Environmental Enrichment does not Disrupt Standardisation of Animal Experiments

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Summary
Environmental enrichment can prevent abnormal behaviours and improve the well-being of laboratory mice, but concerns have been raised that it might disrupt the standardisation of experiments. Based on a multi-laboratory study, I show that animal welfare can be improved by environmental enrichment without disrupting standardisation.

Keywords: ethology, laboratory animals, transgenic animals, enrichment, mice, standardisation, refinement, reduction

Background Information

Standard rodent housing: impaired brain development – abnormal behaviour – poor well-being

Environmental enrichment refers to the addition of structure and resources to the barren standard cages for laboratory rodents with the aim to stimulate active interaction with and exploration of the environment and to facilitate the expression of species-typical behaviour. Accumulating evidence indicates that mice and other rodents housed in barren laboratory cages (which are still "standard" in many laboratories) show impaired brain development due to sensory and motor deprivation (van Praag et al., 2000); develop abnormal repetitive behaviours (i.e. stereotypies), originating from chronically thwarted attempts to gain access to highly valued resources (e.g. shelter) or to perform highly motivated behaviours (Würbel, 2001); and exhibit an anxiogenic behavioural profile compared to mice from more enriched environments (Chapillon et al., 1999). Thus, converging evidence indicates that the well-being and normal brain development of mice housed in barren cages may be seriously impaired, which also questions the validity of research done with these animals (Würbel, 2001). All of these effects could be attenuated, if not eliminated, by adequate environmental enrichment, especially during early ontogeny.

Testing effects of enrichment on standardisation

For many years, concerns have been raised that environmental enrichment might disrupt standardisation by increasing variation in data obtained in animal experiments. Previous studies found variable effects of enrichment on variation in the data depending on the variable studied (Tsai et al. 2003), indicating that enrichment has no consistent effect on variation in data.

However, none of these studies provided conclusive evidence since they were all based on single experiments instead of several independent replicates. In contrast, we used a multi-laboratory approach involving nine independent replicates (three in each of three laboratories) to study the effects of enriched *versus* barren housing on (i) variation in behavioural endpoints and (ii) reproducibility of behavioural differences between three strains of mice across these independent replicates. Each replicate involved eight mice per strain and housing condition, amounting to 48 mice per replicate and 432 mice in total. Enrichment was a combination of more space, additional resources, increased environmental complexity, and novelty (novel items and environmental change). We used mice of two common inbred strains (C57Bl/6J, DBA/2) and their F1-hybrids (B6D2F1). They were housed in either small barren or large enriched cages from weaning to 9 weeks of age (Fig. 1a, 1b).

Behavioural tests

At 10 weeks of age, all mice were subjected to four behavioural tests commonly used in drug screening studies and behavioural phenotyping of mutant mice (Fig. 2). We used identical test systems in all three labs and standardised test conditions as good as possible. Test performance in all tests was video-tracked using EthoVision 3.00 (Noldus Information Technology, Wageningen NL) and the data was analysed using a 4-way factorial ANOVA model with housing (barren versus enriched housing), strain (DBA/2, C57Bl/6, B6D2F1), laboratory (Lipp, Nitsch, Würbel), and replicate (1, 2, 3) as between subject factors.

Variation and reproducibility are unaffected

To test the effects of enriched housing on the detection and reproducibility of strain differences in behaviour, we split the data by housing conditions and calculated for each replicate the propor-

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tion of variance in behavioural measures contributed by within-group variability and by laboratory x strain interactions. Figure 3 presents a synoptic summary of the results. Within-group variability contributed between 40 and 84% (average 60%) to total variance. With an average of 7.6%, the contribution of strain x laboratory interactions was considerably smaller and also less variable. However, within-group variability was unaffected by enriched housing (except for fecal bolus counts on the O-maze). This indicates that enrichment did not decrease the sensitivity of the tests to detect genetic differences. It also shows that barren housing conditions fail to reduce individual variability in behavioural endpoints. Furthermore, enrichment had no significant effect on the proportion of variance contributed by strain x laboratory interactions, and the direction of differences varied across measures, indicating that enrichment did not increase the risk of obtaining conflicting results between laboratories.

Similar to an earlier multi-lab study (Crabbe et al., 1999), we found significant strain x laboratory interactions in many variables. However, closer inspection of the data revealed that these were mainly of quantitative rather than qualitative nature; reflecting differences in effect magnitude rather than direction of the effects (data not shown; see Wolfer et al., 2004).

Is environmental standardisation ineffective?

Between-laboratory effects (contributing on average 5.2% to total variance) and replicate effects (3.1%) made similar contri-

Fig. 1: Housing conditions
a) Barren housing: Makrolon type II cages with sawdust as bedding and food and water ad libitum. b) Enriched housing: Makrolon type IV cages with sawdust, food, water and shelter ("Mouse House"). Twice a week one enrichment item was added, some of which were removed after one week (e.g. paper tissue, straw, shredded paper), while others remained in the cage until the end of the housing period (e.g. tunnel, wooden branches, cardboard house).

Fig. 2: Example of a behavioural test
Water Maze Test: A circular pool (diameter: 150 cm) filled with opaque water, containing a goal platform (14x14 cm) hidden 0.5 cm below the water surface at a constant location. The mice performed 16 training trials (4 per day) from varying start positions. On day 5, they performed a 60 s probe test without the goal platform.

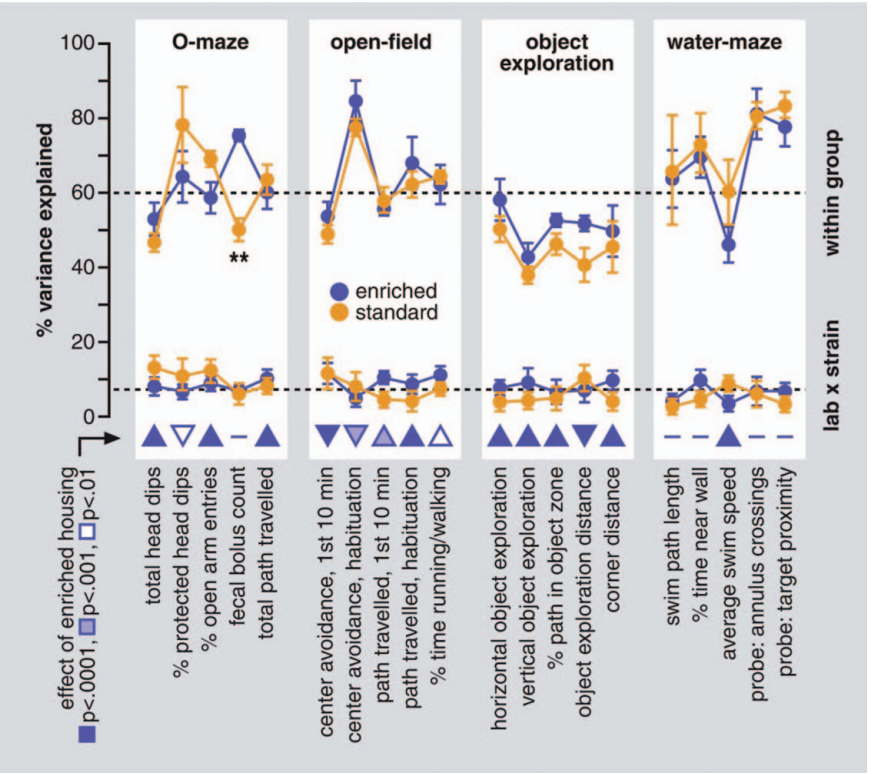

Fig. 3: Effects of enrichment on variation and reproducibility of behavioural endpoints.
Mean (± 1 s.e.) proportion of variance (%) in representative measures of the four behavioural tests contributed by within-group variability and laboratory x strain interactions. Data was pooled for the 3 strains (total N=432). (**: p<0.01). Triangles illustrate direction and significance of enrichment effects on each variable.

butions to total variance, indicating that standardisation between laboratories was nearly as good as standardisation within laboratories. This was surprising since nothing but cage-type, enrichment protocol, light phase, test equipment, and test protocols were equated across labs. This casts doubt on the effectiveness of excessive environmental standardisation to improve between-laboratory replicability of results from animal experiments (Würbel, 2002). On the other hand, it may simply reflect thtat an enriched environment may be as standardised as a barren environment. It says, however, nothing about the external validity of the results. The many significant strain x housing effects (see also below) indicate that barren as well as enriched housing conditions may produce idiosyncratic results that are valid with respect to the specific housing conditions only, and that therefore housing conditions need to be systematically randomised to produce externally valid results (Würbel and Garner, 2007).

More exploratory, less anxious

Enrichment also had significant effects on many measures of exploration and anxiety (Fig. 3). Importantly, enriched mice showed higher exploratory activity and less anxiety-related behaviour in all three tests of exploration. Figure 4 gives an example from the Elevated O-Maze Test, indicating that enrichment effects were consistent across strains and laboratories (see Wolfer et al., 2004 for more details).

Beneficial for animals and research

Our findings reject concerns that environmental enrichment might disrupt standardisation. These are important findings in the light that such concerns have hindered the implementation of enriched housing, despite its known advantages to the animals (Würbel, 2001). Our findings should be generally applicable, for example to drug screening, lesion studies, and the phenotyping of mutant mice. They should also apply to morphological or physiological measures, which are likely to be less sensitive than behavioural measures to environmental perturbations. It remains to be seen whether our findings also apply to male mice who may sometimes respond with enhanced aggression to certain forms of enrichment (Würbel and Garner, 2007). At least for females, however, our results

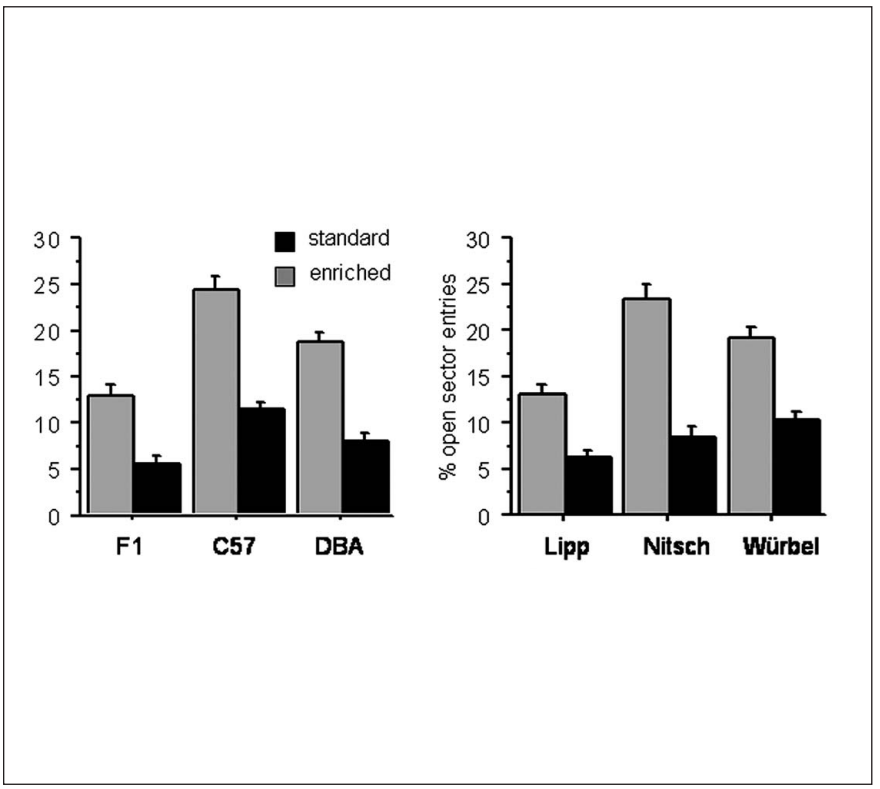

Fig. 4: Enrichment reduces anxiety in all strains and laboratories
Effect of enrichment on mean (± 1 s.e.) proportion (%) of entries to the unprotected sectors on the Elevated O-Maze displayed by strain (left panel: data of the three labs pooled) and lab (right panel; data of the three strains pooled).

demonstrate that environmental enrichment may be used to improve animal welfare without reducing precision and reproducibility of the data, while at the same time attenuating abnormal brain function and anxiety – two potential confounds in animal experiments.

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