

Practical Application of the 3Rs: Enrichment or Standardisation ?

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„Enrichment or Standardisation ?“

- Does enrichment increase variability ?
- Are more animals needed to validate results ?
- Is there a conflict between Refinement and Reduction ?

Point of Reference (1)

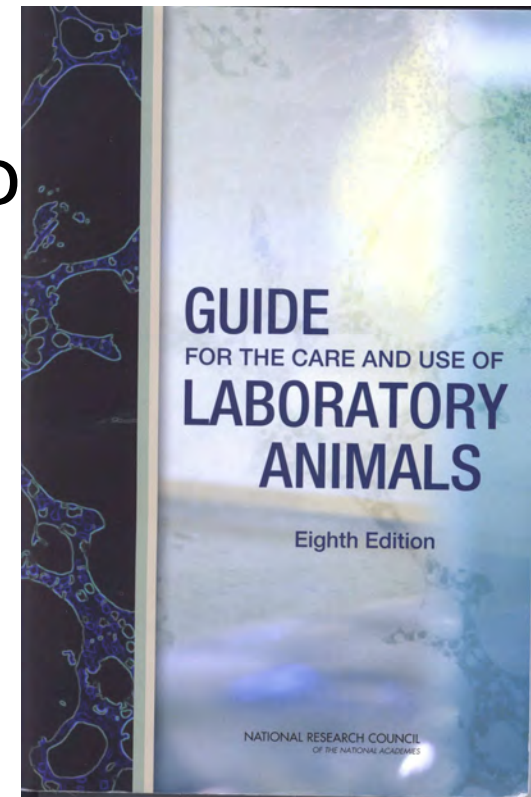
2010/63/EU (3.3 Housing and **enrichment**):

All animals **shall be** provided with space of **sufficient complexity** to allow expression of a **wide range of normal behaviour**. They shall be given a degree of control and choice over their environment to reduce stress-induced behaviour. Establishments **shall have** **appropriate enrichment techniques** in place

Points of Reference (2)

Guide (p. 52/53):

The primary aim of **environmental enrichment** is to enhance animal well-being by providing animals with ... resources that facilitate the **expression of species typical behaviors** and promote **psychological well-being** ...



Key questions:

1. Is enrichment improving animal welfare?
2. Does it affect experimental results?
3. Does it increase variability?



- What enrichment?
- For which animals?

Improvement of welfare?

- Proven ability to correct behavioral abnormalities (e.g. stereotypies, cf. Shepherdson et al. 1999)
- Reduction of anxiety levels - “one of the most robust effect of enrichment” (Sztainberg & Chen 2010)

biology
letters

Animal behaviour

Biol. Lett. (2011) 7, 19–22

doi:10.1098/rsbl.2010.0430

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**Maternal presence and
environmental enrichment
affect food neophobia of
piglets**

Marije Oostindjer*, Julia Mas Muñoz†, Henry Van
den Brand, Bas Kemp and J. Elizabeth Bolhuis



Assessment of welfare impact

Behavioral Needs

- External vs. internal stimuli

Example: Dust bathing chicken on wire-mesh floors (cf. King 2003)

Preferences

Mouse houses: plastic vs. cardboard (van Loo et al. 2005)

Affecting experimental results?

Potential for Unintended Consequences of Environmental Enrichment
for Laboratory Animals and Research Results

ILAR Journal 46, 2 (2005)

Kathryn Bayne

YES!

Abstract

Many aspects of the research animal's housing environment are controlled for quality and/or standardization. Of recent interest is the potential for environmental enrichment to have unexpected consequences such as unintended harm to

program and if so, the reason. The importance of the first question—the appropriateness of the animal model—is obvious. The investigator's response to the second question—regarding enrichment—may not receive the same degree of consideration. The researcher may know that the provision of certain environmental enrichments to the animal's pri-

100+ References



Marashi et al. (2003)*

Housing conditions & stress response

- Sibling male ABG mice
- Standard, Enriched or Super-Enriched housing (PND 22-77)
- Behavioral analysis (videotaped)
- Blood & organ sampling PND 77

*: Hormones and Behavior 43: 281–292

Corticosterone levels (ng/mL)#

Housing Condition (from postnatal day 22-77)	Plasma corticosterone (arithmetic mean \pm SEM)@
Standard	14 \pm 0.3
Enriched*	77 \pm 3.1
Superenriched*	77 \pm 1.5

#Marashi et al.(2003)

@values estimated from Figure 3 of the paper

*significantly different from Standard housing

Verkleinern (Strg+1)



Enriched

Marashi et al. (2003)



Super-enriched

Meaney et al. (1991)*

Prewearing Handling as a Modifier

- Rat pups (PND 1-21) removed from home cage daily for 15 min.
- Corticosterone response to 20 min. immobilization stress at 6-7 mo. of age
- Spatial learning performance at 6, 12 and 24 mo. of age

*Psychoneuroendocrinology 16: 85-103

Meaney et al. (1991)

- In rats with preweaning handling experience, 20 min. immobilization was associated with a significantly faster return (within 120 minutes) of plasma corticosterone to baseline levels as compared to non-handled subjects (with corticosterone levels still increased after 120 minutes).

Meaney et al. (1991)

- Rats with preweaning handling experience performed slightly better at 6 month of age, and significantly better at 12 and 24 month of age as compared to their non-handled counterparts in the Morris water maze (test for spatial memory)

Increasing variability ?*

(Studies in mice)

YES:

Gaertner (1998), Tsai *et al.* (2002, 2003), Pasalic *et al.* (2011).

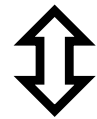
NO (or even decreasing):

Van de Weerd *et al.* (2002), Augustsson *et al.* (2003), Wuerbel *et al.* (2005), Baumans *et al.* (2010)

*For detailed references see: www.felasa.eu/media/uploads/WG_Enrichment_2006_Report-Final.pdf

Interim-Conclusion:

- Enrichment for welfare



- Enrichment as experimental variable
- Effects **may** depend on species, strain, sex, age etc.

FELASA Working Group Standardization of Enrichment (2006)

Understand species needs



Literature search for existing enrichment recommendations for a species



Critically assess likely impact of enrichment (or its absence) on experimental outcomes or variability



Choose enrichment program taking into account social and environmental factors

Guide (2011)

Enrichment **programs** should:

- be regularly reviewed by IACUC, researchers, attending veterinarian
- be updated according to current knowledge
- include training of staff

Mouse standard enrichment: nest + shelter (shelter ≠ shelter)

Baumans et al. (2010); Scand. J. Lab. Anim. Sci. 37: 101-114)



Conclusions

1. Enrichment for welfare

- Has to be practicable
- Needs to be effective
- Effects should be well-known (focus on robust effects !!)

2. Effects on variability inconclusive

3. Standardize enrichment !!!

4. Keep mind: external validity