

Evaluations of environmental enrichment webinar Q&A

- 1. <u>In the first talk the potential for enrichment to cause harm was mentioned. In what cases can enrichment be harmful to animals?</u>
- 2. <u>What approach should be taken if a new physical enrichment item increases aggressive guarding</u> <u>behaviour by dominant animals?</u>
- 3. Can we use common sense for some enrichment items, or must we always evaluate enrichment?
- 4. What if an evaluation shows that there is no demonstrated benefit of the enrichment? Would we decide in this case 1) to remove the enrichment because it is useless, 2) leave it because it was not shown that has a negative impact, or 3) try another enrichment until we see a benefit?
- 5. What type of enrichment can be used for zebrafish?
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- 7. How can cortisol be sampled from zebrafish?
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- 14. <u>I thought standardising everything, including housing conditions, was important. Won't adding</u> <u>environmental enrichment interfere with this?</u>

1. In the first talk the potential for enrichment to cause harm was mentioned. In what cases can enrichment be harmful to animals?

Often this relates to safety concerns like injuries or harm from ingesting materials.

In the second talk, Zoe spoke about an evaluation she carried out on different kinds of nesting material. Part of this evaluation focused on the safety of the mice as tangling of the nesting material on their head implants could be harmful to them. Other negative impacts to consider include increased aggression and the potential for particularly neophobic animals to become stressed by change. In both cases these impacts may be short term, but close monitoring will be necessary.

2. What approach should be taken if a new physical enrichment item increases aggressive guarding behaviour by dominant animals?

When animals try to monopolise enrichment items it suggests that they see the enrichment as a valuable resource. Aggressive behaviour should be monitored closely and evaluated on a case-by-case basis. However, one approach that could resolve this issue is providing more enrichment. This may seem counterintuitive but providing enough enrichment for all group members can reduce the need for animals to compete with each other to access the 'valuable resource'.

Unless urgent action is required, consider providing additional enrichment before you resort to removing items. You should use your judgment on whether this is more of the same item, a wider variety of enrichment, or a combination of these. It will be essential to continue monitoring the animals closely.

See also:

- My study has indicated a negative effect of enrichment on welfare
- NC3Rs webinar: Managing aggression in laboratory animal species

3. Can we use common sense for some enrichment items, or must we always evaluate enrichment?

Some essential enrichment items could be considered 'common sense', for example, nesting material and something to gnaw always need to be provided to rodents. However, if you want to find out which nesting material facilitates mice to build higher quality nests, or which scented chew sticks are preferred by your rats, then an evaluation is required. It is also the case that what seems like 'common sense' to you, may be new information to someone else.

Many refinements start as anecdotal but require data to back them up to before they become widely adopted. By carrying out an evaluation and sharing your findings you can contribute to the wider knowledge base on using environmental enrichment to improve research animal welfare.

4. What if an evaluation shows that there is no demonstrated benefit of the enrichment? Would we decide in this case 1) to remove the enrichment because it is useless, 2) leave it because it was not shown that has a negative impact, or 3) try another enrichment until we see a benefit?

How you respond to an inconclusive result will depend on the purpose of your evaluation and the question that you are aiming to answer.

If you are aiming to address a welfare problem, such as reducing the frequency or severity of a maladaptive behaviour then it would be important to continue trying to address this issue.

A harmless enrichment item that led to an inconclusive result does not necessarily need to be removed, but it might be unlikely that you would roll out use of this enrichment more widely in the facility. It is also possible that the enrichment improves welfare in a way that you have not measured in your assessment and that this could be demonstrated if you changed your question and welfare measure.

See also: Implementing and sharing your findings about enrichment | NC3Rs

5. What type of enrichment can be used for zebrafish?

Visit <u>Choosing appropriate enrichment</u> (see Table 3. examples of enrichment options for zebrafish that have been highlighted in the peer-reviewed literature). You can also find published literature on this subject on our <u>Zebrafish welfare page</u>.

6. How much enrichment is too much enrichment?

Theoretically, there is no limit to how much you can enrich the environment of captive animals, provided that it remains practical. In research facilities space is often limited, which makes it even more important to evaluate enrichment to ensure that the available space is used wisely (i.e. for enrichment that has a positive impact on animal welfare).

If you are concerned about being able to provide a wide variety of enrichment in the home cage, consider rotating or rearranging in-cage enrichment for variety or <u>creating a playroom or playpen</u>. Be aware that these approaches may not be suitable for species and strains that are prone to neophobia (fear of novelty) and in these cases careful introduction and further evaluation will be necessary.

7. How can cortisol be sampled from zebrafish?

Cortisol can be obtained non-invasively by sampling <u>tank water</u> or the <u>mucus layer of the fish by skin</u> <u>swabbing</u>. Note that it is a good idea to make behavioural observations alongside physiological measurements, and to look at multiple parameters to provide the best insight into animal welfare.

8. Is there a record of enrichment ideas that have been tried and not been successful to avoid repetition, or should facilities try what they can in case it does work for them?

In the planning stage of an evaluation, you should look at the published literature and speak to colleagues to learn what is known about the enrichment and the natural behaviours of the animals you intend to provide it for. However, publication bias means that negative results often do not get reported widely enough.

We would suggest that evaluating for your own animals in your own facility is still valuable. This is because animal behaviour is complex and so how they respond to enrichment can be affected by factors

we are unaware of that may differ between facilities. It is also the case that enrichment needs vary for different sexes, strains, life stages and group compositions of animals.

9. Where can I share my findings?

See Implementing and sharing your findings about enrichment for ideas for how to share your findings.

10. Do you have some enrichment tips for reducing aggression in mice?

Please visit our resource on <u>Minimising aggression in group-housed mice</u> for links to literature on this topic and other scientifically-backed approaches for reducing aggression in mice.

11. Does the colour of enrichment influence behaviour?

In some cases, yes, animals can show a preference for enrichment of one colour over another. If you are interested in this, we recommend searching the published literature to find out what is known about this topic for the species you are interested in. See this page for advice on how to carry out a search for published literature: <u>What to consider before you begin evaluating enrichment</u>.

12. How can masking (blinding) be implemented in a small facility?

Masking (blinding) prevents us from unintentionally influencing our results. In an ideal world we would mask all steps of an experiment, but this is not realistic in many experiments. When the difference is easily visible, which will be the case with most enrichment studies, you can mask by involving someone who is unaware of your expectations.

Masking requires at least two people – one who knows the question you are aiming to answer, and which animals will be exposed to which items and one who does not know this information. In small facilities you could find a buddy and ask them to help you with masking your study in return for you helping them with something they need an extra pair of hands for.

One way to mask could be to ask someone who does not know what you are expecting to see to score videos for behaviours or interactions with the enrichment item. Make sure the videos are coded so they cannot accidentally figure out what you are expecting (e.g. you don't want the videos labelled 'enrichment' and 'control'). If they are not aware of the purpose of your experiment their subconscious is less likely to influence how they score the videos.

Often the easiest step to mask is the data analysis so if resources are limited you could start by masking this step. See the 'Data analysis' section of <u>Improving the scientific quality of your enrichment study</u> for how to incorporate masking into your data analysis.

For more information on masking strategies see the NC3Rs resource page on <u>masking *in vivo*</u> <u>experiments</u> and the <u>paper</u> the resource page is based on.

13. What if enrichment has an impact on study parameters?

If this is a concern, an evaluation of environmental enrichment can be a useful approach to find out whether it is suitable for certain research conditions. Parameters could be compared between groups with and without the enrichment to investigate whether there is a reason to avoid a certain type of enrichment. Before you make any changes or begin an evaluation it is important to get all the necessary permissions in place.

For some scientific studies the inclusion of enrichment could interfere with the experimental outcomes. For example research focused on stress and anxiety will require a certain level of stress in the animals. In these cases enrichment could be used to reverse a physical or behavioural state of anxiety or stress in the animals, but careful discussion with the researcher will be necessary.

14. I thought standardising everything, including housing conditions, was important. Won't adding environmental enrichment interfere with this?

Before you make any changes to enrichment it is important to get all the necessary permissions in place. It is difficult to give 'catch all' advice on standardisation as this should be considered on a study-by-study basis and is something to discuss with the researchers running the project.

We would encourage anyone concerned about enrichment and standardisation to read <u>Refinement of</u> <u>rodent research through environmental enrichment and systematic randomization</u> by Hanno Würbel and Joe Garner. The topic of enrichment and standardisation is also covered in this <u>review paper</u> by Vera Baumans. There are questions around how useful standardisation is for generating reproducible and translatable results in animal studies. Although it does not relate directly to enrichment, this video on <u>biological variation and reproducibility</u> may also be useful to learn more about this subject.