase in the total number of entries, of C57BL/6 but not BALB/c mice. Exposure to EE is used to improve animal welfare (Benaroya-Milshtein et al., 2004). Therefore it was expected that the added items of EE would induce an anxiolytic effect in the EPM test, and indeed this was the case with female C57BL/6 mice. In contrast, stress has been associated with anxious behaviour in male NMRI (Hsu et al., 2007) and CD1 mice (Sterlemann et al., 2008) in the elevated EPM test. It has been proposed that increased defecation reflects the stimulation of the autonomic nervous system in response to stress (Swiergiel & Dunn 2006), and that acute stress increases the number of fecal boluses left in the EPM apparatus by mice (Calvo-Torrent et al., 1999). In our study, the number of fecal boluses voided by BALB/c mice was significantly larger than the number voided by C57BL/6 mice. This result can be explained by the fact that C57BL/6 mice are less anxious than BALB/c mice.

The results of this study are similar to those of our previous studies, in which stairs induced an anxiolytic effect in male C57BL/6, but not male BALB/c, mice (Ókva et al., 2007a; Ókva et al., 2007b). It is probable that the anxiolytic effect of stairs is insufficient to overcome the high levels of anxiety expressed by female BALB/c mice.

Overall, these results confirm the results of previous studies, which showed that the effects of EE are strain-specific (Fernández-Teruel et al., 2002). Exposure to stairs had an anxiolytic effect in C57BL/6 mice, but not in BALB/c mice.
Therefore the anxiolytic effect in C57BL/6 mice could represent decreased stress and enhanced animal welfare.

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References
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Figure 2. The effect of stairs on the behaviour of female C57BL/6 and BALB/c mice in the EPM test. Data are presented as mean ± SEM for groups of 12 mice.
* P <0.05, ** P <0.01 vs. control housing in the same strain;
+ P <0.05, ++ P <0.01 vs. same housing type in C57BL/6 mice (contrast analysis)


